

**STUDIES OF PHYSIOLOGICALLY ACTIVE ARTHROPOD SECRETIONS  
IX. MORPHOLOGY AND HISTOLOGY OF THE PHEROMONE-PRODUCING GLANDS  
OF SOME FEMALE LEPIDOPTERA<sup>1</sup>**

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**Abstract**

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The morphology and histology of the sex pheromone-producing gland in the females of *Choristoneura fumiferana* (Clemens), *C. pinus* (Freeman) (Tortricidae), and *Malacosoma disstria* (Hubner) (Lasiocampidae) is reported. A review of the literature relevant to such a study is presented in tabular form.

**Introduction**

Most current studies of pheromones of Lepidoptera are concerned with the isolation, purification, and characterization of the sex attractants and their possible use as control or monitoring agents. Jacobson (1965) reviewed the published work on the anatomy and physiology of the glands that produce sex pheromones in both male and female Lepidoptera, and since then several workers have published results of additional studies (Barnes *et al.* 1966; Jefferson *et al.* 1966, 1968; Roelofs and Feng 1968; Adeesan *et al.* 1969; Weatherston and Percy 1968, 1969, 1970; and Percy *et al.* 1971 and references contained therein).

A comparative account of the morphology and histology of the pheromone-producing glands of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Tortricidae), the jack pine budworm, *Choristoneura pinus* (Freeman) (Tortricidae), and the forest tent caterpillar, *Malacosoma disstria* (Hubner) (Lasiocampidae), is presented in this paper. Also discussed are the bumble bee wax moth, *Vitula edmandsae* (Packard) (Phycitidae), and the white-marked tussock moth, *Orgyia leucostigma* (J. E. Smith) (Lymantriidae), which were reported on earlier (Weatherston and Percy 1968; Percy *et al.* 1971).

**Materials and Methods**

The insects used in this study were reared in the laboratory on artificial diet (McMorran 1965). For histological studies virgin female adults 1 to 3 days old were used. Sections of the pheromone-producing gland were prepared, stained, and photographed using the method previously reported (Percy *et al.* 1971).

To determine whether there was a cyclic appearance and disappearance of vacuoles in the cells of the pheromone gland of the spruce budworm, the following procedure was used. One-day-old adult females were exposed to a 17 hour light (0330 to 2030) and 7 hour dark cycle at room temperature ( $72^{\circ} \pm 2^{\circ}\text{F}$ ) and relative humidity of 45 to 55%.

After 24 hours, and at each hour thereafter over a 48 hour period, the abdominal tip was excised from each of two moths (that is, the females were from 2 to 4 days old) and fixed in alcoholic Bouin's. The tips were processed for histological study as before.

