1973

Morphology of the Internal Reproductive System of the Male and Female European Corn Borer Ostrinia Nubilalis (Hubner)

Miriam F. Shapiro
The College at Brockport

MORPHOLOGY OF THE INTERNAL REPRODUCTIVE SYSTEM OF THE MALE AND FEMALE EUROPEAN CORN BORER *OSTRINIA NUBILALIS* (HÜBNER)

by

Miriam F. Shapiro

Submitted to the Graduate Faculty in the Department of Biological Sciences on partial fulfillment of the requirements for the degree of Master of Science

State University of New York

College at Brockport

1973

TABLE OF CONTENTS

ABSTRACT

The gross morphology of the internal reproductive organs of the adult male and female European corn borer is described. The adults were laboratory reared progeny and descended from wild specimens collected in the Rochester area, Western N.Y.

The male system displayed 2 major variations in the paired vasa deferentia. In 8% of 75 recently emerged males, each of the ducts arising from the testis, had a single expansion before merging with the narrow portions of the ducts. These expansions have been referred to as the seminal vesicles or upper vasa deferentia. In 88% of the 75 males, the ducts showed 2 expansions; the single expansion immediately followed by a second, smaller expansion, shaped somewhat like a tear-drop.

A stable, distinguishing characteristic of the male tract is the large flask-like or ampulliform extensions of the accessory glands. These bodies were found in all males upon dissection.

The female reproductive tract showed fewer variations. However, this species on emergence shows a transparency of the spermathecal complex, the lateral oviducts, the common oviduct and the bursal duct. After insemination, only the bursal duct displays a transparency.

The female emerges with unripe eggs and at this stage of development has the capacity to receive the spermatophore immediately after wing drying. The male was not observed to copulate until after the first or second day.

INTRODUCTION

Progeny of field collected corn borer larvae from the Rochester area were reared on a meridic diet in the laboratory for more than a year and is here referred to as the B strain. The B imagos live for a maximum of 4-15 days, depending on the diet and population density within the cages, and during the adult phase do not feed but do drink water.

Reports on the life spans of corn borer adults differ ranging from 10-17 days in the field (Kuwayama, 1930; MacArthur and Baillee, 1932; Guthrie, 1965; Raros and Chiang, 1968).

Both the adult *Ostrinia nubilalis* seen in the Rochester region and the B strain are quite small. The male imago averages 12.0mm in length and 1.2mm in diameter. The female averages 13.5mm in length and between 2-3mm in width. The diameter in both sexes was taken at the 4th abdominal segment, usually the widest.

Mutuura and Monrow (1970) report amazing lengths for both sexes, 26-30mm for the male and 28-34mm for the female. The specimens they surveyed included field forms from Southeastern New York State (Port Washington, Long Island) and the difference in the geographical environment may possibly account for the variation in size between the local corn borers and the others.

Mutuura and Monroe (1970) further clarified the taxonomic position of the European corn borer, which has presented difficulties. Their revised classification list the moth as *Ostrinia nubilalis* (Marion, 1957), in the *Family Pyralidae*.

Though the reproductive organs of the male and female *Ostrinia nubilalis* resembles those of other Lepidoptera, there are dissimilarities essentially in the male vasa deferentia, and disagreement in classifying both the type of testis observed in *Ostrinia* and the method of spermatophore formation. Other findings in the B corn borer also differed from a previous study of the species (Drecktrah and Brindley, 1967), particularly in the structure of the female spermathecal gland, the structure of the common oviduct, the anatomical positions of the termini of the female accessory and spermathecal glands and the number of constrictions in the male ejaculatory duct. There also appears to be a lag in the potential for copulation in the male B strain after emergence, not noted in other studies (Drecktrah and Brindley, 1967; Loughner and Brindley, 1971).

This study originally began as an attempt to determine the effects of heterosexual gonadal transplants on the hosts as well as on the transplanted organs. However, the need to establish normal criteria for the reproductive organs, resulted in this work, describing the morphology of the male and female systems.

METHODS AND MATERIALS

For a period of 3 months, hand dissections were performed on 150 male and female adults, 80 of which were living (Table I).

TABLE I

Adults dissected

		Preserved				
		70%	10%	Carnoy's	Ages	
Sex	Living	Ethanol	Formalin	(B)	(days)	No.
Males	40	15	10	10	0,1-5	75
Females	40	15	10	10	0,1-5	75
Total	80	30	20	20		150

All specimens, living and preserved, were dissected in saline. The preserved specimens included the same age ranges, zero referring to the moment of emergence, wings still folded. The saline consisted of; 9.0gm sodium chloride, 0.2gm potassium chloride, 0.1gm calcium chloride, to 1000 cc of distilled water.

All dissections were carried out in petri dishes layered with wax which had been blackened by bone black and weighted with small lead shot, and were viewed under a dissecting microscope. except for those tissues or organs which were compressed on a slide and examined by a compound or phase microscope.

The wings and legs were removed, but the head and thorax were kept as a unit attached to the abdomen to aid in pinning and aligning the specimen. Spermatophores were obtained from mated females either disturbed during copulation or post-copulatory. Spermatophores were also collected from males forming the spermatophores in the cuticular segment and from males just prior to copula, interrupted as they assumed tandem mating positions with the female. Most adults were dissected without anesthesia, though on occasion a few were first anesthetized with carbon dioxide.

Newly emerged males and females were paired and caged. Observations of egg-laying and the presence of spermatophores in the females at the time of dissection indicated that mating did not occur until a day or two following caging. Newly emerged females that had been caged with older males were observed copulating. Multiple-inseminated females were not found in the 75 females dissected.

OBSERVATIONS: THE MALE REPRODUCTIVE SYSTEM

The Testis (Fig. 1A,B,C, 3-5; photo. 1-5)

The testis in the corn borer may be found attached dorso-medially or dorso-laterally to the body wall, most often in abdominal segment 5.

Strong trilateral tracheae from a spiracle on each side of a segment bind the testis to the dorsal body wall and to other parts of the male system (Fig. 2A). Numerous fine tracheae so envelop the testis that they may well constitute a form of outer limiting membrane. The true, outer testicular membrane is very fragile and any attempt to remove all the fine tracheae results in fragmenting of the testis and attached ducts. Fat body tissue is in the form of small, white globular bodies particularly in the region of the testis and in the caudal part of the ejaculatory duct.

The size of the testis varies with the stage of development, but at any stage is quite small in the B strain, ranging from 1mm x 1.5mm in the young imago to 0.5mm x 1mm in the older adult.

The color may be pale lemon, but is most often white, which does not seem to be correlated with age or stage of development (photo. 1-2).

In the newly emerged male, the testis is filled with open sperm bundles, some still retaining portions of the sheath, and has a pH value of 5.0 when rubbed on litmus paper.

The Vasa Deferentia and Vesiculae Seminales (Fig. 1A,B,C, 3-5; photo. 1-5)

Two major structural variations were seen in the paired vasa leading from the testis to the duplex portion of the ejaculatory duct.

The first consisted of doubly expanded ducts (Fig. 1A, photo. 1) and occurred in 88% of the males dissected, living and preserved. The second consisted of a single expansion (Fig. 1B, photo. 2) and was seen in 8% of the specimens, but only in the living, newly emerged males. In the remainder, the entire vasa deferentia had a series of expansions and constrictions (Fig. 1C, photo. 3) indicative of peristaltic activity.

In ventral dissections, the vasa deferentia and their expansions and the duplex duct are masked by fat and tracheae, but mostly by the long, convoluted ductus ejaculatorious simplex (Fig. 2A). Part of the left narrow vas can usually be seen as it is frequently looped around the lower simplex.

The vasa deferentia are found to lie twisted or criss-crossed (photo. 4), but the twist can be straightened, revealing that the departure of the ducts from the testis is parallel (photo. 5).

The length of the upper vas deferens or first expansion varies from 2mm in the new imago to 3-4mm in the post-copulatory male--to the point of constriction--which is followed by a second tear-drop shaped expansion, about 1.5mm in length in both young and older males. The narrow vasa range in length from 2mm to 4.5mm. The longer length does not seem to be correlated with age or secretory activity as 13% of newly emerged males displayed the longer vasa deferentia.

The only variation noted in the expanded and narrow portions of the vasa in the B *Ostrinia* was the difference in pH values, registering 6.0 for the expanded regions and 4.5 for the narrow parts, in the one day old male. Grossly, both areas showed an opaque, whitish secretion. The entire vasa become so tenacious in the copulative males that dissection is extremely difficult.

The narrow vas deferens generally lie caudad and dorsal in the abdomen, twist again basally and cross over to enter the duplex part of the ejaculatory duct somewhat ventrad. Entrance of the vas may vary from central or mid-duplex (photo. 5) to a portion of the duplex closer to the single ejaculatory duct.

The Male Accessory Glands (Fig. 1A,2A; photo. 6-8)

The paired accessory glands are most often found in the posterior part of the abdomen and usually can be seen best in ventral dissection.

The paired glands adhere closely and are very short, being at the most 2.5mm in length. They may lie freely in the lower abdomen (Fig. 2A) but their short length increases the difficulties in freeing the vasa, duplex and testis, as there is no leverage, nor can the glands be pinned down without damage.

The terminal portions of the glands are large and flask-like, being one-half the length of the accessory glands, making a total of 3.5-4.0mm in length. These terminalia are brightly transparent--literally gleaming--and grossly appearing as if the membrane is one cell in thickness (photo. 6). They are separated from the rest of the accessory glands by a sort of plicate constriction (photo 7). Only occasionally has some opaque, white secretion been seen in them (photo 8), although the paired, adherent accessory glands contain such a secretion in males of all ages. These terminal bodies will diminish in size if hit accidently by instruments (photo. 8). They also diminish in size in the post-copulatory male.

In the zero hour male, the accessory glands and their ampulliform end bodies have a pH value of 4.5.

<u>The Ductus Ejaculatorious Duplex</u> (Fig. 1A, 3-5; photo. 4-5,9)

The duplex or paired part of the ejaculatory duct is short in the B corn borer, measuring about 2.5mm in length. The duplex is found lying diagonally or transversely in the abdomen and often may be wrapped collar fashion about the testis in living and preserved specimens (Fig. 3B).

The paired ducts grossly appear to be highly secretory. The distal portion--closest to the accessory glands--contain a gleaming white secretion and the proximal portion--closest to the simplex duct--displays an opaque whitish secretion.

In the young male (within 24 hours of emergence) the duplex has a pH value of 5.0, and anatomically merges somewhat gradually into the single duct and the accessory glands. A constriction can be seen between the duplex and simplex duct (photo. 9).

In the copulatory and post-copulatory males the central portions of the ducts may cohere closely and both ends of the ducts may bulge (Fig. 3A). The junction of the duplex and simplex becomes quite abrupt and a slight swelling may be noted at this junction (Fig. 4). The junction of the duplex and accessory glands also becomes more abrupt but less so than the duplex-simplex junction (Fig. 4).

