



Descriptions of the life cycle, external embryogenesis and thoracic gland development of the cottonwood beetle, *Chrysomela semota* Brown (Coleoptera-Chrysomelidae)
by Linda Susan Stewart

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in ENTOMOLOGY
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Abstract:

The life cycle and external embryogenesis of *Chrysomela semota* Brown (Coleoptera, Chrysomelidae) were observed under laboratory and field conditions. Eggs incubated at 25°C hatched in an average of 4.5 days, and the larvae passed through three instars. Pupation required five to seven days. Sexually immature adults overwintered before beginning the reproductive phase of their life cycle.

External embryogenesis was divided into 15 stages, and each stage was defined morphologically. The germ rudiment first appeared at 25 hours at the posterior pole and ventral surface of the egg. No blastokinesis occurred.

Development of thoracic defense glands begins at Stage 7. The glands, with their associated secretory cells, result from invaginations of the ectoderm in the mesothoracic and metathoracic segments. Three pairs of muscles are associated with each gland, two attached to the neck of the reservoir and one attached to the ventral surface of the reservoir. The glands reach definitive differentiation at Stage 15, and begin secreting shortly after the embryo hatches.

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DESCRIPTIONS OF THE LIFE CYCLE, EXTERNAL EMBRYOGENESIS AND THORACIC
GLAND DEVELOPMENT OF THE COTTONWOOD BEETLE, *CHRYSOMELA*
SEMOTA BROWN (COLEOPTERA - CHRYSOMELIDAE)

by

LINDA SUSAN STEWART

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of the requirements for the degree

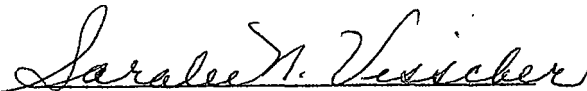
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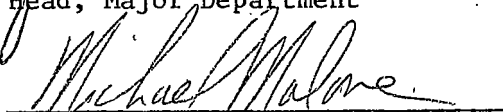
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ABSTRACT

The life cycle and external embryogenesis of *Chrysomela semota* Brown (Coleoptera, Chrysomelidae) were observed under laboratory and field conditions. Eggs incubated at 25°C hatched in an average of 4.5 days, and the larvae passed through three instars. Pupation required five to seven days. Sexually immature adults overwintered before beginning the reproductive phase of their life cycle.

External embryogenesis was divided into 15 stages, and each stage was defined morphologically. The germ rudiment first appeared at 25 hours at the posterior pole and ventral surface of the egg. No blastokinesis occurred.

Development of thoracic defense glands begins at Stage 7. The glands, with their associated secretory cells, result from invaginations of the ectoderm in the mesothoracic and metathoracic segments. Three pairs of muscles are associated with each gland, two attached to the neck of the reservoir and one attached to the ventral surface of the reservoir. The glands reach definitive differentiation at Stage 15, and begin secreting shortly after the embryo hatches.

INTRODUCTION

Chrysomela semota Brown was recognized as a new species in 1956 (Brown, 1956). Prior to this time members of the species were either misidentified or considered to be varieties of closely related species. Since many *Chrysomela* species are morphologically similar, classification standards require the collection of a series of specimens with their host plant data and localities.

Chrysomela semota ranges from northwestern Montana to east-central British Columbia, the Mackenzie Valley, and Great Slave Lake. It is considered to be economically unimportant, feeding on *Salix* and *Populus* species, with those in Montana known to feed only on *P. trichocarpa* T & G (Brown, 1956).

Like other species of *Chrysomela*, the larvae of *C. semota* exhibit segmentally arranged eversible defense glands. These glands secrete a whitish, highly volatile fluid, possibly salicylaldehyde, which is thought to have a repugnant odor to would-be predators (Hinton, 1951; Garb, 1915).

The life histories of all species of *Chrysomela* are thought to be similar, but most of the literature concerning the American species consists of fragmentary notes (Brown, 1956). Search of the literature failed to reveal any information concerning the embryological development of *C. semota*, and very little concerning any other species of *Chrysomela*.

The present investigation was undertaken to describe the life cycle of *C. semota*, to describe the external embryogenesis, and to trace the internal development of the larval defense glands.

LITERATURE REVIEW

Few complete works of coleopteran embryology appear in the literature. The most complete recent works were done on *Lytta viridana* Le Conte (Meloidae) (Rempel and Church, 1969, 1971; Church and Rempel, 1971). These articles are almost entirely devoted to internal embryogenesis, as are Ullmann's (1964, 1967) works on *Tenebrio molitor* L. (Tenebrionidae).

There are few complete works on the embryology of chrysomelid species. The internal embryogenesis of *Euryope terminalis* Baly (Paterson, 1931, 1932) and *Corynodes pusis* (Paterson, 1935) has been described, but notes concerning external development are fragmentary. Krysan (1976) has studied the early internal and external development of *Diabrotica undecimpunctata howardi* Barber in detail. The early external development of *Chalcoides aurata* and *C. fulvicornis* F. was described by Zakhvatkin, 1967, but the later stages were not included. In another article Zakhvatkin (1968) has compared, among other characteristics, the differentiation of the blastoderm and the position of the embryonic region among seventeen species of *Chrysomela*. A complete set of staging criteria was developed for the external embryogenesis of *Atrachya menetries*; Faldermann (Miya, 1965).

Brown (1956) described a general life history for chrysomelids, and Garb (1915) and Hinton (1951) both described the structure of the dorsolateral defense glands in the larval forms. Analysis of the

defense gland exudate by gas chromatography and mass spectral analysis of closely related *C. scripta* showed it to be salicylaldehyde (Wallace and Blum, 1969).

MATERIALS AND METHODS

Rearing

Chrysomela semota was collected within a two square mile area around Pierce Lake, 17 miles north of Seeley Lake, Missoula County, Montana. Eighteen pairs of copulating beetles were collected on May 27 and 28, 1978. Each pair was placed in a separate vial with several leaves of the host plant, *Populus trichocarpa*. They were transported to the laboratory on ice in a cooler chest and kept in a cold room maintained at 1 - 3°C until they were caged.

Each pair was housed in a 200 ml glass container covered with a nylon net top secured by a rubber band. Fresh leaves of *P. trichocarpa* were provided daily. Rearing temperature was 16 - 20°C. Collection data, food consumption, mating and egg deposition were recorded for each pair.

During the egg laying period, cages were examined at four-hour intervals, from 6:00 a.m. until 11:00 p.m. Leaves with attached eggs were transferred to moistened filter paper in a 3.5 cm plastic tissue culture dish. The eggs were incubated at 25°C until they hatched.

Larvae were removed from the incubator and were provided with fresh leaves daily. They were maintained in the culture dish until the second instar when they were transferred to glass cages and reared as described above for the adults. Larvae were allowed to pupate in the cages. Following emergence from the pupa, adults were

kept for further observation.

