

ABDOMINAL MUSCULATURE IN RELATION TO SEX PHEROMONE GLAND EVERSION IN FEMALES OF THREE SPECIES OF LEPIDOPTERA

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Abstract

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The posterior apophyses in terminal abdominal segments of female moths form part of the sex pheromone gland in each of three species examined (*Choristoneura fumiferana* (Clem.) (Tortricidae), *Trichoplusia ni* (Hübner) (Noctuidae), *Orgyia leucostigma* (J.E. Smith) (Lymantriidae)). Four groups of paired dorsolateral muscles are attached to the anterior or posterior apophysis and the integument. An additional group is attached to the anterior and posterior apophyses. The probable relationship of these muscles to the eversion, or protrusion, and inversion of sex pheromone glands is discussed.

Introduction

The sex pheromone gland in female moths is a modified intersegmental membrane located between the 8th and 9th abdominal segments (Percy and Weatherston 1974). The species used in the present study were *Choristoneura fumiferana*, *Trichoplusia ni*, and *Orgyia leucostigma*. In each the gland is a modification of only the dorsal part of the intersegmental membrane. The gland and terminal abdominal segments are telescoped within segment 7 while the females rest.

Females of each species have distinctive calling behaviour in which the pheromone gland is exposed to release sex pheromone which attracts males for mating. Females of *C. fumiferana* call continuously for several hours (Sanders and Lucuik 1972) and those of *T. ni* call for about 20 min (Sower *et al.* 1971). In both species the everted gland resembles a bulbous saddle (Jefferson *et al.* 1966; Sanders 1969; Weatherston and Percy 1970). Females of *O. leucostigma* also call for several hours; however, in this species the gland does not evert, rather it protrudes by a rhythmic protraction and retraction of segments 8, 9, and 10 (Percy *et al.* 1971).

The terminal abdominal segments of female moths contain paired anterior and posterior apophyses (Matsuda 1976). Apophyses are also an integral part of the sex pheromone glands (Percy *et al.* 1971; Percy and Weatherston 1971). The present study was undertaken to identify muscles associated with these apophyses and to attempt to relate the roles of these muscles in the control of eversion or protrusion of the sex pheromone glands.

Materials and Methods

The spruce budworm (*C. fumiferana*), the white-marked tussock moth (*O. leucostigma*), and the cabbage looper (*T. ni*) were reared in the laboratory on a synthetic diet (Grisdale 1973). Larvae and pupae of the spruce budworm and the tussock moth were kept at 24°C and 55–60% humidity. Adults were kept at ambient temperature. All stages of the cabbage looper were maintained at 27°C and 50% humidity.

Whole insects were fixed for 1 h by injection with 70% ethanol. The ventral surface of an insect was then opened to expose the dorsally-situated gland and the associated muscles. The gut and other organs in the region were carefully removed and the last four abdominal segments separated from the body. Scales were removed from each segment. The terminal segments were usually bisected sagittally into left and right portions prior to staining.

The tissue was immediately stained with Grenacher's borax carmine (Humason 1967) by immersing the tissue in the stain for 5 min after which 4 drops of concentrated hydrochloric acid were added. The tissue was left in the latter solution overnight at room temperature and subsequently differentiated with 3% concentrated hydrochloric acid in 70% ethanol, dehydrated, cleared and mounted.

Observations

In all three species examined, the anterior apophyses (aa) originate on the anterior borders of the 8th tergite either dorsolaterally (Figs. 1, 2, 4) or laterally (Fig. 3). The apophyses project anteriorly and fork immediately anterior to the 8th tergite in the spruce budworm and the tussock moth. The ventral arm of each apophysis surrounds the body to form part of the copulatory opening while the dorsal arm continues anteriorly to terminate blindly in the 7th segment (double arrows in Figs. 1, 2, 4). In the cabbage looper the anterior apophyses are straight and do not fork (double arrows in Fig. 3).