The Ductus Ejaculatorious Simplex; The Primary Segment (Fig. 1A,2A, 3-5; photo. 10-11)

On opening the abdomen of the male *Ostrinia* whether dorsally or ventrally, the most prominent part of the reproductive tract is the single ejaculatory duct, measuring about 80mm in length. It lies convoluted in the abdomen from the first to the seventh abdominal segments, with a good portion entwined under and above the testis dorsally, and is attached to the testis by fat and tracheae. Also, the fat and tracheae attach the duct to itself along its length and to other parts of the system.

Long nerves extending cephalad from the last abdominal ganglion (the 4th) tie the lower portion of the simplex to the single vas looped around it.

Rhythmic contractions varying in intensity occur all along the ducts from the testis down during dissection of living specimens.

Constrictions can be seen along the simplex duct giving it a segmented appearance. Usually, in the zero hour male, before secretions appear along the duct, 3 constrictions can be counted (Fig. 1A). The duct in the just emerged male is transparent, though with shades of gleaming yellow and blue. Initially the constrictions are located, when going caudally, between the simplex-duplex junction (photo. 9), midway along the primary segment, which is moderate, and one between the cuticular and primary segments, which is quite strong (photo. 10).

The number of constrictions increase in the secretory and copulatory males (Fig. 3A, 4-5; photo. 11). Nine constrictions have been counted and these equal what grossly seem to be 9 secretory areas in the simplex duct (Fig. 5).

<u>The Cuticular Segment and Aedeagus (Penis)</u> (Fig. 1A,2A, 3-6; photo. 10, 12-15)

The cuticular segment portion of the single ejaculatory duct is about 5mm long not including the thick heavy muscular part which is looped around the lower simplex duct (Fig. 1A; photo. 10).

Caudad from the loop, the duct flattens and makes a second loop before entering the aedeagus dorsally (photo. 12). This loop is hidden by the lower abdominal segments.

Triple strands of tracheae tightly spiralled appear to run through the cuticular segment to the point of constriction with the rest of the simplex. The tracheae press against the left wall within the segment and resemble a tube or spiral wall of dense fibrous tissue.

Both the cuticular segment and the aedeagus have a pH value of 5-6 in the young imago.

The aedeagus varies from 1.5mm to 3mm in length (if the term is all inclusive, instead of just referring to the penis proper). Several regions can be discerned (Fig. 6, photo. 13).

The ejaculatory duct opens terminally in the membranous part of the penis, extending within the penis through all its length, and at the distal part, a spoon-shaped sclerotic piece (cornutus) is present.

Complete spermatophores, consisting of the corpus or body and collum or neck, have been found in the cuticular segment, with the corpus extending into the caecum penis (photo. 14-15). The spermatophores are smaller in size, in the corpus region, and have less volume than spermatophores seen in the female duct. A male, rejected by the female he was caged with, extruded a small but perfectly shaped spermatophore with secretions external to the spermatophore (photo. 16).

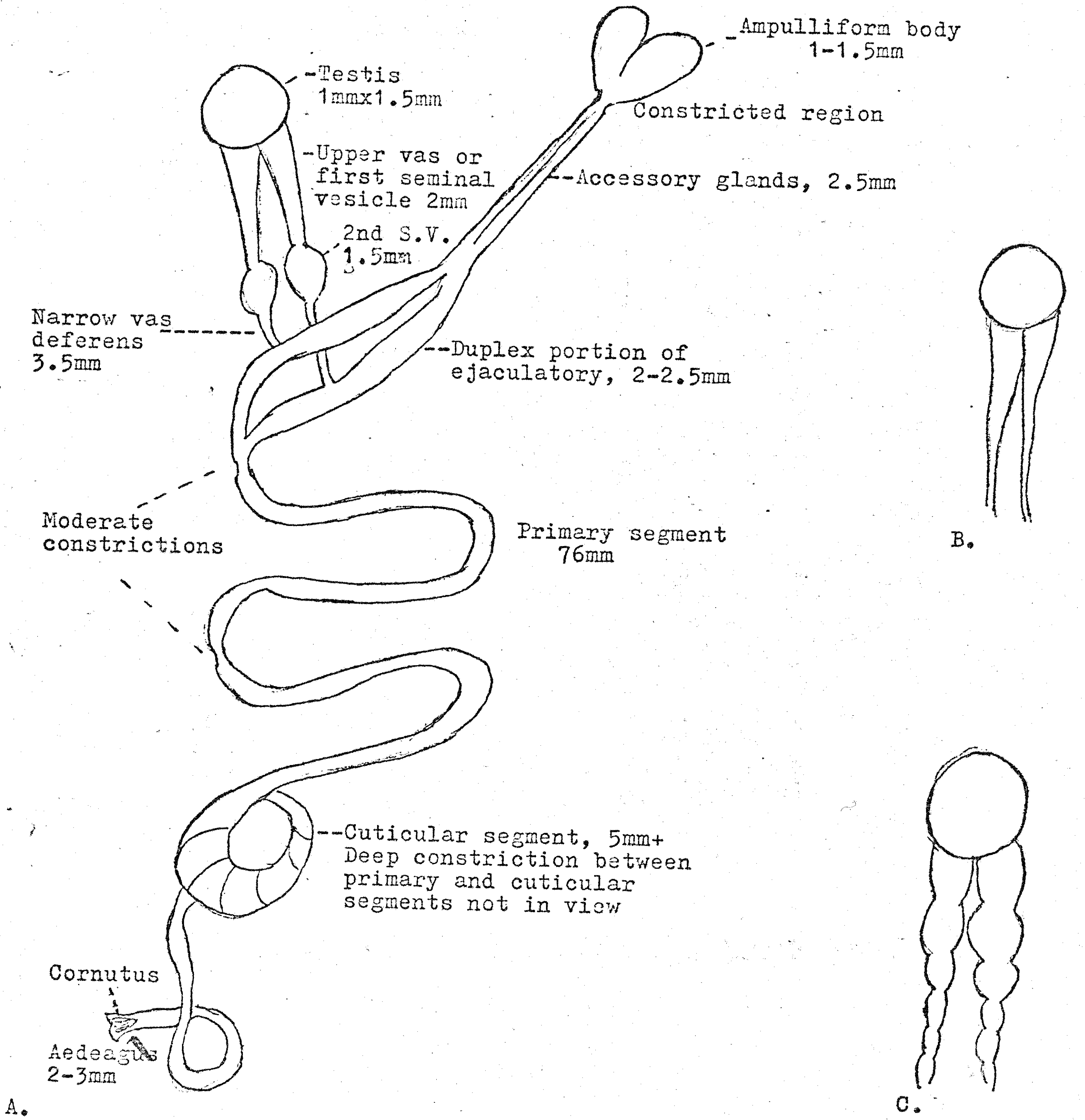


Figure 1A. Male reproductive system. The double expansions or seminal vesicles (first and 2nd S.V.) found in 88% of males dissected.
B. Single expansion, 8% of specimens, but only in newly emerged males.
C. Series of contractions and expansions in 24 hour old pre-copulative male. Possible incipient stage.

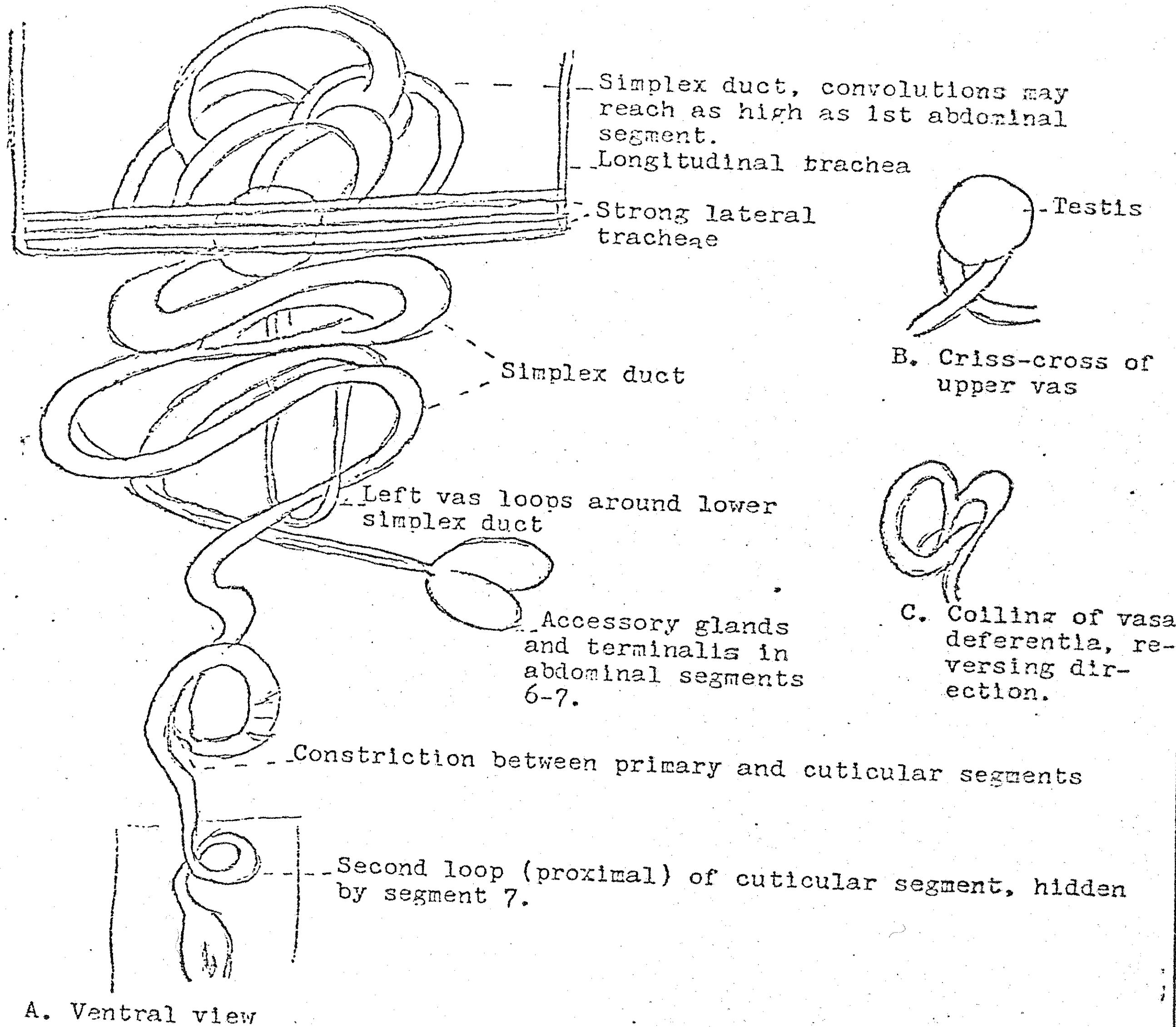


Figure 2A. Appearance of male reproductive system in the abdomen with most of fat and tracheae removed. Ventrally, the testis, seminal ducts deferent and duplex ducts may not be seen until most of fat and tracheae removed.