Fixation and Preservation of Materials

For each hour of development from zero hour to eclosion, eggs were removed from the incubator and fixed in hot alcoholic Bouin's solution followed by storage in 70% ethanol. Eggs in the early stages were fixed at fifteen minute to one-half hour intervals. After removal of the chorion, vitelline membrane, and serosa, embryos stained with eosin were drawn to scale with the aid of a 0.1 mm grid in the eyepiece of a Wild dissecting scope. From these drawings staging criteria were developed based on the distinct external morphological characteristics.

Histological Technique and Procedures

Embryos to be sectioned were fixed in alcoholic Bouin's as previously described, then double-embedded in methyl - benzoate - cellodine followed with paraffin (Humason, 1962). For each stage of development three embryos were serially sectioned at eight microns both transversely and longitudinally. Sections were stained with Harris' hematoxylin followed by eosin counterstain, then mounted in Histoclad (Clay-Adams, Inc.).

Photographic Techniques

Photographs of the serial sections were made using a 35 mm Carl Zeiss camera, photometer, and microscope. The objectives used were: a 10 X apochromatic, a 16 X neofluar, a 25 X planapochromatic, and a

40 X oil immersion apochromatic. Kodak 35 mm Panatomic-X B & W film was used for all the photographs, and it was developed commercially.

RESULTS

Life History

Mating and Egg Stage. Only mating pairs of beetles were collected for rearing in the laboratory. Sexes were readily determined by size, the female being 1 to 1.5 mm larger than the male. Upon removal from the cold room some beetles resumed mating immediately. Others did not resume mating for up to six days, the average being four days. Females mated for two to three days prior to egg deposition, and again immediately thereafter. Frequently the male was mounted on the female while she laid her eggs. In one instance the male was observed to mate with the female after the deposition of each egg. However, such frequent mating does not seem to be required to produce viable eggs. One female produced nine masses of eggs within the month following the male's escape from the cage, and some of the eggs in the ninth mass hatched.

Eggs were usually deposited on the undersurface of a leaf, always with the posterior end of the egg adhering to the leaf. Occasionally eggs were found on the upper leaf surface, on the sides of the glass cage, or on the nylon netting covering the mouth of the cage. The females appeared to "pat" each egg with their ovipositors immediately following deposition, apparently to insure its adherence to the leaf surface and the other eggs in the mass. Eggs within a mass were usually arranged in rows of six to ten, with the long axis

of each egg perpendicular to the surface of the leaf. The number of eggs in a mass ranged from 10 to 41, the most common numbers being between 32 and 36. Some females consistently laid the same number of eggs in successive masses, while others laid egg masses varying in number by as many as five eggs. Females deposited up to nine masses during the course of the summer, with the average being 5.2.

On the average, eggs were 1.4 mm long and 0.6 mm wide. When first laid, they appeared bright orange-red and were covered with a thin film of sticky material. Within 24-36 hours they developed yellow tips. This lighter coloration gradually spread over the surface until it became entirely yellow just prior to hatching. At the definitive stage, embryos were visible through the semi-transparent egg membranes. When incubated at 25°C the eggs usually hatched in an average of 4.5 days, and ranged from 3 to 6 days.

Larval and Pupal Stages. Newly hatched and molted larvae were transparent yellow in color, but they darkened to black within approximately ten minutes. Upon hatching the larvae consumed at least a portion of the egg membranes before moving off in search of food.

Three larval instars were observed prior to pupation. In the first instar the larvae from a single egg mass remained tightly grouped. At this stage only the mesothoracic and metathoracic defense glands appeared functional. In the second instar the seven pairs of abdominal defense glands became functional, and the larvae began to disperse.

Bright white or yellow spots appeared laterally to the defense glands following ecdysis to the third instar, and during this stage the larvae were widely dispersed.

Larvae in all instars possessed a flattened caudal disc used to anchor themselves to the host plant during feeding, molting and pupation. Prior to pupation the third instar larvae attached themselves head downward to a leaf, branch or the side of the glass cage. The prepupal stage lasted for an average of 2.2 days. The subsequent pupal stage hung from the caudal portion of the third larval exuvium.

Adults. Adult beetles emerged in five to seven days, and were light yellow in color, but gradually darkened as the cuticle hardened. This color change took about an hour.

Younger beetles were easily distinguished from older ones as the former had shiny elytra, while the elytra of the latter were dull with orange colored edges.

Feeding. Various portions of the leaves of the host plant were utilized during development. First instar larvae appeared to feed only on the upper and lower epidermal portion of the leaf, but by the third instar feeding activities destroyed all of the leaf except for the vascular tissues. Newly emerged adults fed voraciously for three to five days, consuming all portions of the leaf, including vascular tissue. This was followed by a rapid decline to a non-feeding state and apparent dormancy. Beetles emerging from hibernation in the

spring fed heavily until the first egg masses were deposited, again utilizing all portions of the leaf. Feeding declined gradually thereafter, with females continuing to feed longer than males.

Development in Nature. Notes were taken on the natural population of *C. semota* at Pierce Lake during the summers of 1976-80. Adult beetles first appeared during the last part of May, just as *P. trichocarpa* began to leaf out. At this time many of the beetles were found in the unfurled leaves. Beetles were almost always found on the terminal leaves of immature plants less than two meters in height. During the course of the summer the females laid at least two or possibly three masses of eggs, always on the undersurface of the terminal leaves. Larvae utilized these and surrounding leaves until just prior to pupation. Pupae were usually found hanging from leaves or leaf petioles of the host plant, but some were observed on other plants adjacent to the host such as *Larix occidentalis* and *Salix* species. In nature the life cycle required a longer period for development than was observed in the laboratory.

Predation in Nature. Various predators of *C. semota* were observed. That most frequently encountered was the larvae of an unidentified species of Syrphidae. These were observed consuming eggs of *C. semota* in all stages of development. Individuals from wild populations were observed in the laboratory consuming first instar larvae. A nymphal predator of the larval and prepupal stages was

identified as a pentatomid, *Podisus modestus* Dallas. These would pierce the larvae or prepupae and extract the body juices. Adult beetles were observed caught and wrapped in spider webs, but whether or not they were actually utilized by the spiders was not observed.

External Embryogenesis

At Stage 1 the first visible portion of the embryonic rudiment appears at hour 25 (Plate 1, fig. 1, er). It is located on the ventral and posterior surfaces of the egg, and represents approximately the middle third portion of the future embryo. A portion of the protocorm lies immersed in the yolk on the dorsal surface of the egg. Gastrulation has begun, forming a gastral groove on the ventral surface of the embryonic rudiment (Plate 1, fig. 1, gg).

The embryonic head, or procephalon, is visible at Stage 2 (Plate 1, fig. 2, prc), and the stomodaeum (Plate 1, fig. 2, st) has begun to invaginate. The posterior portion of the protocorm has risen from the yolk and is located on the surface of the egg.

Stage 3 is characterized by the elongation of the germ band, resulting in the head region being pushed forward to the anterior end of the egg (Plate 1, fig. 3). Differentiation of the gnathal and thoracic regions is evidenced by the rippling of the lateral edges of the germ band.