The posterior apophyses (pa) in all three species originate (encircled area in Figs. 5, 6) from the ovipositors (o). Immediately anterior to the ovipositors they form part of the lateral cuticular wall of the sex pheromone glands (arrows in Figs. 5, 6, 7). The posterior apophyses lie medial to the anterior apophyses within the abdomen and terminate freely in the 8th segment of the tussock moth (Fig. 4) and in the 7th segment of the cabbage looper (Fig. 3) and the resting spruce budworm (Fig. 1). In calling spruce budworm the posterior apophyses terminate in the 8th segment and are curved rather than straight as in the resting insect.

Muscles associated with the apophyses and therefore with the pheromone glands are positioned dorsally or laterally within abdominal segments 7 and 8 in all three species (Figs. 8-10). There are 8 groups of these bilaterally paired muscles which, in the following description, are designated as groups 1, 2, 3, 4, 5 (each member of a pair attached to an anterior apophysis) and groups 1P, 2P, 3P (each member of a pair attached to a posterior apophysis). Groups 1 and 3 consist of more than one pair of muscles in which case each pair has been assigned a letter, i.e., 1a, 1b, 1c, 3a, 3b. The musculature of the three species differs in details. Individual variations are listed after the general description for each group.

Group 1. These muscles originate on the anterior margin of the 7th tergite immediately posterior to the intersegmental membrane between the 6th and 7th segments. They insert at the anterior free end of the anterior apophyses.

Spruce budworm — a single pair of muscles (1)

Cabbage looper — 2 distinct pairs of muscles (1a, 1b)

Tussock moth — 4 distinct pairs of muscles (1a, 1b, 1c, 1d) with 1a and 1b oblique and mediodorsal and 1c and 1d laterodorsal

Group 1P. These muscles originate immediately posterior to the intersegmental membrane between the 6th and 7th segments and insert at the anterior end of the posterior apophyses.