2B. The upper vas or first seminal vesicles always lie criss-cross.

2C. The vas often coils between testis, duplex and expansions.

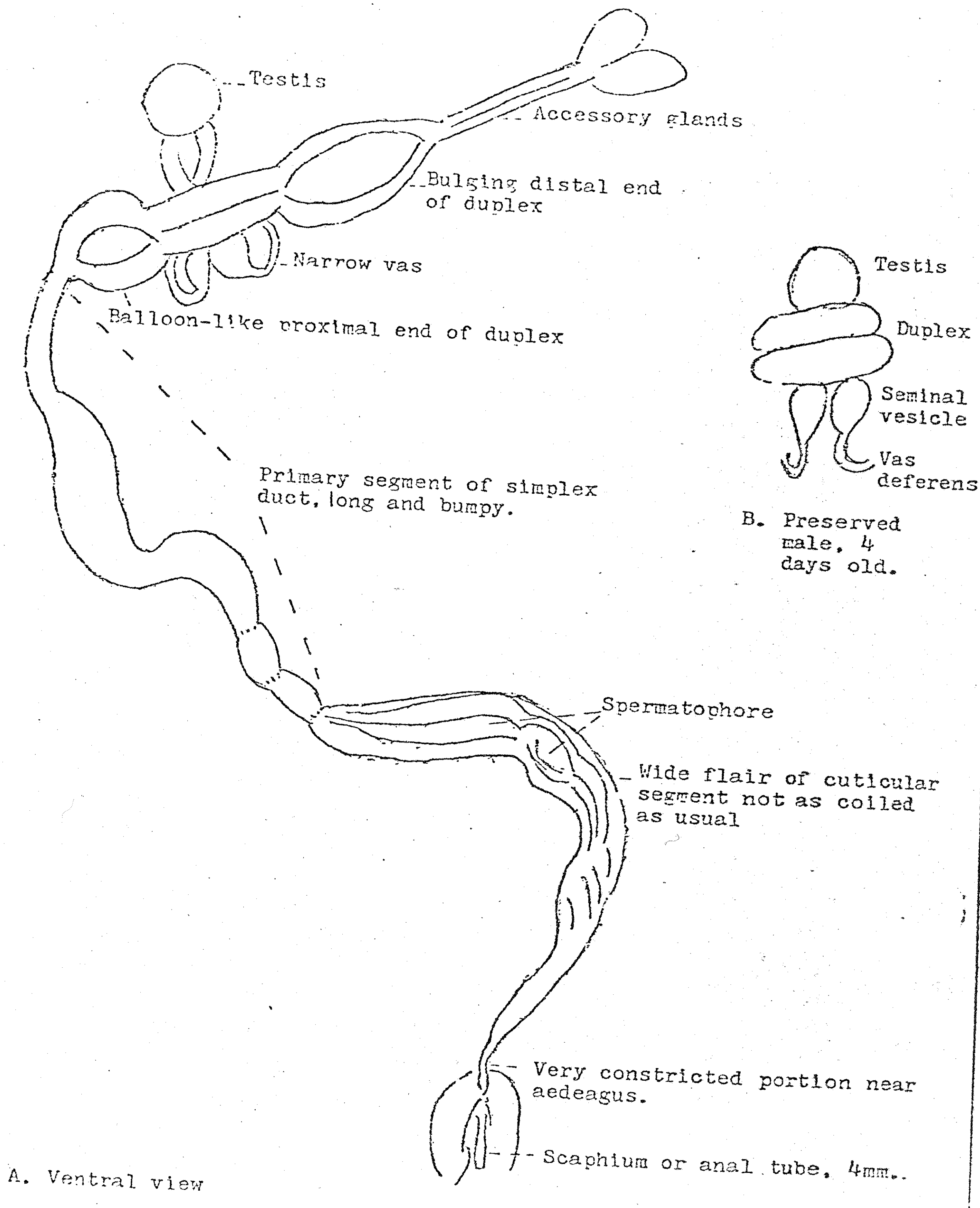


Figure 3 A. Four day old male with spermatophore forming.

B. Duplex duct wrapped collar fashion around testis as it appears in the abdomen of most preserved specimens and in many living specimens.

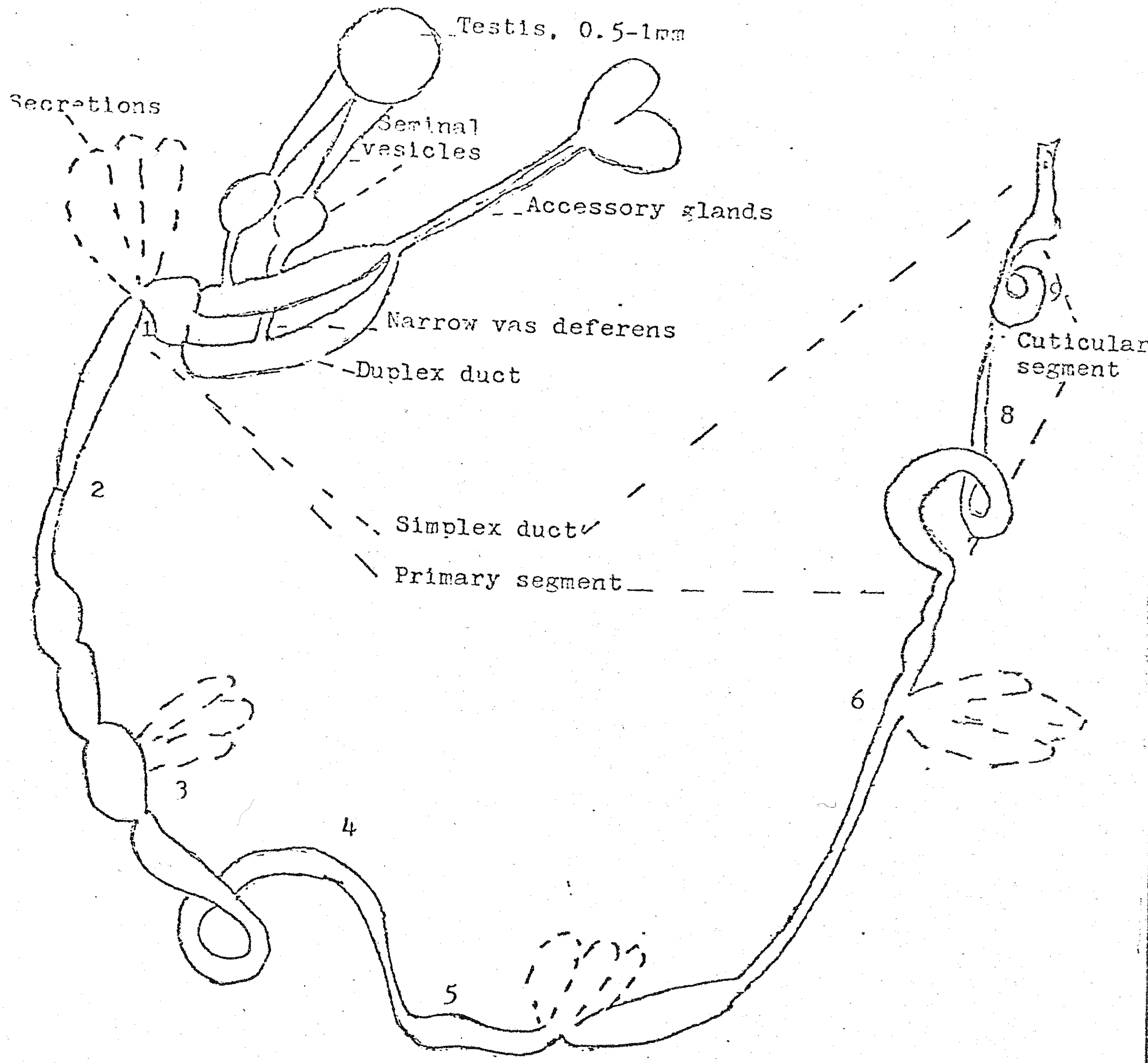


Figure 4. Ventral view. Recently emerged male, pre-copulative, but ejaculative. Spermatophore secretions ejected into abdomen which may be due to surgical trauma or powerful peristaltic action. These secretions are also ejaculated mimicing the shape of the ejaculatory ducts and lying close to them.

Microscopic investigation of these secretions showed the presence of closed or sheathed sperm bundles.

The numerals indicate 9 secretory areas grossly observed varying from bluish, yellowish, white to opaque white.

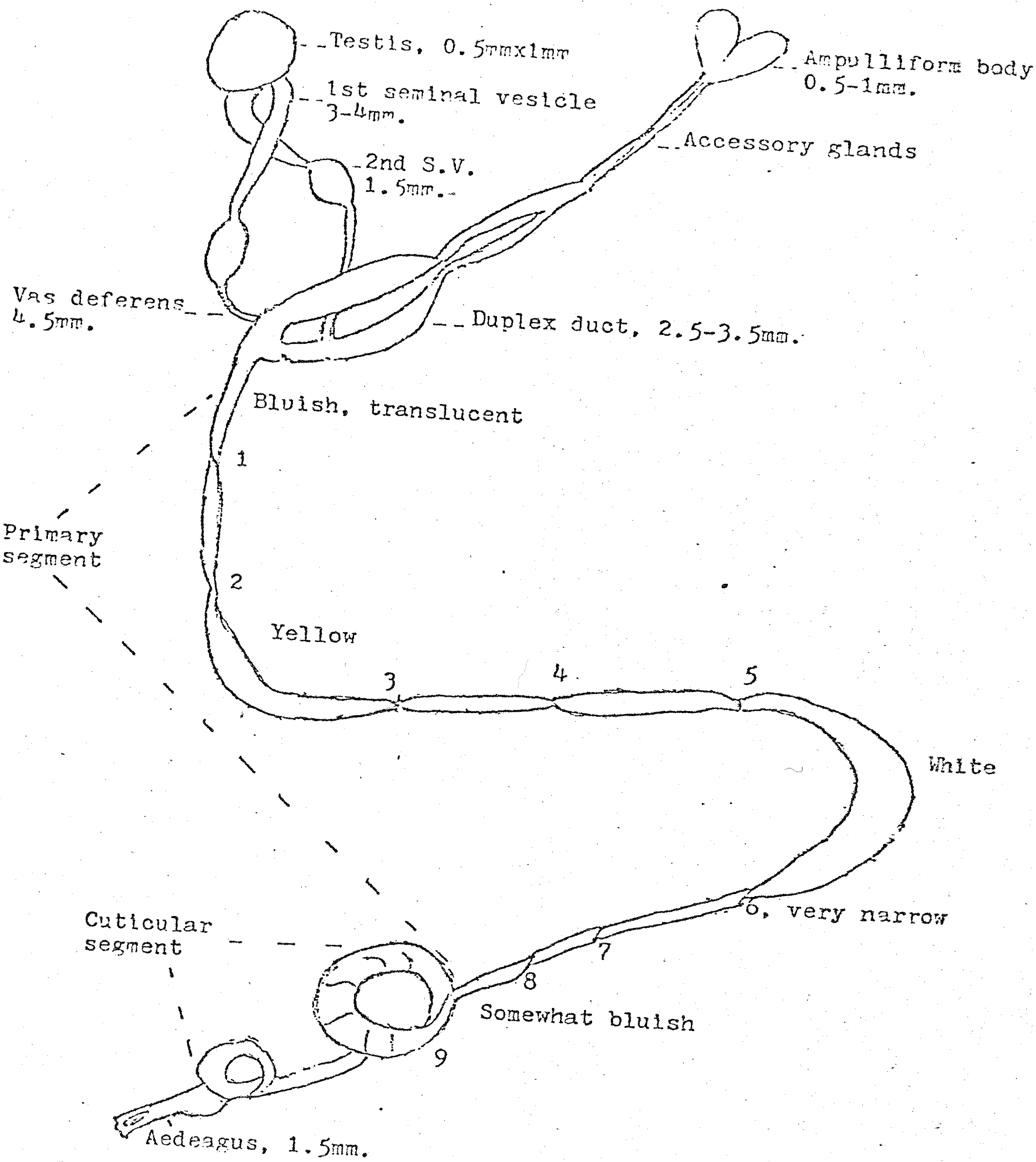
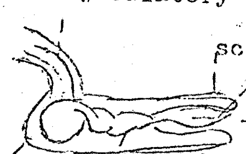