At Stage 4 (Plate 2, fig. 4) the gastral groove is no longer

visible on the surface of the germ band. Transverse furrows segment the germ band into distinct gnathal and thoracic regions. Three distinct segments comprise the gnathal region, the mandible, the maxilla and the labium (Plate 2, fig. 4, md, mx, lb). Segmentation in the head region also results in the formation of the antennary segment at the posterior edge of the protocephalic lobe (Plate 2, fig. 4, at). The prothorax, mesothorax and metathorax (Plate 2, fig. 4, p, m, mt) constitute the three segments of the thoracic region. The abdominal region (Plate 2, fig. 4, ab) has elongated anteriorly on the dorsal surface of the egg, resulting in the germ band encircling slightly more than three-fourths of the yolk.

Further elongation of the abdominal region occurs at Stage 5 (Plate 2, fig. 5). Transverse grooves demarcate ten abdominal segments, and the telson (Plate 2, fig. 5, te). Limb buds appear on all three thoracic segments (Plate 2, fig. 5, le). New developments in the protocephalic region include the appearance of the labrum (Plate 2, fig. 5, lr) anterior to the stomodaeum, and paired swellings on the gnathal segments which constitute rudiments of the gnathal appendages.

Stage 6 (Plate 2, fig. 6) is characterized by three major events. First, the lateral edges of the embryo begin to grow toward the dorsal surface of the egg. This will eventually result in dorsal closure when the two edges meet on the dorsal surface of the egg prior to

hatching. Second, after reaching maximum length at Stage 5, the embryo begins to shorten and thicken in the abdominal region. This will continue through Stage 7. Third, the thoracic appendages lengthen and now appear tripartite even though they have not yet undergone complete segmentation.

Other events which characterize embryos at Stage 6 of development include a slight migration of the labrum (Plate 2, fig. 6, lr) to a more terminal position on the head, and a slight shift of the antennary rudiments (Plate 2, fig. 6, at) so that they appear perpendicular to the longitudinal axis of the egg.

Spiracles first appear at Stage 7 (Plate 3, fig. 7, sp), resulting from invaginations of the ectoderm. The thoracic region develops two pairs of spiracles, one pair located on the mesothorax and the other pair on the metathorax. Additionally, one pair of spiracles appears on each of the first seven abdominal segments. On the thorax the spiracles are located anteriorly to the appendages and have a slit-like appearance, while in the abdomen they are located near the antero-lateral margin of each segment and appear as pinpoint invaginations. No spiracles are found on the prothorax or on abdominal segments eight, nine and ten.

Other developments at Stage 7 include the previously mentioned shortening and thickening of the abdominal region, and further dorsal growth of the lateral walls of the embryo so that the edges occupy a

medial position in the egg. The gnathal appendages lengthen and the mandible and maxilla begin to differentiate from one another (Plate 3, fig. 7, md, mx, lb). The antennal rudiments (Plate 3, fig. 7, at) move forward to lie anteriorly to the gnathal appendages.

At Stage 8 (Plate 3, fig. 8) the most significant developmental changes occur in the head and thorax. The gnathal segments appear to undergo a reduction in size, resulting in the gnathal appendages coming to lie in a closer proximity to the protocephalic lobes (Plate 3, fig. 8, prc). The labium (Plate 3, fig. 8, lb) lengthens and begins to take on its definitive embryonic shape. The thoracic appendages lengthen, extending over two abdominal segments posteriorly, and they begin to show definite segmentation between the future coxa and femoral regions (Plate 3, fig. 8, cx and fe). Stage 8 is the last stage in which the telson is readily visible.

Most of the externally visible changes at Stage 9 (Plate 3, fig. 9) are due to growth and continued maturation of features already discussed. The gnathal segments become encompassed within the cephalon, so that three concise body regions are visible, the head, thorax, and abdomen. The antennal rudiments (Plate 3, fig. 9, at), after their movement anteriorly, project in an antero-ventral position, just lateral to the labrum (Plate 3, fig. 9, lr). Concerning the thoracic appendages, the coxal segments become delineated from their respective thoracic segments, and the femur and tibial segments have

become narrowed and tapered. Movement of the lateral edges toward dorsal closure continues, so that only slightly less than half of the yolk is still visible (Plate 3, fig. 9, ylk).

Two new features of the Stage 9 embryo result from external invaginations. In the abdominal region the final pair of spiracles (Plate 3, fig. 9, sp8) appears on segment eight, lying in line with the placement of spiracles previously described. A single pair of invaginations occurs on both the mesothoracic and metathoracic segments lying posterior and dorsal to the spiracles (Plate 3, fig. 9, gl). These are the external openings leading to the developing thoracic defense glands. The labium (Plate 3, fig. 9, lb) has moved anteriorly and ventrally, so that it lies in the region of the maxilla (Plate 3, fig. 9, mx).

At Stage 10 (Plate 4, fig. 10) dorsal growth of the lateral body walls along with the shortening of each abdominal segment has finally eliminated the s-shaped curve of the embryo at the posterior end of the egg. Other changes are less distinct. Gnathal appendages begin to show evidence of their future lobed condition. Thoracic appendages continue to elongate, tapering at their distal ends, although the rate of growth has decreased. The openings of the thoracic defense glands exceed those of the spiracles in size, while the thoracic spiracles appear larger than the abdominal spiracles.

Most of the changes at Stage 11 (Plate 4, fig. 11) are also quite subtle. Dorsal growth of the body walls leaves only about one-fourth of the yolk still visible on the dorsal surface. In the cephalic region, the labrum (Plate 4, fig. 11, lr) has begun to move to a more ventral position just anterior to the gnathal appendages. The gnathal appendages appear more distinctly lobed. In the thoracic region, all three pairs of appendages are further segmented, with a new groove separating the femur and tibia (Plate 4, fig. 11, fe and ti). The openings of the thoracic defense glands are smaller now, and have moved dorsad with the lateral growth of the body walls.

Stage 12 (Plate 4, fig. 12) is marked by continued lateral growth of the body walls toward dorsal closure. As a result, the conspicuous thoracic and abdominal features have moved dorsad. The thoracic spiracles have moved little, but the abdominal spiracles have changed from a ventro-lateral position to a medio-lateral position. They now lie almost in line with the thoracic defense glands, which also have shifted slightly dorsad. Only the mesothoracic spiracles are visible; the metathoracic spiracles presumably are hidden in the fold between the coxa of the two appendages. The dorsal edge of the mesothoracic and metathoracic coxae is delineated from the segments themselves. Minor features of Stage 12 include the segmentation of the antennae (Plate 4, fig. 12, at) from the protocephalic lobes, and the reduction in size of the ninth abdominal segment.

Stage 13 (Plate 5, fig. 13) occurs just prior to complete dorsal closure. The mesothoracic and metathoracic segments alone have not yet undergone dorsal closure. The thoracic defense glands (Plate 5, fig. 13, gl) are now positioned at the dorsal edge of the coxal segments. In the head region, the labrum extends more ventrally, and the antennal appendages (Plate 5, fig. 13, at) have become segmented from the cephalon. Small ridges appear in each abdominal segment ventral to the spiracles, precursors to the mound of ectodermal tissue that will eventually surround each spiracle.