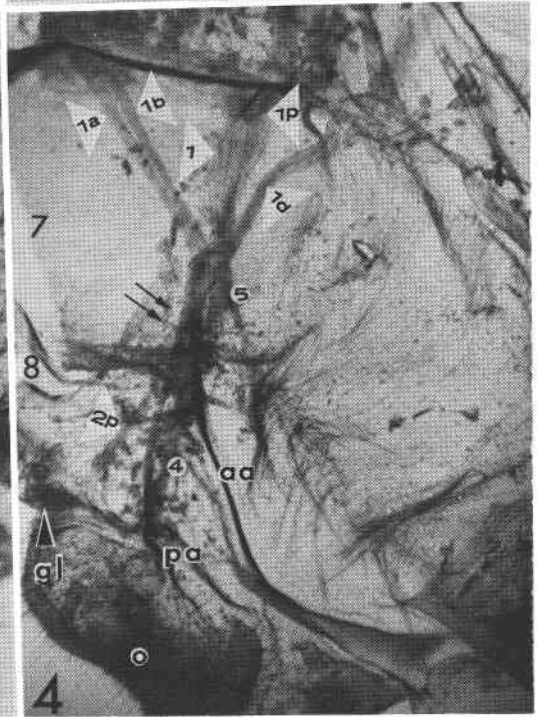
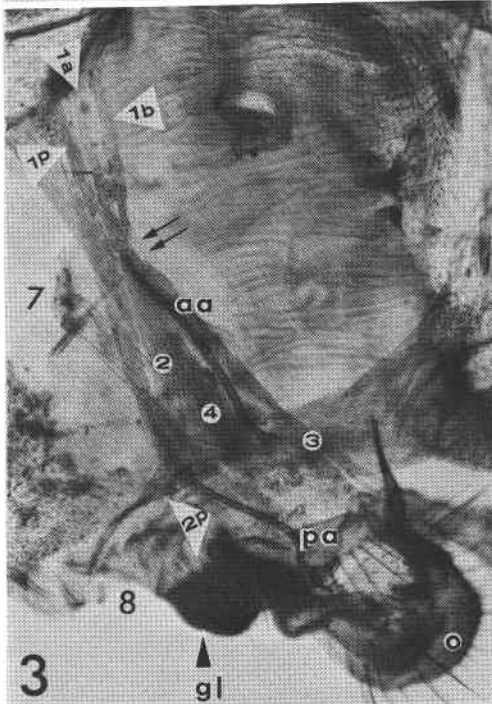
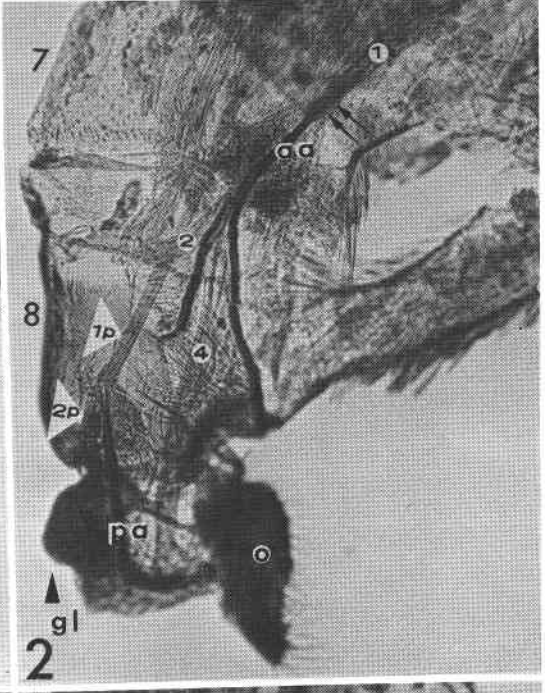
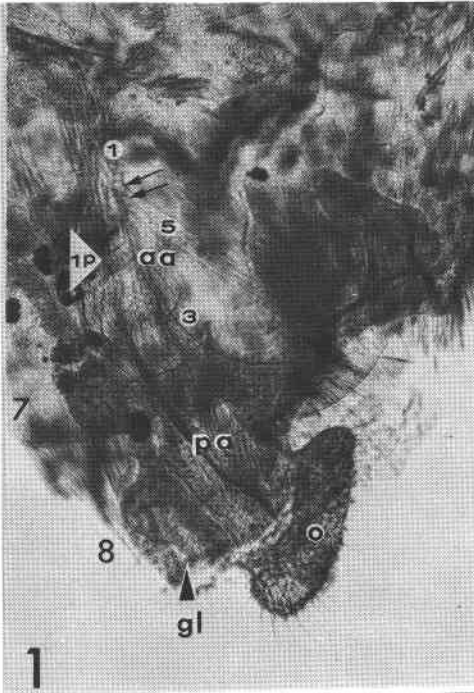
Figs. 1-4. Whole mounts of terminal abdominal segments illustrating apophyses and muscles associated with the glands. These whole mounts represent one side of each insect thus illustrating only one member of each pair of muscles. The black numbers on white background designate the groups of muscles. Anterior apophysis, **aa**; gland, **gl**; ovipositors, **o**; posterior apophysis, **pa**; **7** and **8** designate the 7th and 8th abdominal segments.

(1) Spruce budworm with gland inverted. The groups of muscles illustrated are 1, 1P, 3, 5. 40×.

(2) Spruce budworm with gland everted. The groups of muscles illustrated are 1, 1P, 2, 2P, 4. The anterior termination of the anterior apophyses (aa) is designated by double arrows. 40×.

(3) Cabbage looper with gland everted. The groups of muscles illustrated are 1 (a, b), 1P, 2, 2P, 3, 4. The anterior termination of the anterior apophyses (aa) is designated by double arrows. 40×.

(4) Tussock moth with gland inverted. The groups of muscles illustrated are 1 (a, b, c, d), 1P, 2P, 4. (Muscle 3P lies adjacent to 4 and cannot be distinguished in this figure — see Fig. 6). 20×.