Figure 5. Ventral view. Three day old male, post-copulatory. The simplex duct to aedeagus equals 81mm. The diameter of the simplex duct is usually not this narrow in most post-copulatory males. Note changes in size of testis, duplex and terminal bodies of accessory glands.

The numerals refer to 9 constrictions seen. The variation in color of the duct is also indicated.



Aedeagus, lateral view

Figure 6.

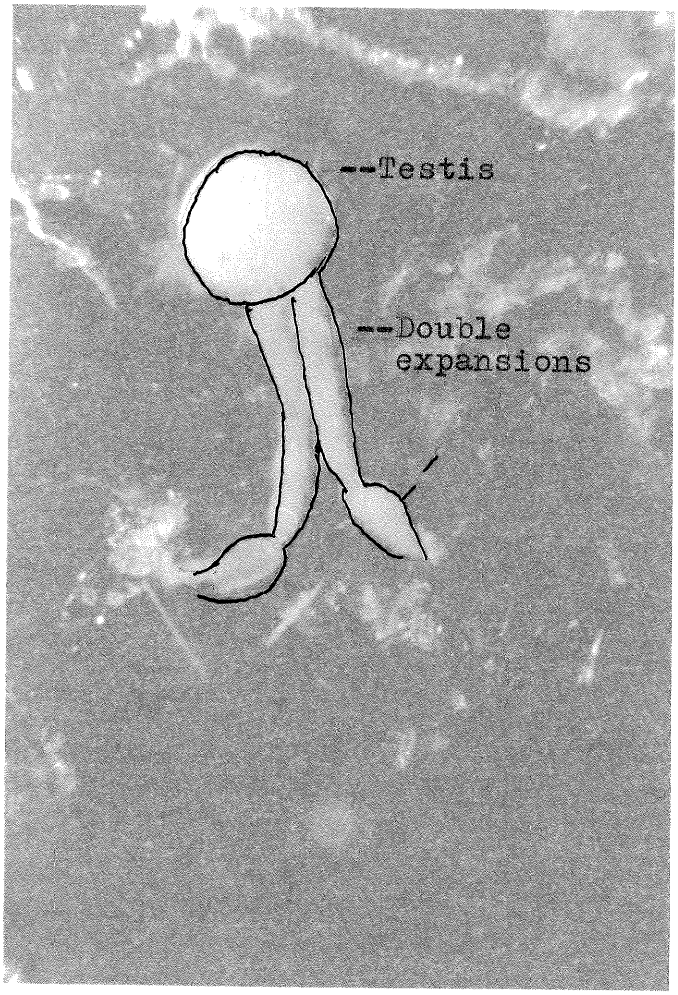


Photo. 1. Specimen damaged, but the 2 expansions of the vasa deferentia can be visualized.

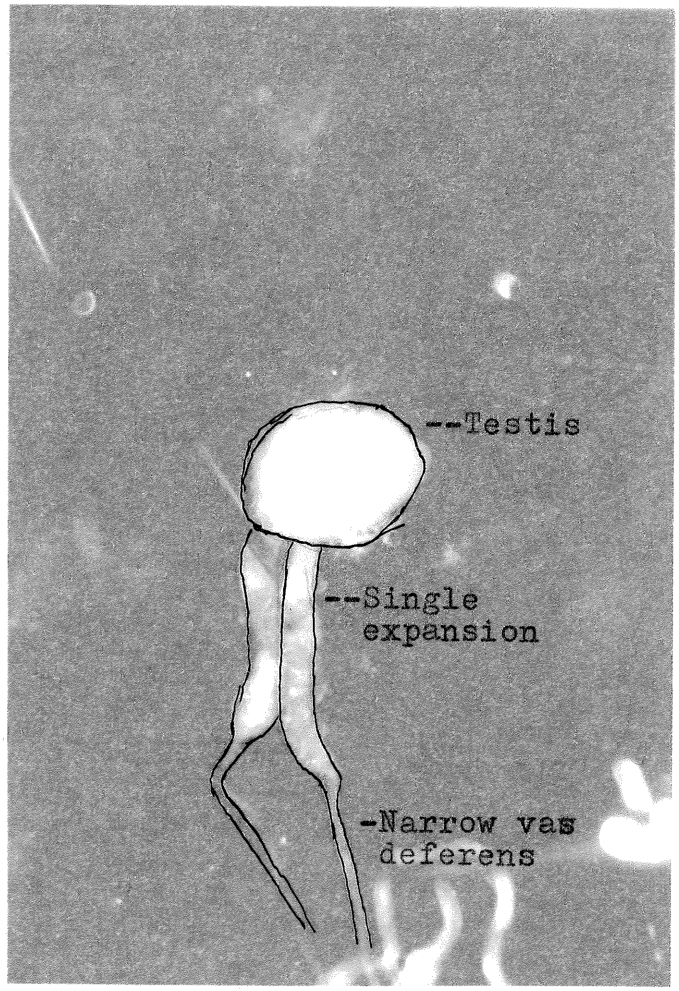


Photo. 2. Single expansion of the vas deferens in a recently emerged male. The color of the testis is usually white.

Alcian blue used to aid in viewing reproductive system.

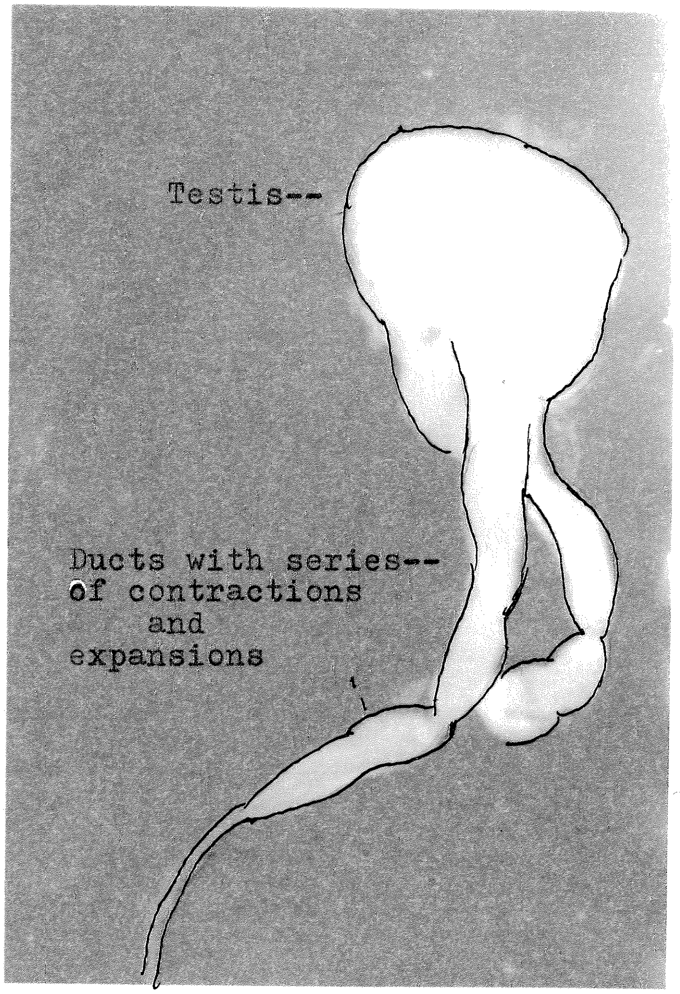


Photo. 3. Possible incipient stage in which the singly expanded vasa deferentia develop a second tear-drop shaped expansion.

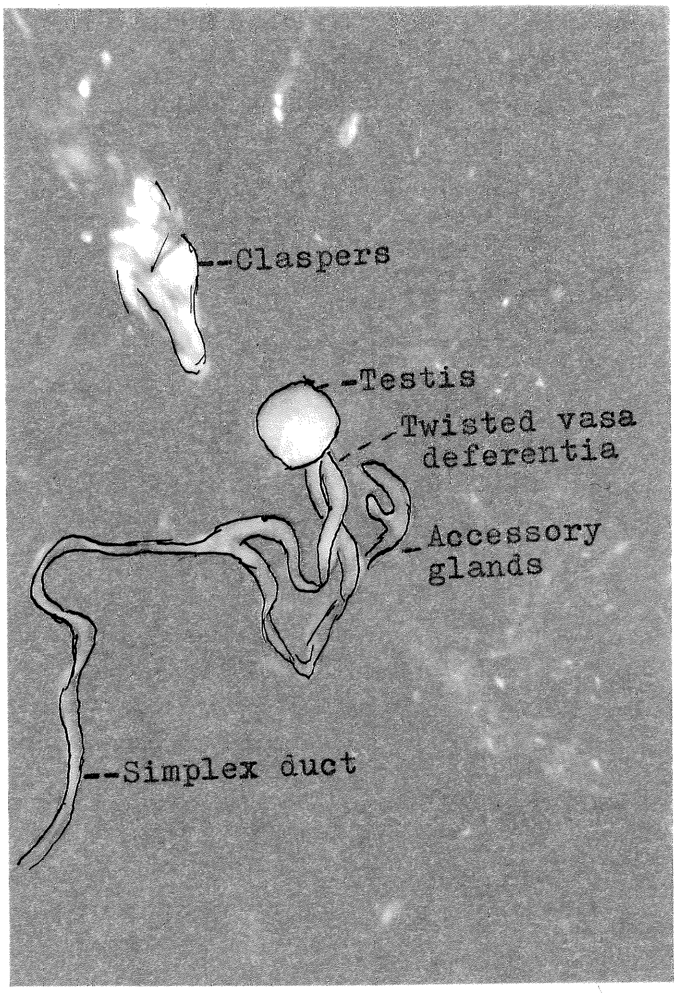


Photo. 4. The vasa deferentia are found to lie twisted or criss-crossed in the abodomen.