Two major events occur at Stage 14 (Plate 5, fig. 14). The most obvious event is the completion of dorsal closure, thus totally enclosing the remaining yolk within the body walls. Further leg segmentation now divides the distal portion of each leg into the tibia and tarsus (Plate 5, fig. 15, ti and ta).

Three events of lesser importance occur at Stage 14. In the cephalic region, the labrum (Plate 5, fig. 14, lr) curves ventrally and falls in line with the curvature of the head. Gnathal segments, as well as the antennae, have become proportionately reduced in size.

Stage 15 (Plate 5, fig. 15) is the definitive stage. In the thoracic region the defense gland openings are located in a dorso-lateral position within an elevated tubercle (Plate 5, fig. 15, gl), the opening of which is sclerotized. Infoldings at the base of the tubercle separate it from the coxa. The mesothoracic spiracles have

moved to a medial position on the pleura and are now sclerotized. Abdominal spiracles protrude from the surface in an antero-dorsal position. A sclerotized claw is now present at the distal end of the tarsus of each thoracic appendage (Plate 5, fig. 15, cl). This may be difficult to see from a lateral view because the appendages fold around and lie on the ventral surface. In the cephalic region the mandibles are sclerotized (Plate 5, fig. 15, md), the maxillae are distinctly lobed, and the antennae show rings of segmentation (Plate 5, fig. 15, at).

Setae appear on all three body regions of the definitive embryo. Cephalically they tend to form a ring pattern just dorsal to each antenna. Two setae are located anterior to the ring pattern, and one is seen on the dorsal surface of the head. In the prothorax there is again a ring pattern formed by the setae, this time in a dorso-lateral position. Each defense gland tubercle has two setae pointing in a postero-dorsal direction, while the mesothoracic and metathoracic coxae have three setae pointing antero-ventrally. At least the first seven abdominal segments have two sets of setae on each side. The first set, consisting of a single seta, is located dorsally to the spiracle and points in a posterior direction, while the second set, consisting of three setae, is located ventrally to the spiracles. These point in a postero-ventral direction. Various pigmented spots,

perhaps trichogen cells, appear in rows on the tibia and tarsus of all thoracic appendages.

Internal Development of Thoracic Defense Glands

The first evidence of defense gland development occurs at Stage 7. At this time, coelomic sacs have formed, neural ridges are present and begin to segment, and ectodermal invaginations have formed spiracles on the mesothorax and metathorax (Table 1).

Differentiation of the defense glands begins in the mesothorax, just posterior and dorsal to the spiracles. Two oval, lightly staining cells (Plate 6, fig. 1, gc), 2.5 microns in diameter, appear in a region of thickened ectoderm near the basement membrane. These are anlagen of the defense glands. A faint separation appears in the body wall above the differentiating cells, indicating the zone of future invagination. By Stage 8 similar gland cells also appear just posterior and dorsal to the metathoracic spiracles.

Gland development at Stages 9 and 10 appears similar for both pairs of glands. A distinct invagination of the ectoderm appears by Stage 9, and becomes more pronounced by Stage 10 (Plate 6, fig. 2, in). The number of differentiating cells associated with each developing gland increases from at least four at Stage 9 to approximately seven at Stage 10 (Plate 6, fig. 2, gc).

Three major developmental changes take place at Stages 11 and 12.

Table 1. Major developments occurring during embryogenesis.

Stage of Development	Characteristics
1	Embryonic rudiment appears on ventral and posterior surfaces.
2	Protocephalic lobes appear, gastral groove and amniotic folds present, invagination of stomodaeum.
3	Lower layer formed. Entire embryo on the surface. Gnathal and thoracic regions undergoing differentiation.
4	Differentiation of middle and lateral plates, segmentation of gnathal and thoracic regions, invagination of proctodaeum.
5	Differentiation of mesoderm and endoderm rudiments, appearance of coelomic sacs and Malpighian tubules, formation of neural ridges and neural groove, development of thoracic appendages.
6	Invagination of thoracic spiracles, protocerebrum and deutocerebrum forming.
7	Differentiation of mesothoracic defense glands, splanchnic mesoderm rudiment appears, subesophageal and thoracic ganglia forming.
8	Invagination of abdominal spiracles. Appearance of metathoracic glands.
9	Eight segmental ganglia evident, gonad appears.
10	No major internal changes.
11	Invagination of posterior tentorial arms, appearance of muscles controlling the defense glands. Gonads in second and third abdominal segments.
12	Invagination of anterior tentorial arms, gonads in third, fourth, and fifth abdominal segments.
13	No major internal changes.
14	Dorsal closure complete, cardioblast cells in thoracic and abdominal segments, gonad in sixth and seventh abdominal segment.
15	Dorsal closure of midgut wall, formation of heart, secretion of chitin.

With continued invagination of the ectoderm through Stage 12 a very distinct sac-like structure develops, which will become the reservoir of the defense gland (Plate 6, fig. 3, r). The gland cells, now in larger numbers, begin to separate from the body wall, but maintain cytoplasmic connections with it (Plate 6, fig. 3, cc). A muscle appears at Stage 12, extending from the ventral neck of the reservoir to an apodeme dorsal to the coxa. This is the first of three sets of muscles which appear to control the gland secretion.

Similar developments occur at Stages 13 and 14. Up to ten gland cells are associated with each reservoir, each approximately 8.1 microns in diameter. Two new sets of muscles develop, one extending from the dorsal neck of the invagination to a ventral apodeme, and the other, consisting of two muscle strands (Plate 6, fig. 4, mu), extending from the ventral portion of the invagination to an apodeme ventral to the coxa, possibly the same apodeme previously mentioned.

In the definitive stage the reservoir has changed from a circular to an elongate sac. The wall of the reservoir has villi projecting into the lumen (Plate 6, fig. 5, l). The gland cells (Plate 6, fig. 5, gc) have enlarged to 11.24 microns, and the central nuclei are surrounded by large vacuoles (Plate 6, fig. 6, v). Cytoplasmic projections lead from the cells to the reservoir, and two small nuclei surround each projection (Plate 6, fig. 7, n). One of the muscles

associated with the gland is shown in figures 5 and 6 of Plate 6.

Figure 5 shows its attachment to the neck of the reservoir, and figure 6 shows its attachment to the apodeme dorsal to the coxa.

DISCUSSION

Life History

The life cycle of *Chrysomela semota* reported herein corresponds closely to observations of others concerning *C. semota* and other closely related species reared in the laboratory and observed in nature. Brown (1956) observed that all North American chrysomelids develop similarly in the laboratory, requiring four or five days for eggs to hatch, ten to 15 days for pupation to occur and three to five days for emergence from the pupa. These results correspond with the time intervals observed in this investigation, except that the required pupation time observed was five to seven days. Brown (1956) also indicated that the color of *C. semota* eggs was "either pink or deep yellow". Observation of the Pierce Lake population showed the color to be a deep orange, gradually changing to yellow prior to hatching. The orange edge observed on the elytra of older beetles is, according to Brown (1956) found only in breeding adults. In this study similar coloration was observed only on beetles that had emerged from hibernation and were becoming sexually active.