Spruce budworm — 1 pair of muscles (1P) which originate mediodorsally to muscle 1

Cabbage looper — 1 pair of muscles (1P) which originate dorsally to muscle 1a

Tussock moth — 1 pair of muscles (1P) which originate ventrolaterally to muscles 1a, 1b, 1c and mediodorsally to muscles 1d. Some of the fibers are attached to termination of anterior apophysis adjacent to muscles 1a, 1b, 1c, 1d

Group 2. These relatively short muscles originate at the posterior laterodorsal margins of the 7th tergite and insert on the anterior apophyses.

Spruce budworm — 1 pair of muscles (2) which insert medially at the fork of the apophyses

Cabbage looper — 1 pair of muscles (2) which insert externally at the terminations of the apophyses

Tussock moth — These muscles are not present. Muscles originate from a similar position but their insertion is on the laterodorsal cuticle in the anterior half of the 7th segment

Group 2P. These originate at the posterior laterodorsal margins of the 8th tergite and insert externally at the anterior terminations of the posterior apophyses. There is 1 pair of muscles (2P) in each of three species with no variation.

Group 3. These muscles originate on the anterior apophyses and insert lateroventrally at the anterior edge of the ventral intersegmental membrane between the 8th and 9th abdominal segments.

Spruce budworm — 1 pair of muscles (3) originating ventrally at the fork of the apophyses and insert posteriorly and dorsolaterally to the copulatory opening

Cabbage looper — 2 muscle pairs (3a, 3b); they originate ventrally at the anterior end of the apophyses and insert lateral and posterior to the ovipore

Tussock moth — There are no comparable muscles in this insect

Group 3P. These originate near the anterior ends of the posterior apophyses and run posteriorly with different insertions in each insect.

Spruce budworm — 1 pair of muscles which insert on the rectum

Cabbage looper — 1 pair of muscles (3P) which run dorsally to insert lateral to the anus

Tussock moth — 1 pair of muscles which insert ventrolaterally on the intersegmental membrane between the 8th and 9th segments immediately posterior to the ventral arms of the anterior apophyses, that is, insertion similar to those of group 3 in the spruce budworm and the cabbage looper but the origin differs

Group 4. These muscles originate on the anterior apophyses and insert on the portions of the posterior apophyses which form part of the walls of the glands.

Spruce budworm — 1 pair of muscles (4) which originate medially near the forks of the anterior apophyses and insert ventrolaterally on the posterior apophyses

Cabbage looper — 1 pair of muscles (4) which originate externally at the anterior termination of the apophyses and insert ventrolaterally on the posterior apophyses

Tussock moth — 1 pair of muscles (4) which originate externally and anteriorly to the forks of the anterior apophyses and insert medially and externally on the posterior apophyses

Group 5. These originate at the anterior terminations of the anterior apophyses and insert ventrolaterally at the posterior margin of the 7th segment.

Spruce budworm — 1 pair of muscles (5) which insert anteriorly and dorsally to the copulatory opening

FIGS. 5-7. Whole mounts of terminal abdominal segments showing points of attachment ($\rightarrow \leftarrow$) of posterior apophyses (pa) to the glands (gl). The encircled area in each figure represents the origin of the posterior apophyses on the ovipositors (o). 7 and 8 designate the 7th and 8th abdominal segments. Only the posterior apophyses for the right half of each insect is shown although there are identical structures on left half.