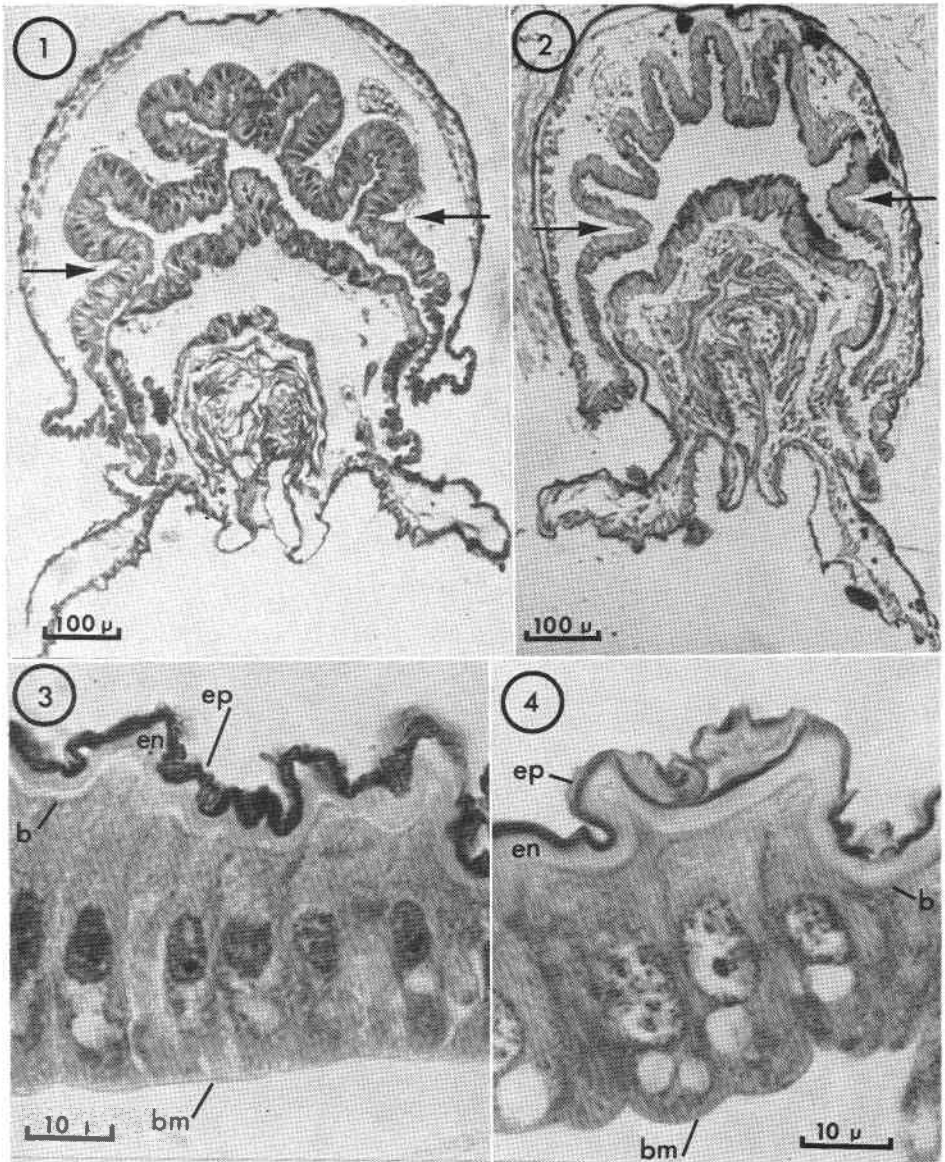
**Results**

*Morphology and Histology of Glands*

*Choristoneura fumiferana*

A female spruce budworm, when calling the male, exposes the sex pheromone-producing gland by flexing the abdomen ventrally and evaginating the modified intersegmental membrane which appears as a dorsal saddle between the eighth and

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FIGS. 1-2. Cross section through the invaginated sex pheromone-producing gland of *C. fumiferana* (Fig. 1) and of *C. pinus* (Fig. 2). The anterior convoluted portion is that part of the gland between the arrows.

FIG. 3. Columnar cells from the anterior convoluted portion of the sex pheromone-producing gland of *C. fumiferana*. ep, epicuticle; en, endocuticle; b, brush border; bm, basement membrane.

FIG. 4. Columnar cells from the lateral portion of the sex pheromone-producing gland of *C. pinus* (beneath arrows Fig. 2). ep, epicuticle; en, endocuticle; b, brush border; bm, basement membrane.

ninth abdominal segments (Sanders 1969; Weatherston and Percy 1970). In a cross section of the invaginated gland most of the secretory cells are seen in the dorsal convoluted area (Fig. 1). This area represents the most deeply invaginated portion of the intersegmental membrane and is particularly well seen under the scanning electron microscope (Weatherston and Percy 1970).

The gland consists of columnar epithelial cells (Fig. 3) which on the interior are limited by a thin basement membrane (bm). Basally, each cell membrane appears to have numerous projections (not shown in Fig. 3). A large ovoid nucleus is located in the central area of the cell. Below the nucleus are usually 1 or 2 large vacuoles; above the nucleus numerous tiny vacuoles are seen.