To the author's knowledge no predators of *C. semota* have been reported in the literature. In closely related chrysomelid species, various species of jewel wasps appear to be the only naturally occurring predators. Predators of the population at Pierce Lake included a nymphal pentatomid, *Podisus modestus*, and larvae of an unidentified

species belonging to the Syrphidae.

External Embryogenesis

The age of an egg is often unsatisfactory for use in determining the stage of development because eggs of the same age may show considerable variation (Krause and Ryan, 1953). This was found to be true for *C. semota* (Table 2), so stages were determined by certain characteristic features present, rather than by age.

According to Zakhvatkin (1968) the position of the embryonic region can be in one of three general categories occupying (1) the ventral surface of the egg, (2) the posterior pole, or (3) the posterior pole and part of the ventral side. The protocephalic lobes may be present from the first appearance of the germ band, or they may develop later. Submergence within the yolk also varies as to amount and duration. Members of the same genera do not necessarily follow the same pattern. In *C. semota* the embryonic region is located on the posterior pole and part of the ventral side, and the protocephalic lobes appear later in development. The caudal portion is submerged in the yolk until after invagination of the stomodaeum. Other chrysomelids following a similar pattern of development are *Atrachya menetriesi* Falderman (Miya, 1965) and *Diabrotica undecimpunctata howardi* Barber (Krysan, 1976).

Morphological Stages of Embryonic Development

Age in Hours

Euryope terminalis Baly is similar to *C. semota* in that both have the greater portion of the germ band lying on the surface with only the caudal extremity submerged in the yolk. Consequently little or no blastokinesis occurs (Paterson, 1931) because there is no change in orientation as the caudal portion rises to the surface.

Segmentation of the germ band of *C. semota* can be seen in longitudinal sections prior to its appearance externally. Miya (1965) states that segmentation seems to appear first in the mesoderm and then in the ectoderm, which would account for the observed discrepancy. This is in agreement with the usual differentiation sequence in insects (Johannsen and Butt, 1941). Similarly, spiracle formation is observed in cross section one stage earlier than it is observed externally. While originating in the ectoderm, the actual opening on the exterior is very small and therefore not immediately visible externally.

No pleuropodia were found on the abdomen of *C. semota*, although they do occur in other chrysomelids (Miya and Abe, 1966), as well as in some Tenebrionidae (Stanley and Grundmann, 1970; Ullman, 1967) and Meloidae (Church and Rempel, 1971).

The external development generally proceeds similarly to those descriptions of development reported for other Chrysomelidae except for the duration of development. *Chrysomela semota* requires 4.5 days at 25°C to complete development; *Euryope terminalis* requires 18 days

(Paterson, 1932); and *Atrachya menetriesi* requires five days at 26°C before diapause, and an additional eight to nine days at 23°C after diapause (Miya, 1965).

Internal Development of Thoracic Defense Glands

The structure of the eversible glands of chrysomelid larvae has been described by two authors within this century, Garb (1915) and Hinton (1951). Garb describes the glands of *Chrysomela lapponica* as developing from an invagination of the cutaneous epithelium, resulting in the formation of a reservoir. Fifteen large gland cells secrete a fluid which is stored in the reservoir. Three sets of muscles, two in the tubercle and one at the base of the reservoir are said to control the eversion of the glands, and hence, the secretion of the repugnant fluid. Hinton, in his drawing of the defense gland of *C. tremula* F., shows the same general structure, but with only two sets of muscles attached to the gland, one in the tubercle and one at the base of the reservoir. He also pictures well over 50 gland cells associated with the reservoir.

While these descriptions refer to glands in the larval stage, much is applicable to the embryonic glands described in this investigation. The glands of *C. semota* also originate by an invagination of the ectoderm, and certain cells differentiate from this invagination and become the large gland cells. Three groups of muscles may control

the glandular secretions, two in the tubercle and one located ventrally, as first described by Garb.

Based on their figures, the question arises as to whether Garb and Hinton are describing the same large glandular cells. The cells of *C. lapponica* have large, darkly staining nuclei, are heavily vacuolated, and have two guard cells surrounding the chitin-lined canal connecting them to the reservoir (Garb, 1915). These are similar in appearance to those cells of *C. semota*, although no chitin is visible in the canal, probably because of its embryological state. Hinton's diagram describing *C. tremulus* is unlike this structure. The large gland cells appear to be smaller and more numerous than those of *C. lapponica* and *C. semota*. It is impossible to compare actual sizes of the cells due to what appears to be a misprint in Garb's article stating that the size of the cells is as large as the whole reservoir. The large gland cells described in *C. tremulus* (Hinton, 1951) resemble smaller cells seen on the surface of the reservoir in *C. semota*, and these have no known secretory function.

The descriptions of the points of insertion of the muscles associated with the glands are necessarily vague because not all three complete sets of muscles were seen in association with one particular reservoir. Further, no references could be found which described the internal skeleton of a larval coleopteran thorax.

SUMMARY

Chrysomela semota Brown closely resembles other described North American chrysomelids in all phases of its life cycle. Sexually mature adults emerge in the spring, and, after several days of heavy feeding, begin mating and egg laying. In the laboratory, eggs hatch in an average of 4.5 days, and the larvae pass through three instars before pupation occurs. After five to seven days in the pupal stage, adults emerge and feed voraciously, then enter a period of dormancy.

Similarly, the external embryogenesis of *C. semota* appears very similar to the development of other chrysomelids. The development is herein divided into fifteen stages from the first appearance of the germ band at the posterior pole and ventral surface. The protocephalic lobes appear at Stage 2, followed by the rising of the submerged caudal region to the surface of the egg. No true blastokinesis occurs as there is no change in the orientation of the embryo during development.

Development of the thoracic defense glands first appears at Stage 7 with the differentiation of two cells in a region of thickened ectoderm. As development continues, an invagination of the ectoderm develops, and the differentiating cells increase in number before coming to lie beneath the invaginated ectoderm. They act as secretory cells, providing fluid for the reservoir formed by the invaginating ectoderm. Precursors of the three sets of muscles controlling the glands appear at Stages 12 and 13, and are complete by Stage 14.

EXPLANATION OF PLATES

The following list is a key to the plates illustrated in this manuscript:

ab, abdomen; at, antenna; cc, cytoplasmic connection; cl, claw; cx, coxa; er, embryonic rudiment; fe, femur; gc, gland cells; gg, gastral groove; gl, thoracic defense gland opening; in, invagination; l, lumen; lb, labium; le, limb buds; lr, labrum; m, mesothorax; md, mandible; mt, metathorax; mu, muscle; mx, maxilla; n, nucleus; p, prothorax; prc, procephalon; r, reservoir; sp, spiracle; sp8, eighth abdominal spiracle; st, stomodaeum; ta, tarsus; te, telson; ti, tibia; ylk, yolk; v, vacuoles.