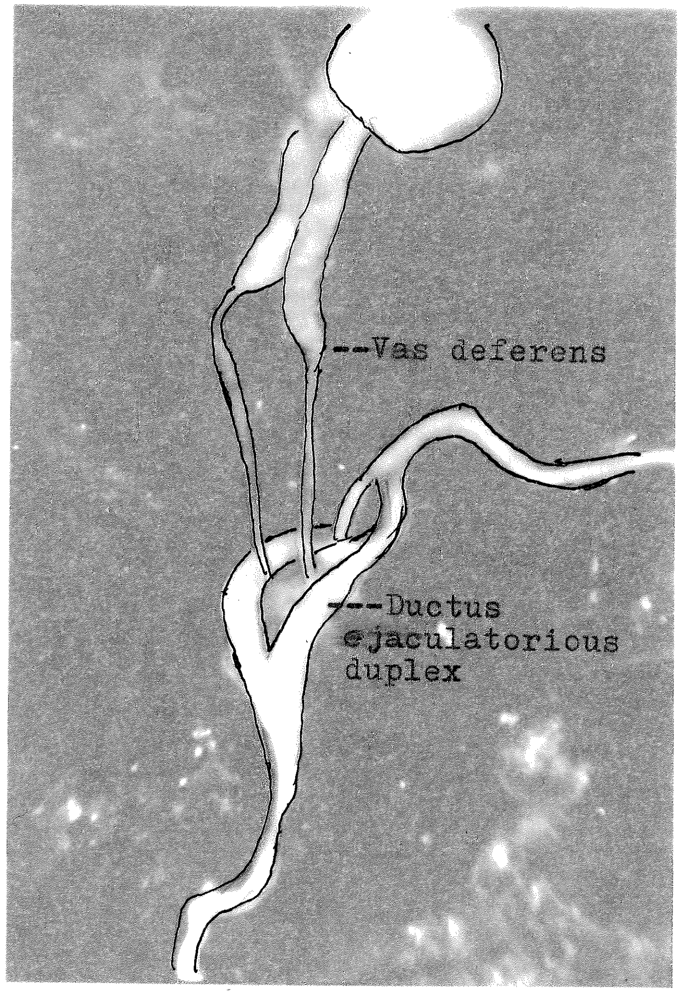


Photo. 5. The ductus ejaculatorious duplex and the parallel departure of the vasa deferentia from the testis.

The vasa enter the duplex centrally or mid-duplex.

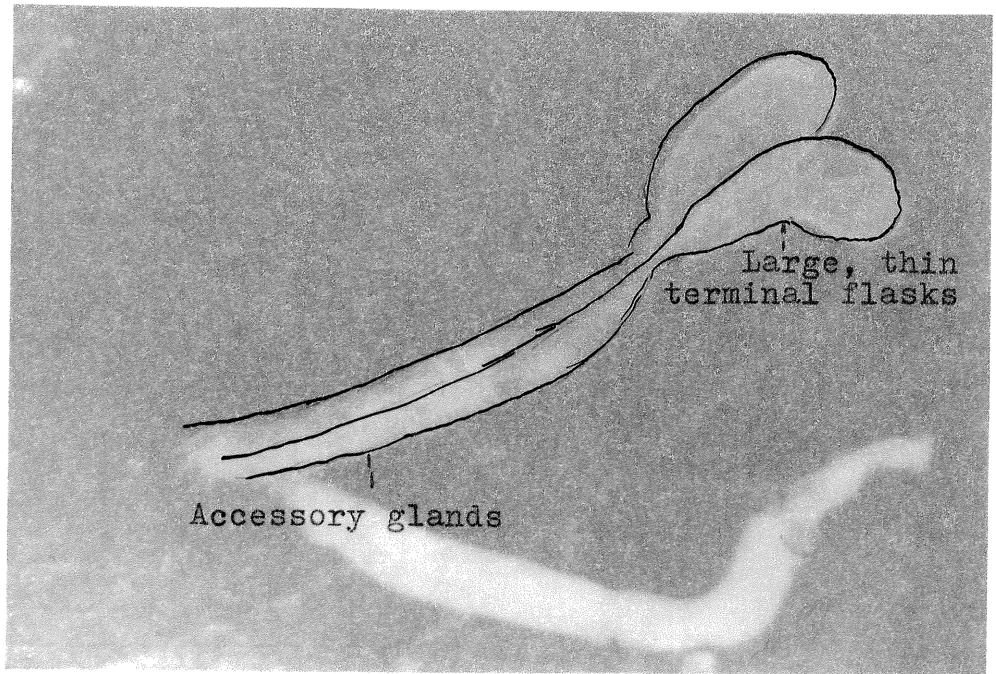


Photo. 6. Large, flask-like terminalia of the accessory glands, appear one-cell thick. Seldom contain secretion, but have a bright, gleaming appearance.

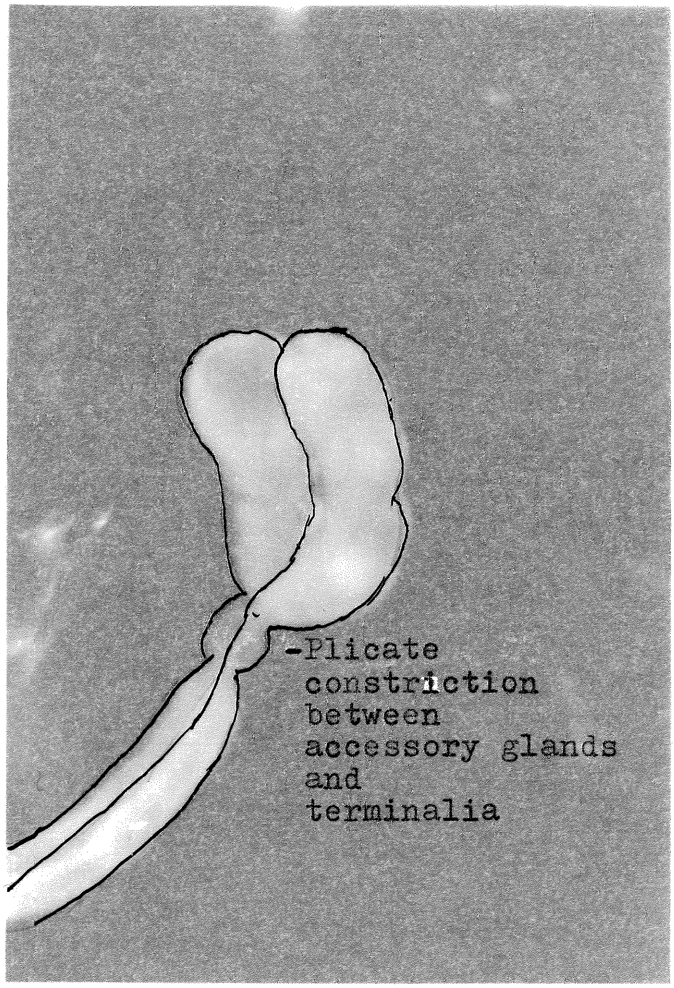


Photo. 7. The terminal bodies are separated from the rest of the accessory glands by a plicate constriction.

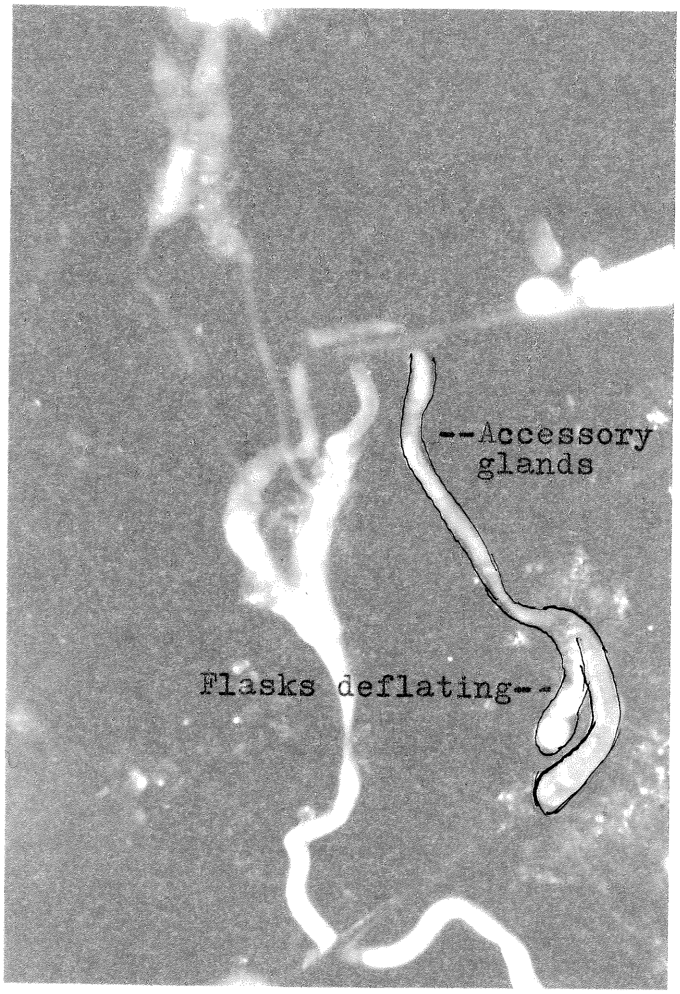


Photo. 8. The terminal bodies of the accessory glands deflating due to trauma, but their size in relation to the glands still apparent.

This specimen had a small amount of secretion in one of the end bodies.

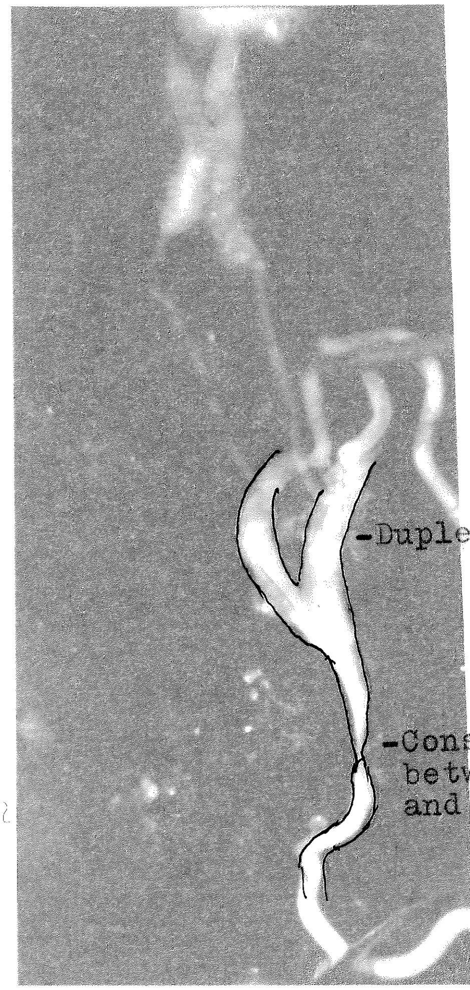


Photo. 9. Constriction between the ductus ejaculatorius duplex and the primary segment of the ductus ejaculatorius simplex, going caudally.