Study of the time-series slides indicated that although the size and number of the vacuoles varied, there was no definite cycle of appearance and disappearance. Further, there seemed to be no correlation between the size or number of small vacuoles and the size or presence of large vacuoles. The variability of the vacuoles also did not have any relationship to the apparent changing nature of the brush border (b) which for the most part remained as an indistinct band at the apical edge of the cell. When the brush border was observed under the electron microscope, it proved to consist of distinct microvilli. The thick endocuticle (en) is loosely laminated; the thin epicuticle (ep) is corrugated and is known to possess many small mammiform projections—'spikes.' These are very obvious under the scanning electron microscope (Weatherston and Percy 1970).

#### *Choristoneura pinus*

A calling female jack pine budworm evaginates the gland in the same manner as the spruce budworm (Sanders, pers. comm.). A cross section through the invaginated gland reveals that it too is a dorsal saddle very similar to that of the spruce budworm except that the dorsal folds have a different arrangement (Fig. 2). Here again the anterior convoluted portion contains most of the secretory cells.

The columnar epithelial cells of the gland are histologically identical to those of the spruce budworm except for a slight variation in size, those of the jack pine budworm being, on the average, a little larger. The cells seen in Fig. 4 occur in the lateral portion of the gland which explains their near-cuboidal appearance; they also illustrate that cells can vary in size depending on the area of the gland being observed. This variation in size probably results from the cells being more numerous and tightly packed in the anterior convoluted portion. Consequently they appear more columnar. In the invaginated gland this portion penetrates the body most deeply whereas in a completely evaginated gland the large columnar cells and their secretion will be more exposed than those less columnar and more laterally situated.

#### *Malacosoma disstria*

The presence of a sex attractant in the terminal abdominal segments of the forest tent caterpillar was reported by Struble (1970). Females adopt the calling position (Fig. 5) shortly after eclosion and, if undisturbed, remain calling for about half an hour. In the laboratory they are usually found hanging from the upper surface of the container with their wings spread slightly and abdominal segments 8 to 10 protruding. The sex pheromone-producing gland (the intersegmental membrane between segments 8 and 9) appears to the unaided eye as a large, smooth dorsal saddle (Fig. 5 arrow). There is no visible pumping of the gland as has been reported for other Lepidoptera (Richards and Thomson 1932; Dickens 1936; Gotz 1951; Brady and Smithwick 1968; Percy *et al.* 1971).