Plate VI: Stages of Thoracic Gland Development.

Figs. 1, 2, 4, and 7 - X2500.

Fig. 3 - X1000.

Figs. 5 and 6 - X1560.

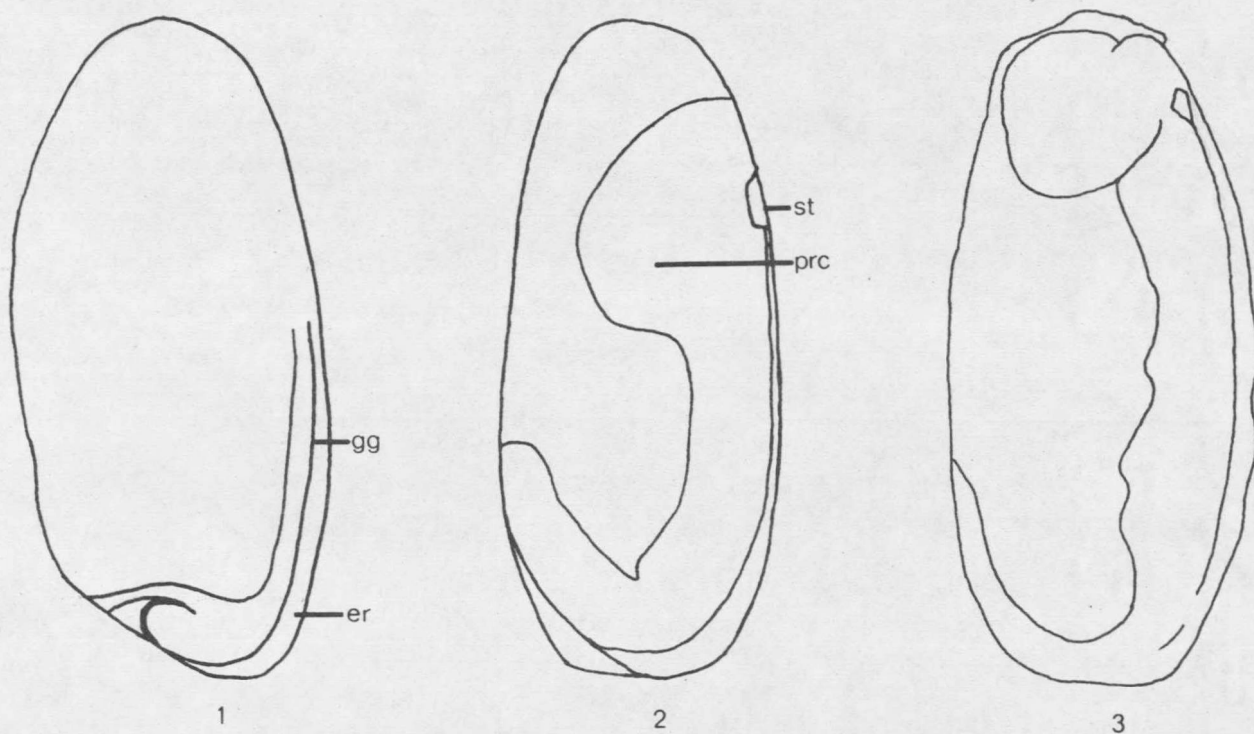
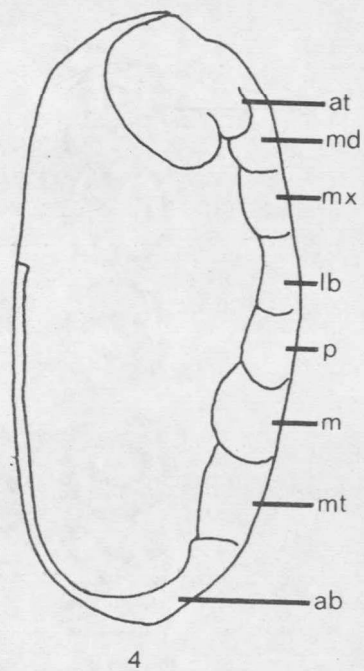
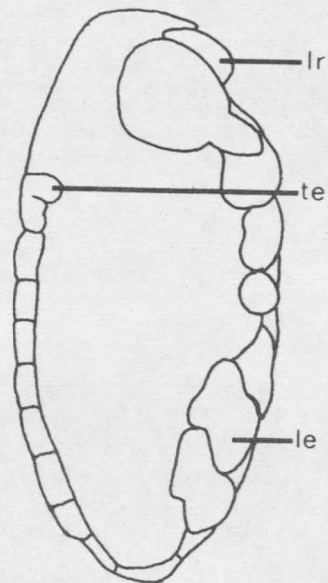


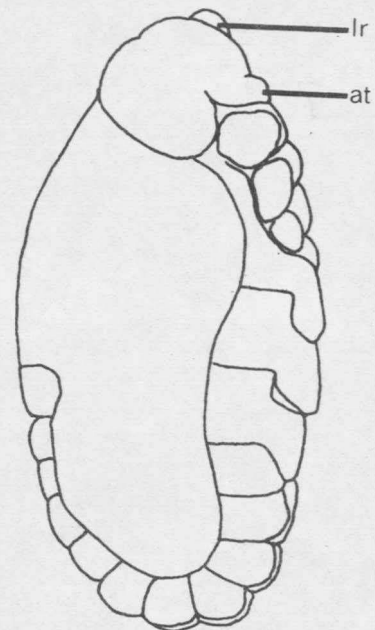
Plate 1. Stages of Development 1, 2, and 3.