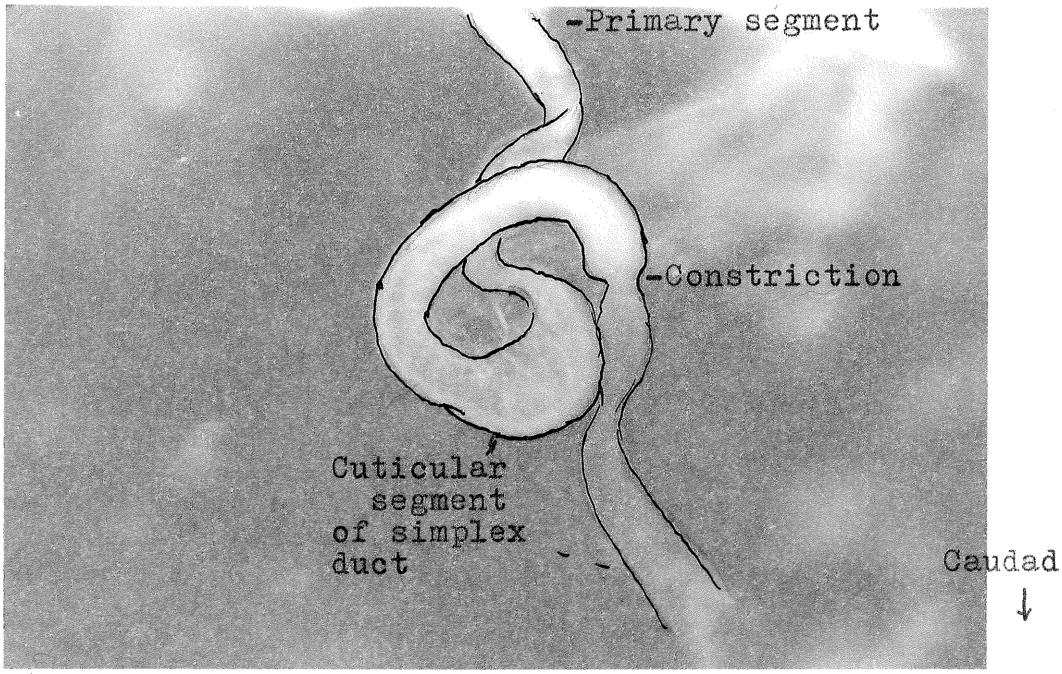


Photo. 10. Strong constriction between the cuticular segment and primary segment in newly emerged male. Ducts still transparent.

The muscular portion of the cuticular segment is looped around the lower primary segment.

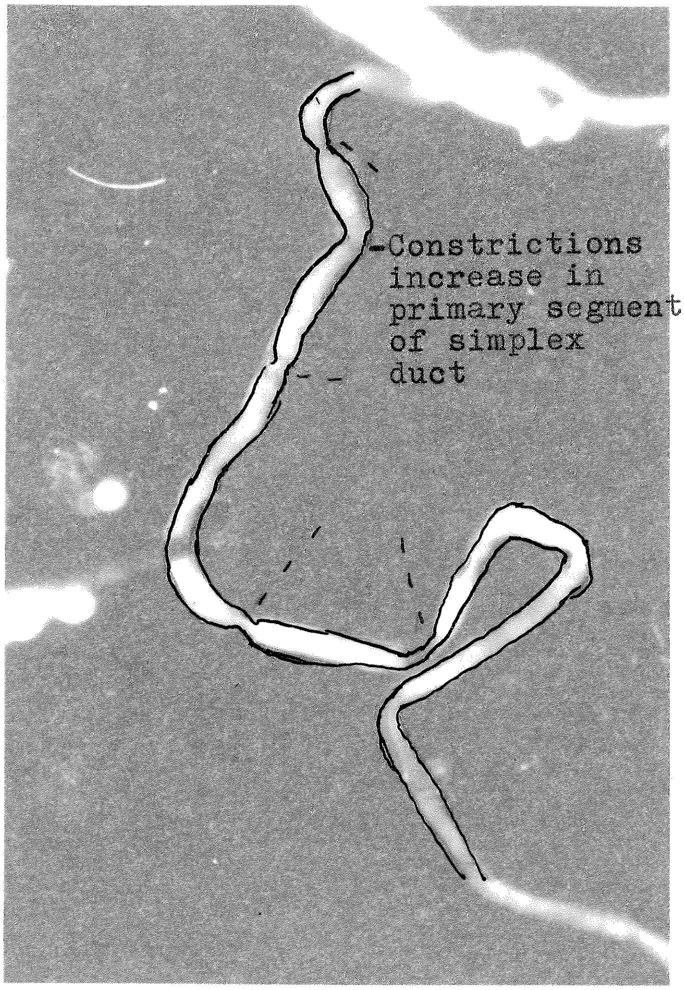


Photo. 11. The number of constrictions increase in the primary segment as secretions appear.

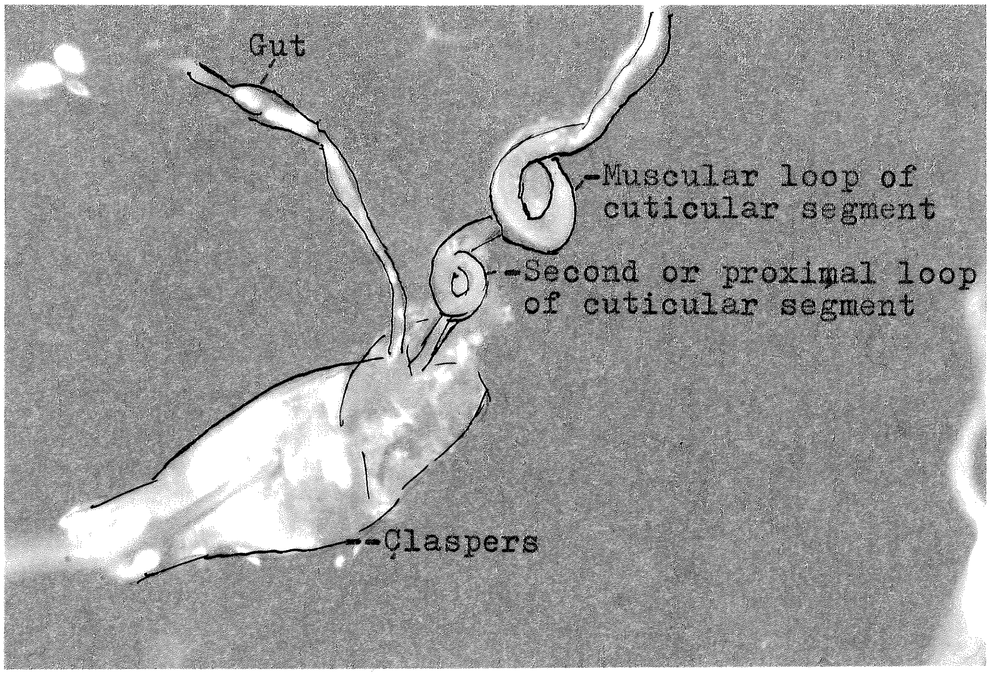


Photo. 12. The second loop of the cuticular segment below the muscular loop. Seen when lower abdominal segments are removed.

The claspers and part of the gut are in lateral view.

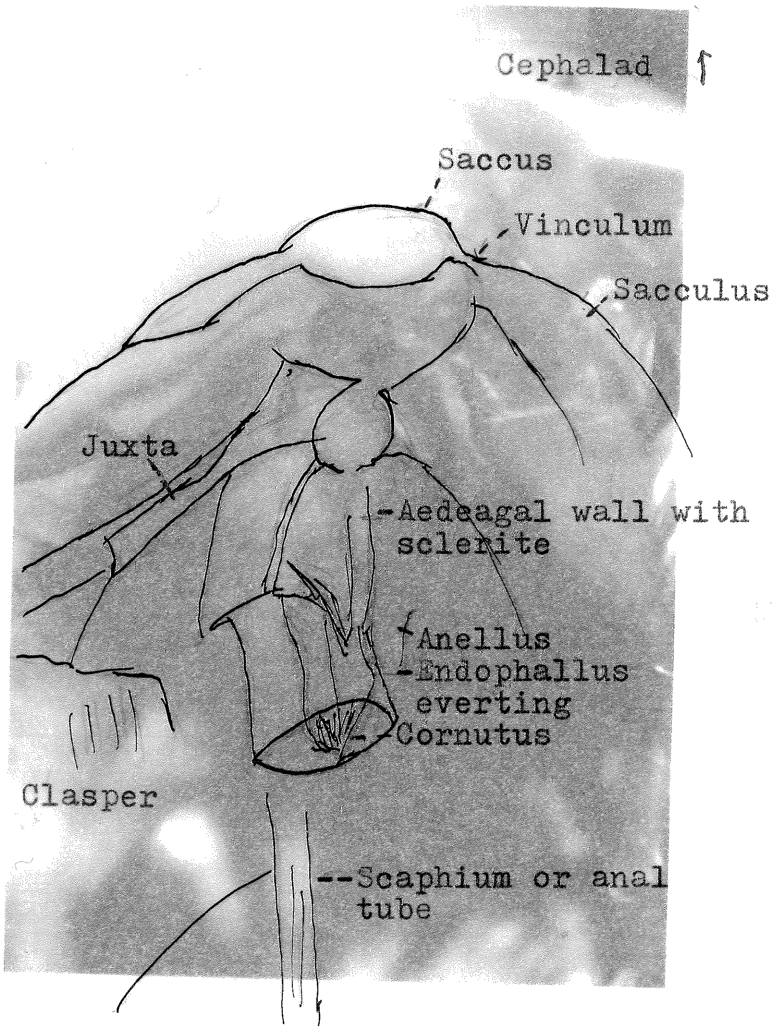


Photo. 13. Ventral view of aedeagus and claspers. The lower portion of the cuticular was compressed to force the endophallus to evert.