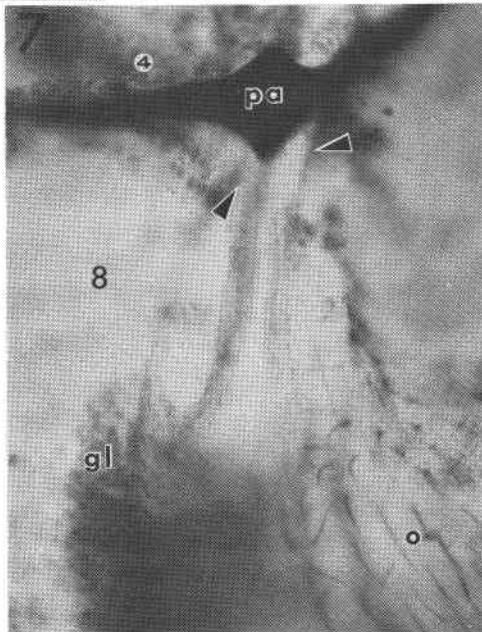
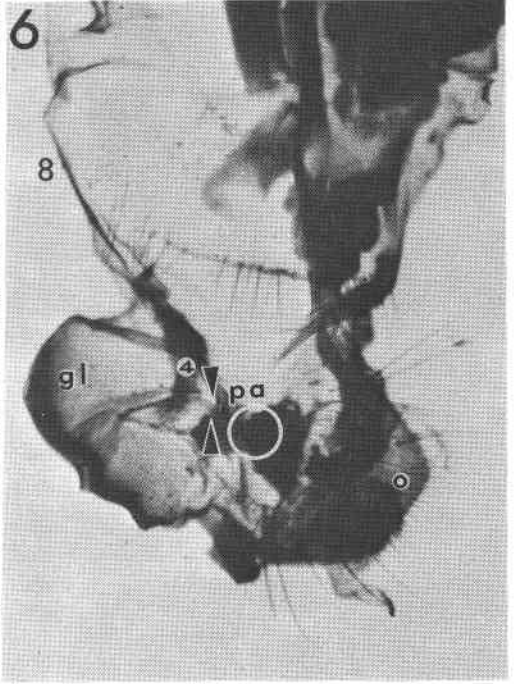
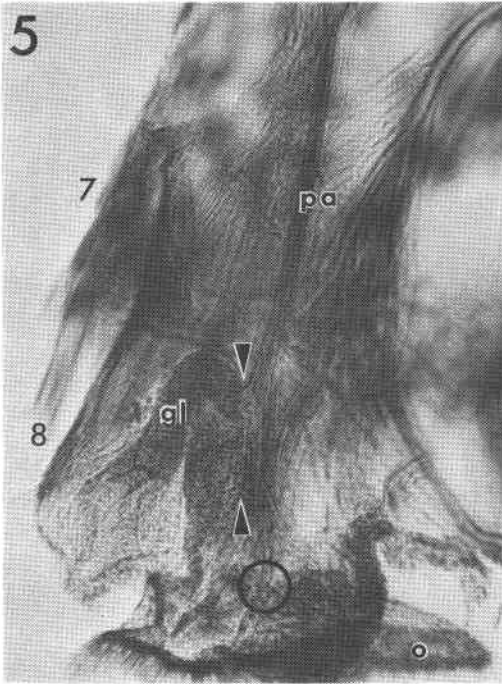
(5) Spruce budworm. Muscles of group 4 are illustrated (black number on white background). Stained with borax carmine. 80 \times .

(6) Cabbage looper. Muscles of group 4 are illustrated. Reacted with diaminobenzidine and with gland cells removed. 50 \times .

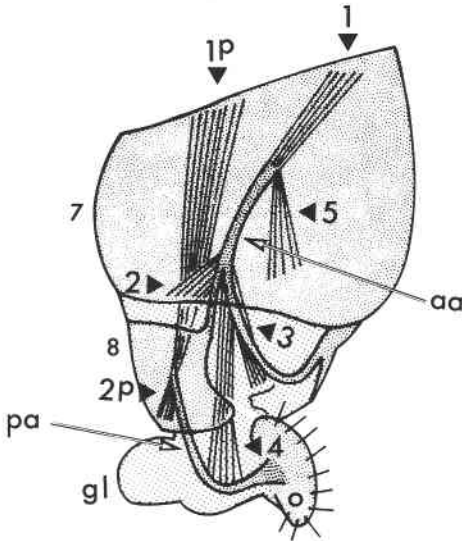
(7) Tussock moth. Muscles of group 4 are illustrated. Stained with borax carmine. 100 \times .