4



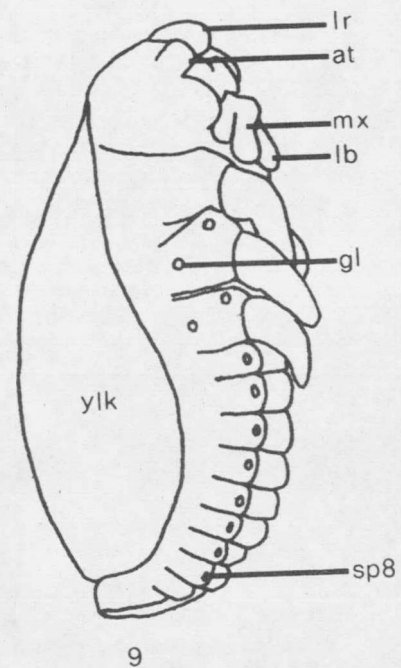
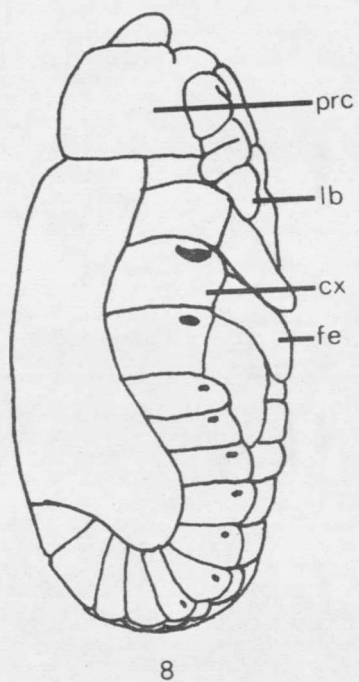
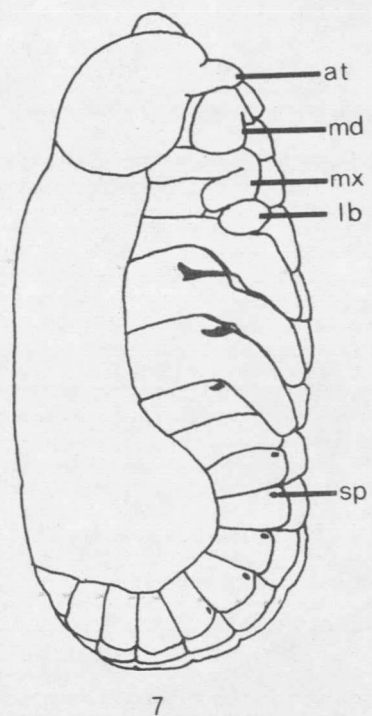
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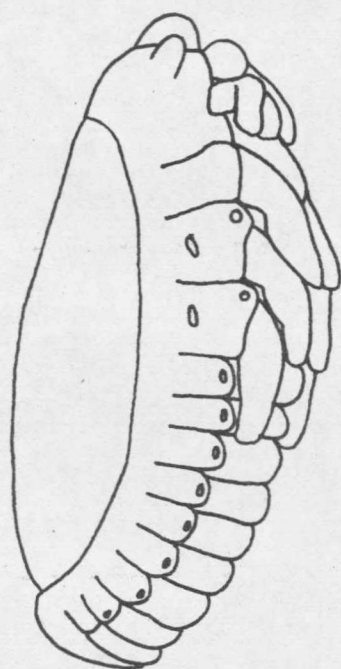
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Plate 2. Stages of Development 4, 5, and 6.

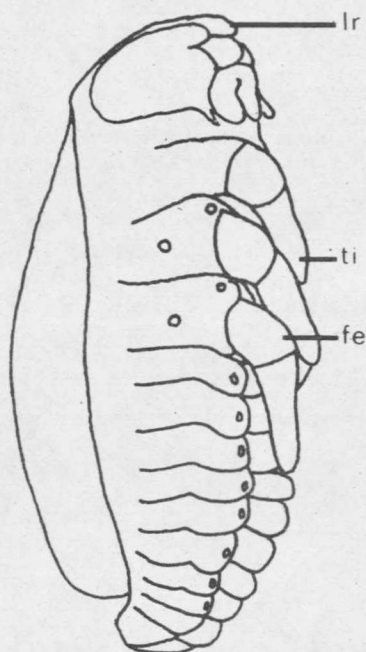


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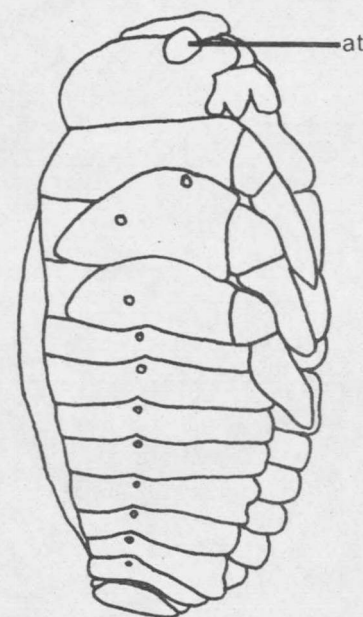
Plate 3. Stages of Development 7, 8, and 9.



10



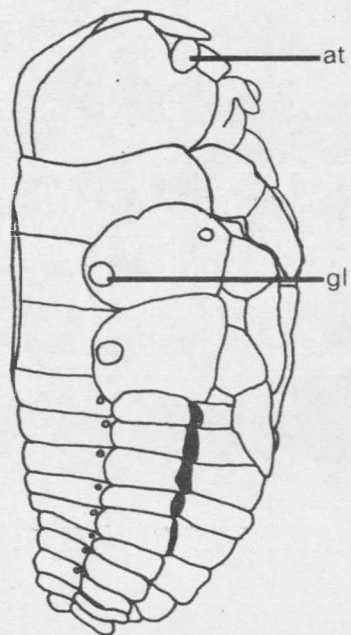
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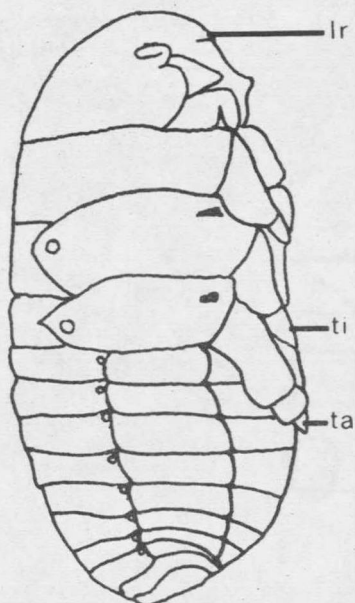
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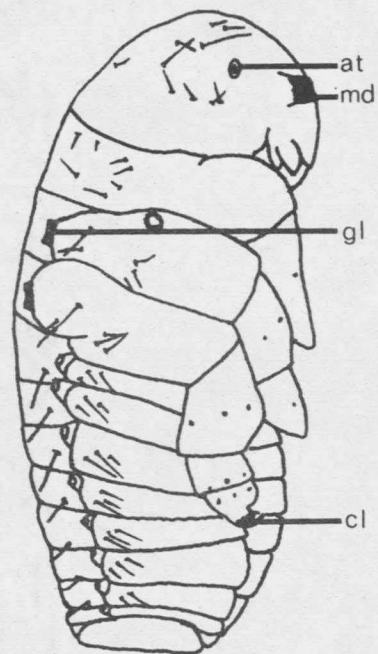
Plate 4. Stages of Development 10, 11, and 12.



13



14



15

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Plate 5. Stages of Development 13, 14, and 15.