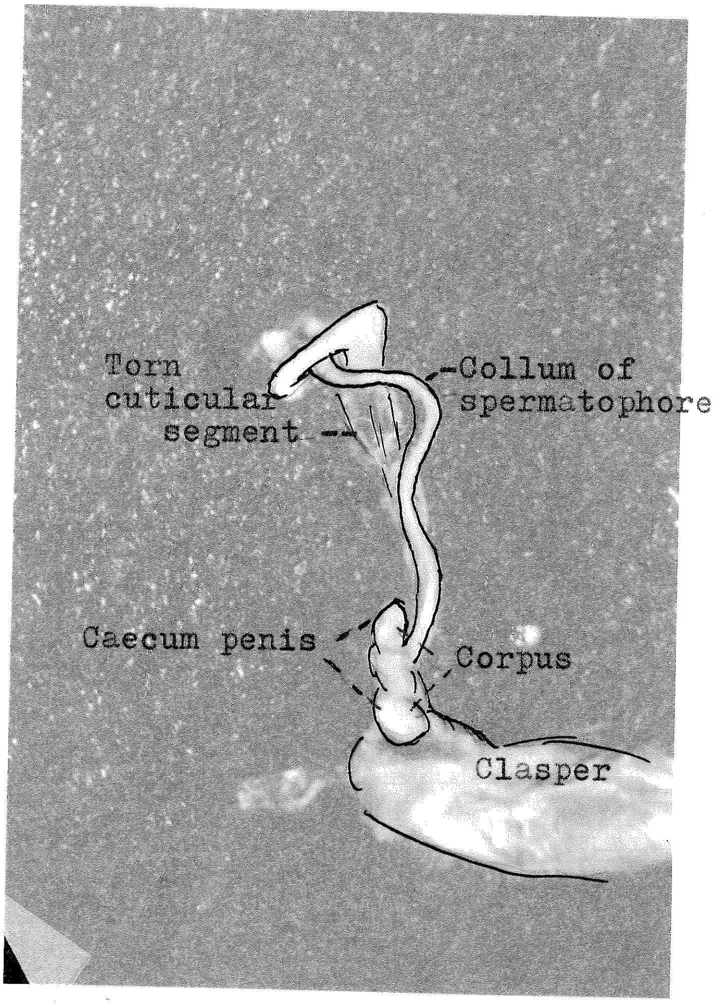
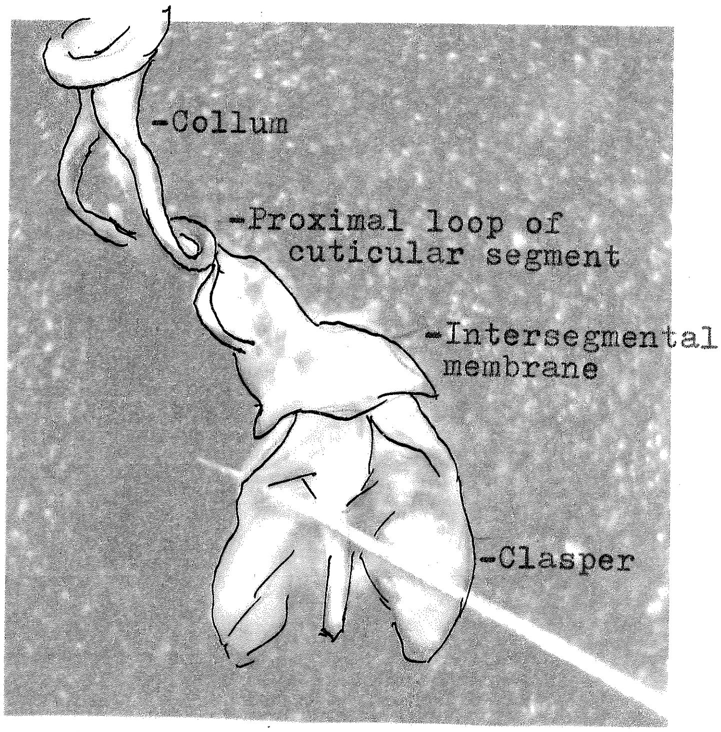


Photo. 14 and 15.
Lateral and ventral views of spermatophore in cuticlar segment. Segment is torn, male disturbed during copula.



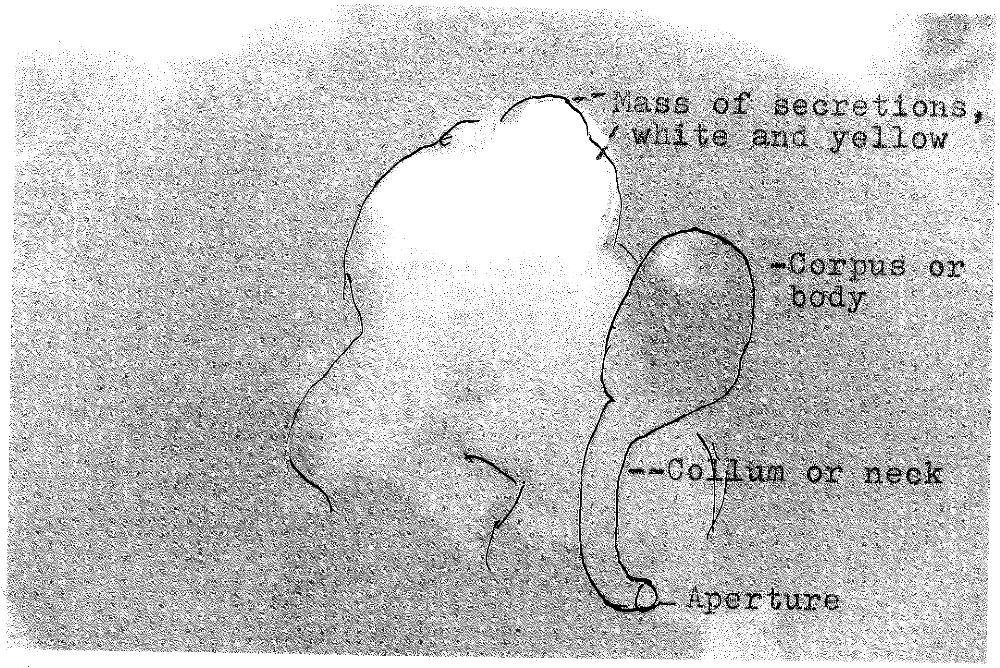


Photo. 16. A rejected male ejected spermatophore and secretions. Part of claspers seen in upper right corner.

OBSERVATIONS: THE FEMALE REPRODUCTIVE SYSTEM

The complete female reproductive system, except for the ovaries is diagrammed in Figure 7.

Ovaries and Oviducts

The paired polytrophic ovaries in Lepidoptera and the linear sequence of oocytes in each tubular ovarioles that reflects their progressive development have been frequently and adequately described and will be omitted here.

The ovaries are the most prominent organs in the female abdomen and even in the recently emerged female reach from the second to the seventh abdominal segment.

The female imago emerges with unripe eggs but can be inseminated after wing-drying.

In the zero hour female, the lateral oviducts appear flattened, long and transparent, and lie folded midway along their length. The common oviduct also appears long, thin and flattened, but not folded. It is also transparent (photo. 17). As the oviducts expand, the empty calyx and pedicel are clearly distinguished. Ventral dissection best visualizes the appearance ot the ducts and the vertical looping and reverse looping of the ovaries on each side of the abdomen.

The narrow, anterior ends of the germaria may be found crossed or uncrossed and floating free either in the upper or lower abdomen and on occasion have been found embedded in the dorsal wall between the second and third abdominal segments.

As the eggs ripen, the ovaries become bulky masses obliterating other organs, the lateral oviducts foreshorten, and the calyx and pedicel become indistinguishable. The common oviduct becomes a prominent, thick structure asymmetrically positioned toward the right side, and the widened portion of the common duct at the apex, called the vestibulum, is distinguished (Fig. 8). The membrane or the follicular sacs become fragile as the eggs mature.

<u>The Bursae Copulatrix</u> (Fig. 7,9,11,14; Photo. 18, 20-22)

The copulatory pouch is the second most prominent organ lying dorsally between the third and fourth abdominal segments, measuring 3mm in diameter and 2mm in length in the unmated female. The pouch is peculiarly shaped like a saddle and not flattened except medially where the large rhomboid signum is located (Fig. 7). The signum is reddish-brown in color and visible, though the bursa is opaque on emergence. Beneath the thinner, outer membrane of the bursal pouch, lies a layer of heavy muscle, wheel-shaped in appearance.

From the dorsal, proximal portion of the bursa, a short duct extends to a thin sac, called the accessory bursa or appendix bursa. The accessory bursa is empty in unmated females (photo. 18). It becomes filled with a white secretion when the female is inseminated (photo. 19).

Upon insemination, the bursal pouch may elongate to 5mm and becomes more opaque. During the first 12 hours following insemination, a transverse groove develops along the wall to which the signum is being pushed by the increasing volume of substances in the bursa (Fig. 9; photo. 20). Later, the groove is obliterated.

Spermatophores within the bursa appear to undergo a tanning process in females that are 3-5 days post-copulatory (photo. 21-22). The spermatophore, on removal from the bursa from females more than 48 hours post-copulatory, appears to develop clefts or openings in the chitinous-like covering of the corpus (Fig. 10).

The Ductus Bursae (Fig.7,9,11,14; photo. 23)

The bursal duct is fairly long in the B corn borer, being 4mm in length. It extends from the copulatory pouch making 1 or 2 spirals along the dorsal wall until the seventh segment where it projects ventrally in a half circle continuing caudally to the ostium bursa or vulva (Fig. 11 D.B.).

Grossly, there is no distinct morphological cervix bursa, but the part of the duct extending basally from the pouch may possibly be considered one.

The duct is transparent in emerging and non-mated females (photo. 23), though the transparency is somewhat greater following insemination.

Caudally and close to the copulatory aperture, a cuff-like sclerotization occurs in the duct.

Connecting the bursal duct to the common oviduct is a short ductus seminis (or seminalis) ranging from 1.5mm in the non-mated female to 2.5mm in the mated. The duct extends ventro-laterally from near the base of the ductus bursa and opens almost dorsally into the vestibulum. There is an enlargement of the duct near its entry into the ductus bursa. In unmated females the seminal duct may not be visible as it lies closely against the intersegmental margins of the seventh and eighth abdominal segments (Fig. 12). After insemination the duct is raised (Fig. 13).

The Accessory Glands (Fig. 7-8, 13-14; photo. 24)

The paired reservoirs of the accessory glands are the third most prominent organs in the female system. They are 4-5mm in length, ovoid or flask-like in shape and filled with a golden yellow fluid in females of all ages, mated and non-mated (photo. 24). They are easily damaged on dissection. A ventral approach may prevent trauma to the reservoirs, but the specimen has to be turned dorsal side up for better viewing of these organs.

The common duct of the reservoirs, 0.4mm-0.6mm long, enters the common oviduct dorsally and caudal to the entrances of the ducti seminis and spermatheca (Fig. 7,13).