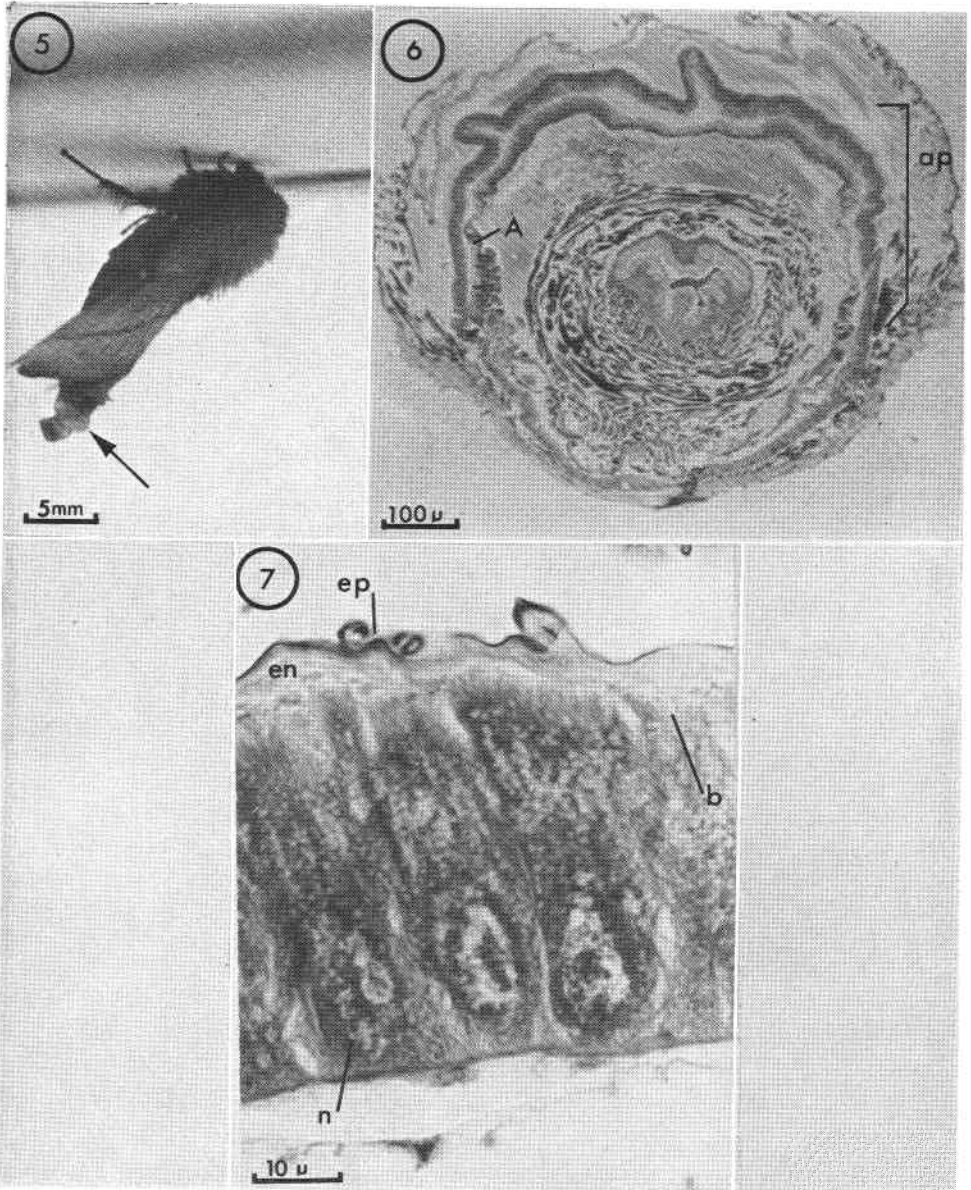


FIG. 5. Calling female *M. disstria*. Arrow indicates dorsal portion of sex pheromone-producing gland.

FIG. 6. Cross section through invaginated sex pheromone-producing gland of *M. disstria*. **ap**, anterior convoluted portion; **A**, antecosta.

FIG. 7. Columnar cells from sex pheromone-producing gland of *M. disstria*. **ep**, epicuticle; **en**, endocuticle; **b**, brush border; **n**, nucleus.

In a cross section of the invaginated gland (Fig. 6) it is seen that, unlike the *Choristoneura* species, the secretory cells completely encircle the abdomen in the anterior portion (ap) of the intersegmental membrane. Ventrally, the cells are somewhat smaller and less numerous but in other respects are identical to those on the dorsal surface which also contains longitudinal folds. In the posterior portion of the intersegmental membrane, on the other hand, the dorsal glandular epithelium does not extend further than the laterally situated antecostae (A).

Once again the secretory cells are columnar (Fig. 7). However, in *M. disstria* the large ovoid nuclei (n) are basally situated and the brush border (b) is more distinct. The remainder of the cell is filled with tiny vacuoles. The overlying endocuticle (en) is relatively thick and loosely laminated whereas the epicuticle (ep) is thin.

### Discussion

The sex pheromone-producing glands of female Lepidoptera studied thus far, with but two exceptions, consist of a modified intersegmental membrane situated between the eighth and ninth abdominal segments. In the butterfly, *Argynnis adippe*, the gland is situated on the intersegmental membrane between the seventh and eighth abdominal segments. In the salt-marsh caterpillar moth, *Estigmene acrea*, the gland is a complex structure located on the ninth abdominal segment and the papillae anales (MacFarlane and Earle 1970).