Cabbage looper — The muscles are not present in this insect

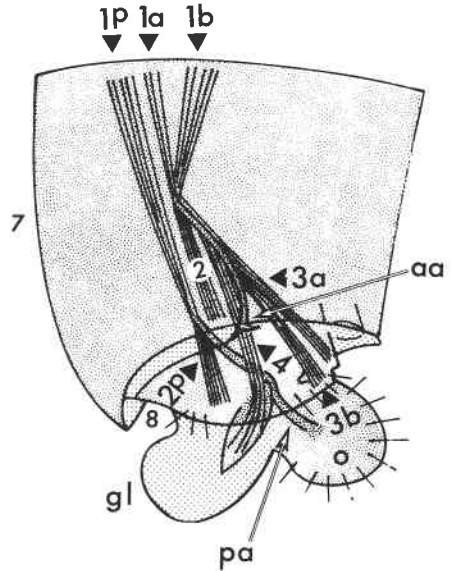
Tussock moth — 1 pair of muscles (5) which insert anteriorly and dorsally to the copulatory opening



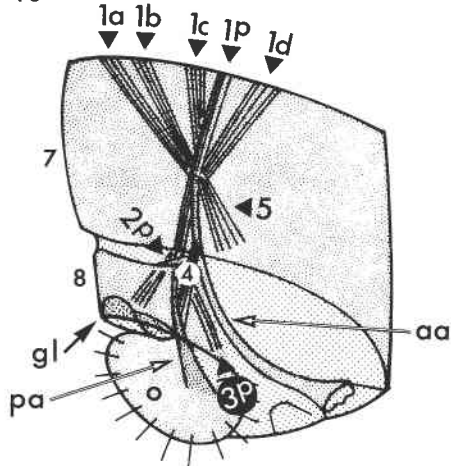
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FIGS. 8-10. Semidiagrammatic interpretation of muscles and apophyses (aa, pa) associated with sex pheromone glands (gl) as viewed laterally and illustrating only the right member of each pair. Anterior apophysis, aa; gland, gl; ovipositor, o; posterior apophysis, pa; 7 and 8 designate the 7th and 8th abdominal segments.

(8) Spruce budworm. Groups of muscles illustrated are 1, 1P, 2, 2P, 3, 4, 5.

(9) Cabbage looper. Groups of muscles illustrated are 1 (a, b), 1P, 2, 2P, 3 (a, b), 4.

(10) Tussock moth. Groups of muscles illustrated are 1 (a, b, c, d), 1P, 2P, 3P, 4, 5.

Other muscles. These may not be as closely related to eversion or protrusion of the gland but nevertheless may play a role.

Spruce budworm — muscles which originate on the anterior apophyses and terminate on the gut and common oviduct

Cabbage looper — muscles which originate on the anterior apophyses and terminate on the common oviduct

— dorsal muscles which originate near the intersegmental membrane between the 6th and 7th segments and insert near the membrane between the 7th and 8th segments

Tussock moth — dorsal muscles which originate near the intersegmental membrane between the 6th and 7th segments and insert near the membrane between the 7th and 8th segments

Discussion

The muscles associated with the sex pheromone glands in all three species are attached to the two pairs of apophyses which have as their origin tergites of the segments, 8 and 9. Therefore, these muscles represent some of the musculature from two abdominal segments and can (perhaps) be homologized to those groups of muscles listed and described by Snodgrass (1935). These groups of muscles are summarized in Table I and include the internal dorsals, the reversed external dorsals, and the laterals.

The internal dorsals are composed primarily of longitudinal fibres of segmental length and are attached near successive intersegmental folds or may shift their origins to the postcostal regions of the folds. Functionally, they act as abdominal retractors (Snodgrass 1935). In the present study groups 1, 1P and 4 are internal dorsals. As abdominal retractors they could also cause inversion of the glands by withdrawing segments 9 and 10 into segment 8.