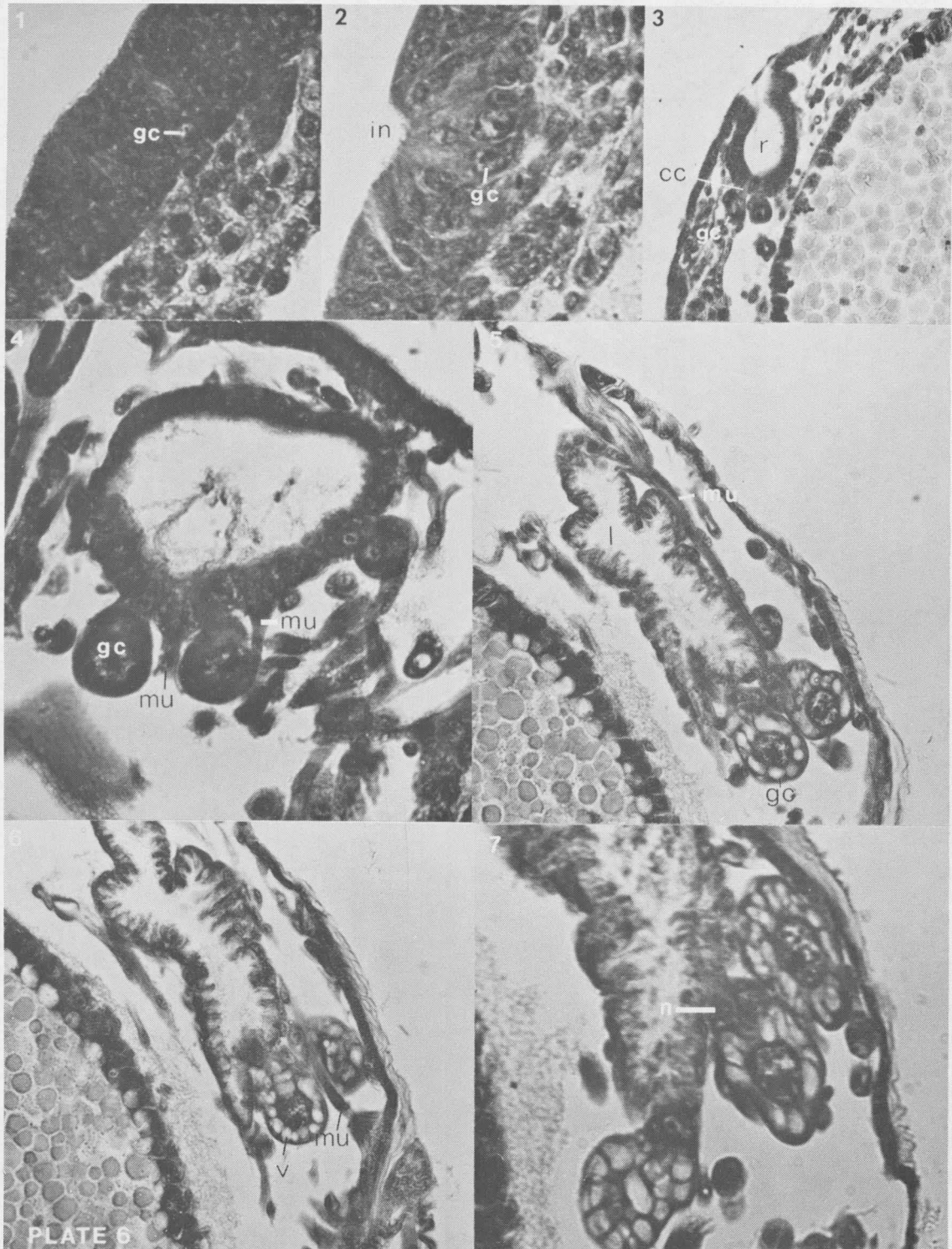


Plate 6. Stages in Thoracic Gland Development.

LITERATURE CITED

- Brown, W. J. 1956. The new world species of *Chrysomela* L. (Coleoptera: Chrysomelidae). Can. Entomol. Suppl. 3. 88:3-54.
- Church, N. S. and J. G. Rempel. 1971. The embryology of *Lytta viridana* Le Conte (Coleoptera, Meloidae). VI. The appendiculate, 72-h embryo. Can. J. Zool. 49:1563-1570.
- Garb, Gerson. 1915. The eversible glands of a chrysomelid larvae, *Melasoma lapponica*. J. Entomol. Zool. 7:88-95.
- Hinton, H. E. 1951. On a little known protective device of some chrysomelid pupae (Coleoptera). Trans. R. Entomol. Soc. Lond. A, 26:67-73.
- Humason, Gretchen L. 1962. Animal Tissue Techniques. San Francisco: W. H. Freeman & Co.
- Johannsen, O. A. and F. H. Butt. 1941. The Embryology of Insects and Myriapods. New York: McGraw-Hill.
- Krause, James B. and Marie Therese Ryan. 1953. The stages of development in the embryology of the horned passalus beetle, *Popilius disjunctus* Illiger. Ann. Entomol. Soc. Amer. 47:1-20.
- Krysan, James L. 1976. The early embryology of *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae). J. Morph. 149: 121-126.
- Miya, K. 1965. The embryonic development of a chrysomelid beetle, *Atrachya mentriresi* Faldermann (Coleoptera: Chrysomelidae), I. The stages of development and changes of external form. J. Fac. Agr., Iwate Univ. 7:155-166.
- Miya, K. and T. Abe. 1966. The early embryology of *Epilachna vigintioctomaculata* Motschulsky (Coleoptera, Coccinelidae), including some observations on the later development. J. Fac. Agr., Iwate Univ. 7:277-289.
- Paterson, N. F. 1931. A contribution to the embryological development of *Euryope terminalis* Baly, (Coleoptera, Phytophaga, Chrysomelidae). Part I: The early embryological development. S. African J. Sci. 28:344-371.

- Paterson, N. F. 1932. A contribution to the embryological development of *Euryope terminalis* Baly, (Coleoptera, Phytophaga, Chrysomelidae). Part 2: Organogeny. S. African J. Sci. 29: 414-448.
- Paterson, N. F. 1935. Observations on the embryology of *Corynodes pusis* (Coleoptera, Chrysomelidae). Quart. J. Microsc. Sci. 78:91-132.
- Rempel, J. G. and N. S. Church. 1969. The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae). V. The blastoderm, germ layers, and body segments. Can. J. Zool. 47:1157-1171.
- Rempel, J. G. and N. S. Church. 1971. The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae). VII. Eighty-eight to 132 h: the appendages, the cephalic apodemes, and head segmentation. Can. J. Zool. 49:1571-1581.
- Stanley, M. S. and A. W. Grundmann. 1970. The embryonic development of *Tribolium confusum*. Ann. Entomol. Soc. Amer. 63:1248-1256.
- Ullmann, Suzanne L. 1964. The origin and structure of the mesoderm and the formation of the coelomic sacs in *Tenebrio molitor* L. (Insecta, Coleoptera). Phil. Trans. R. Ent. Soc. Lond. B, 248: 245-277.
- Ullmann, Suzanne L. 1967. The development of the nervous system and other ectodermal derivatives in *Tenebrio molitor* L. (Insecta, Coleoptera). Phil. Trans. R. Ent. Soc. Lond. B, 252:1-25.
- Wallace, J. B. and M. S. Blum. 1969. Refined defensive mechanisms in *Chrysomela scripta*. Ann. Entomol. Soc. Amer. 62:503-506.
- Zakhvatkin, Y. A. 1967. Some features of the embryogenesis of *Chalcoides aurata* Marsh. and *Chalcoides fulvicornis* F. (Coleoptera, Chrysomelidae). Biol. Nauk. 10(8):24-28.
- Zakhvatkin, Y. A. 1968. Comparative embryology of Chrysomelidae. Zool. Zh. 47:1333-1342.

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Descriptions of the life cycle, external



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