In Table I we present, in summary form, a comparison of the morphological and histological descriptions of sex pheromone-producing glands of female Lepidoptera. Five of the species were studied in this laboratory whereas the information about the other species was taken from the studies of other workers.

As can be seen from the table there is no consistent pattern of cell type with the particular kind of gland in the representative species of the eight families so far examined. In the case of the Gelechiidae, specifically *Pectinophora gossypiella*, El-Sawaf *et al.* (1968) stated that the gland is of the ring type, is situated on the ninth abdominal segment, and consists of columnar cells. On the other hand, Jefferson *et al.* (1971) indicate that the gland is a dorsal sac, is situated in the usual place, and consists of columnar cells. The only other species in this family which has been examined is the potato tuberworm moth, *Phthorimaea operculella*, and it is reported to have a dorsal sac-type gland with closely packed cuboidal cells (Adeesan *et al.* 1969).

Within the Phycitidae five species have been examined and they are all reported to possess ring glands. In the case of *Plodia interpunctella* the observations of Smithwick and Brady (1968) differ from those of Dickens (1936); the former authors indicate that the gland is situated only in the ventral part of the intersegmental membrane. Considering that the four other species show great similarity in possessing a ring gland which is deeper ventrally than dorsally, it may well be that Smithwick and Brady are in error.

In the Tortricidae the table lists five species of the sub-family Olethreutinae as having been examined and possessing a dorsal sac type of gland. However, in one of these species, namely, the strawberry leaf roller, *Ancylis comptana fragariae*, no glandular epithelial cells were found (Roelofs and Feng 1968).

Percy *et al.* (1971) reported that the cells of the sex pheromone-producing gland of the lymantriid *Orgyia leucostigma* are goblet-shaped. The number of cells is increased by a U-shaped arrangement and this unusual cell formation is thought to provide the female with a greater potential for pheromone production to com-

Table I. Morphological and histological description of sex pheromone-producing glands of female Lepidoptera

Family	No. of species examined	Gland area or type	Type of cell	Reference
Noctuidae				
Heliothinae	3	Ring	Cuboidal to slightly columnar	Jefferson <i>et al.</i> (1968)
Plusiinae	4	Dorsal sac	Columnar	Jefferson <i>et al.</i> (1968)
Amphipyridae	4	Ventral sac	Columnar	Jefferson <i>et al.</i> (1968) Jefferson and Rubin (1970)
Bombycidae	1	Sacculi laterales	Cuboidal	Steinbrecht (1964)
Gelechiidae	2	Dorsal sac*	Cuboidal	El-Sawaf <i>et al.</i> (1968) Adeesan <i>et al.</i> (1969) Jefferson <i>et al.</i> (1971)
Phycitidae	5	Ring gland deeper ventrally*	Columnar	Dickens (1936) Weatherston and Percy (1968) Smithwick and Brady (1968)
Tortricidae				
Olethreutinae	5*	Dorsal sac	Columnar	George (1965) Barnes <i>et al.</i> (1966) Roelofs and Feng (1968)
Tortricinae	8	Dorsal sac	Columnar	Roelofs and Feng (1968) Percy and Weatherston (this paper)
Lasiocampidae	1	Ring gland deeper dorsally	Columnar	Percy and Weatherston (this paper)
Lymantriidae	1	Dorsal fold	Goblet-shaped	Percy <i>et al.</i> (1971)
Arctiidae	1	Across dorsal part of 9th abdominal segment, ventral surface of dorsal papillae anales, and ventral surface of lateral papillae anales	Columnar and cuboidal	MacFarlane and Earle (1970)

\*See text for explanation.

pensate for the lack of depth of the gland and the insect's immobility—the female tussock is wingless.

The 50 or so families of Lepidoptera comprise many thousands of species. The pheromone-producing glands of only a very few have been examined critically and it is obvious that much work is still to be done before a pattern of gland structure will become apparent. Many more species representing more families must be examined before a clear understanding of the methods of pheromone production and release are understood.

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