Reversed external dorsals are short longitudinal muscles seldom of segmental length and are often oblique. When their origins are transposed to the posterior margins of the terga they become functionally antagonistic to the internal dorsals

Table I. Muscles associated with the sex pheromone glands and their probable functions in eversion (or protrusion) and inversion of the glands

Group	Type of abdominal muscle*	Function in abdomen (Snodgrass 1935)	Presumed function as related to gland	No. of pairs in each group		
				Spruce budworm	Cabbage looper	Tussock moth
1	Internal dorsals	Retractor	Inversion	1	2	4
1P	Internal dorsals	Retractor	Inversion	1	1	1
2	Reversed external dorsals	Protractor	Eversion (or protrusion)	1	1	Absent
2P	Reversed external dorsals	Protractor	Eversion (or protrusion)	1	1	1
3	Laterals	Compressor	Eversion	1	2	Absent
3P	Laterals	Compressor	Eversion (or protrusion)	1	1	1
4	Internal dorsals	Retractor	Inversion	1	1	1
5	Laterals	Compressor	Eversion (or protrusion)	1	Absent	1

*Accepted terminology for these muscles of abdominal segments (Snodgrass 1935).

and become abdominal protractors (Snodgrass 1935). In the present study, groups 2 and 2P are reversed external dorsals whose origins are located on the posterior margins of terga. Therefore, these muscles as abdominal protractors may cause eversion (or protrusion) of the pheromone glands by decreasing the overlap of the segments and allowing the gland to be exposed.

The laterals are usually intrasegmental in position and tergo-sternal in attachments but they may cross obliquely from one segment to the next. Functionally, they act as compressors of the abdomen (Snodgrass 1935). In the present study group 3, 3P and 5 are laterals. These muscles may assist in eversion, or protrusion, of the dorsal glands by compressing the abdomen laterally and causing the abdomen to bulge at a weak point, i.e., the glandular intersegmental membrane.

The literature contains a single report of musculature relating to sex pheromone gland eversion in a female moth, the cabbage looper (Jefferson *et al.* 1966). As reported by these authors the gland is everted by two groups of longitudinal muscles. One set "has its origin on the posterior margin of the seventh tergum and is inserted on the anterior end of the apophyses anteriors and the other set originates on the posterior margin of the eighth tergum and is inserted on the anterior end of the apophyses posteriors." These muscles can be related to those of the present study as follows: the first set represents group 2 while the second set represents group 2P. However, Jefferson *et al.* (1966) may have overlooked the lateral muscles (groups 3 and 3P), which may presumably help cause eversion of the pheromone gland.

Inversion of the gland of the cabbage looper has been reported to occur by means of "longitudinal muscles which have their origin in the seventh segment and insert on the valves, the common oviduct, the apophyses anteriors and the apophyses posteriors" (Jefferson *et al.* 1966). These muscles can be related to those of the present study as follows: those which insert on the valves (= ovipositors) actually insert on the lateral edges of the gland as group 4, those which insert on the posterior apophyses are group 1P.

As determined from the present study, the number of muscles controlling the eversion, or protrusion, of the sex pheromone gland varies with the species. The spruce budworm has five groups of muscles (2, 2P, 3, 3P, 5), the cabbage looper has four groups (2, 2P, 3P, 5), and the tussock moth has three groups (2P, 3P, 5). In the last species the surface of the gland is exposed only by rhythmic protraction and retraction of the abdominal segments, that is, the gland is not completely everted, therefore, one would expect fewer muscles to be involved in its protrusion. Inversion of the glands appears to be controlled by three groups of muscles which are present in all three species.

These muscles may also participate in other physiological activities of the terminal abdominal segments. For example, they are almost certainly used during oviposition when the terminal abdominal segments are also extended. The gland is not everted during oviposition, in either the spruce budworm or the cabbage looper although it is exposed in the tussock moth (J. Percy, pers. obs.).

The presence of muscles associated with the gland does not exclude the possibility that eversion and inversion may be influenced by the presence of, or factors from, other tissues. The circulating haemolymph may contain factors (hormones?) necessary for gland eversion and this in turn may be assisted by changes in haemolymph pressure.

In summary, it appears that the musculature of the terminal abdominal segments of female Lepidoptera can, at least partially, control eversion of the sex pheromone glands. However, physiological experiments are necessary to accurately determine the functions of these muscles.

Acknowledgment

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