The accessory glands are fairly long, extending 14mm or more from odd finger-like projections laterally positioned near the distal ends of the reservoirs (Fig. 14). The glands may extend cephalad around the left ovary but usually winds anteriad through the ovary, reversing directions with the ovary and is bound to it by fat and tracheae. The glands terminate dorsally on the bursa copulatrix adjacent to or between the accessory bursa and the bursa copulatrix, and it may loop back against the bursa as it terminates.

The anterior ends of the glands adhere firmly to the bursa being tied to it by fat and tracheae which also masks them. The caudal portions of the glands have to be extricated and separated from the spermathecal gland and the nerves extending from the fourth ganglion.

The Spermatheca (Fig. 7,13-15; photo. 24-27)

The spermatheca consists of a spiral canal which enters the vestibulum of the common oviduct at one end, and at the other end extends to a bilobed structure, the larger lobe being the receptaculum seminis and the smaller lobe, the lagena. The lobes do not appear to be completely separated (Fig. 14-15, photo. 25). A long (29mm) glandula receptaculi extends from the lagena (Fig. 14). The spiral canal contains a sclerotic region which divides it into 2 ducts for part of its length (Fig. 15).

On dorsal dissection, the glandula receptaculi is intertwined caudally with the accessory glands, and then usually winds through the right ovary laterally and anteriad. It also positions terminally with the accessory glands to the dorsal bursa copulatrix. The gland remains single throughout its length.

The spermatheca in unmated females of any age is somewhat transparent (photo. 25). When the female is inseminated, the spermatheca is found to be filled with an opaque white substance (photo. 26).

In non-mated females, the glandula receptaculi as it extends from the lagena, is moderately narrow for 2-3mm before widening. After insemination, the moderately narrow portion becomes highly constricted, has a plastic-like appearance (photo. 26) and acts resilient to touch.

Observations on squashed slides of the spermatheca, stained with fast green, revealed spermatozoa and open sperm bundles, with the bundle walls collapsed (photo. 27).

<u>The Ostium Bursae and Ovipositor</u> (Fig. 16; photo. 28-30)

The ostium bursa (copulatory aperture or vulva) is near the pre-segmental margins of the eighth sternite and is surrounded by intersegmental sclerites (Fig. 16, photo. 28-29).

The sclerites appear to fuse with the anterior margin of the eighth sternite. The anterior apophyses seem to arise from the border of the eighth sternite and extend anteriad into the body cavity. The posterior apophyses emerge from the ninth segment and extend anteriad into the abdomen.

The ovipositor is an extension of the abdomen itself. The seventh, eighth and fused ninth-tenth segments taper and are capable of being protruded (photo. 28-29) and retracted telescopically. The ninth-tenth segments form a ring, covered with hairs, for the deposition of eggs on the smooth underside of leaves. The ring is called the papillae anales (photo. 30).

Mating

The female calls the male by protruding the caudal segments and releasing a sex pheromone. The chemical nature of the pheromone was analysed in 1968 (Roelofs and Arn). The female was not observed to take the calling position except in the presence of the male.

Mating occurs end to end, dorsal side up (photo. 31). At the time of copulation, the elaborate male armature angles to allow penetration of the aedeagus into the ductus bursa. The inner faces of the valves clasp the lower segments of the female abdomen. Large, lateral bundles of bristles spread widely at their proximal ends, possibly to aid in maintaining the copulatory position.

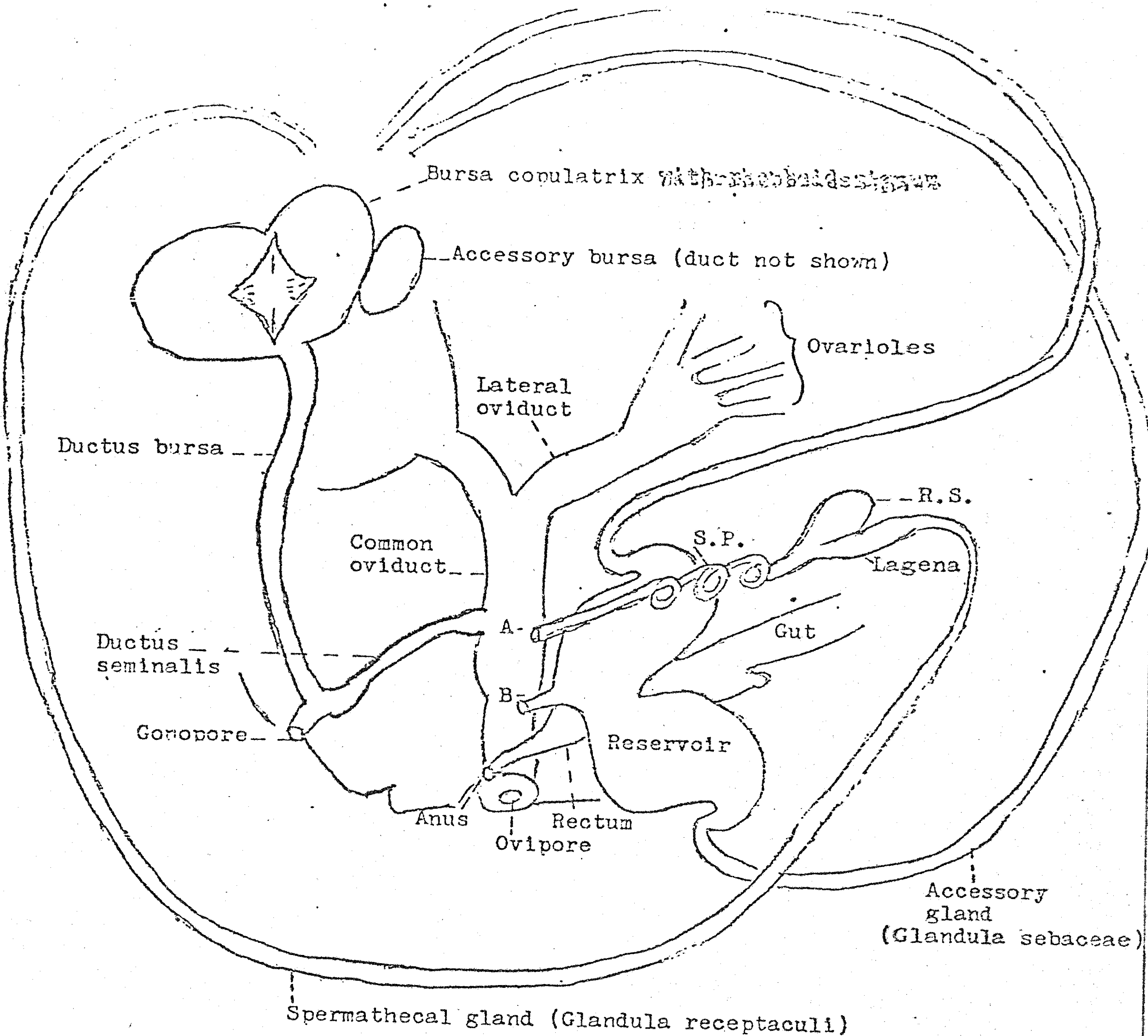


Figure 7. Dorsal view. Stylized diagram of female reproductive system.
S.P. = spiral canal
R.S. = receptaculum seminis
A = duct of spermatheca
B = common duct of reservoirs of accessory glands

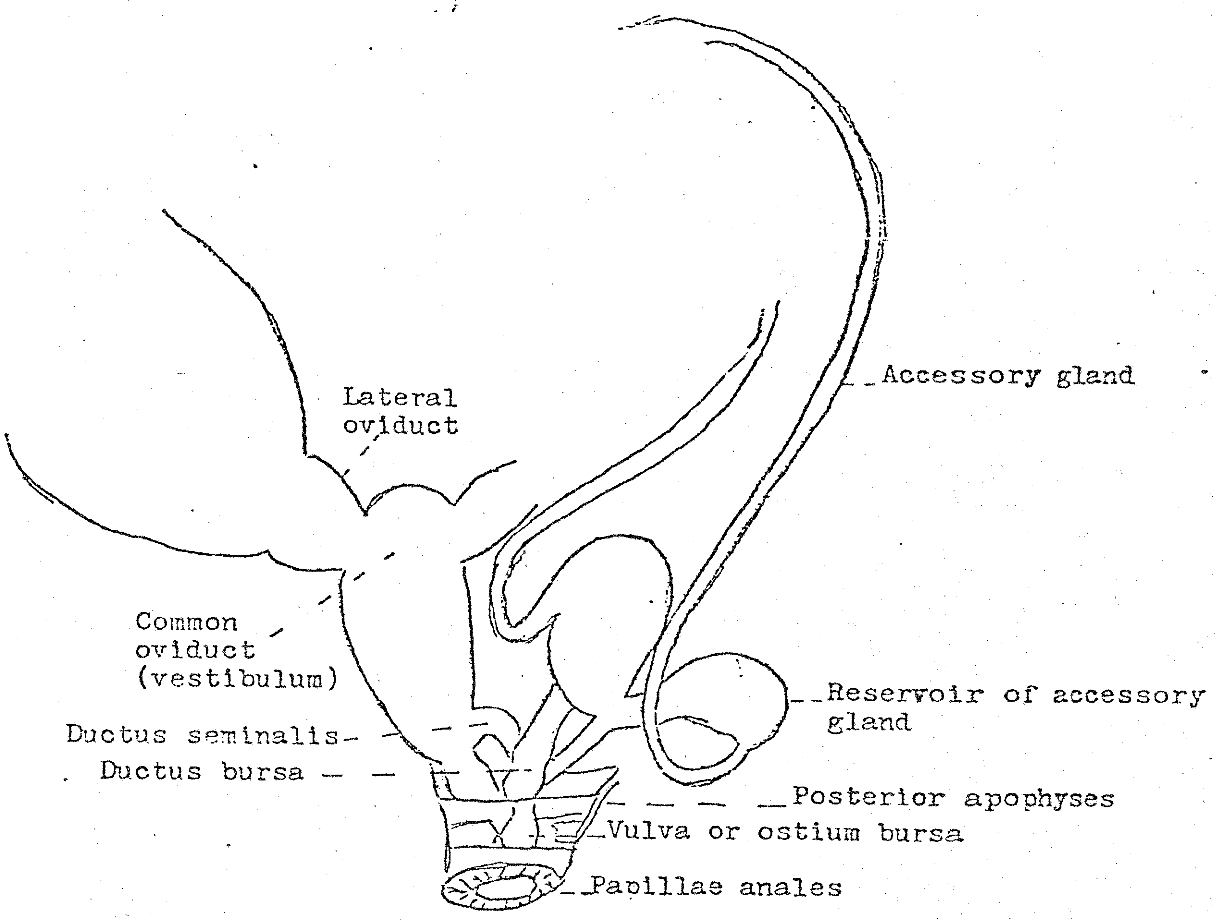


Figure 8. Ventral view. Female with mature eggs. Note size of common oviduct, its asymmetric position, and the foreshortening of the lateral oviducts.

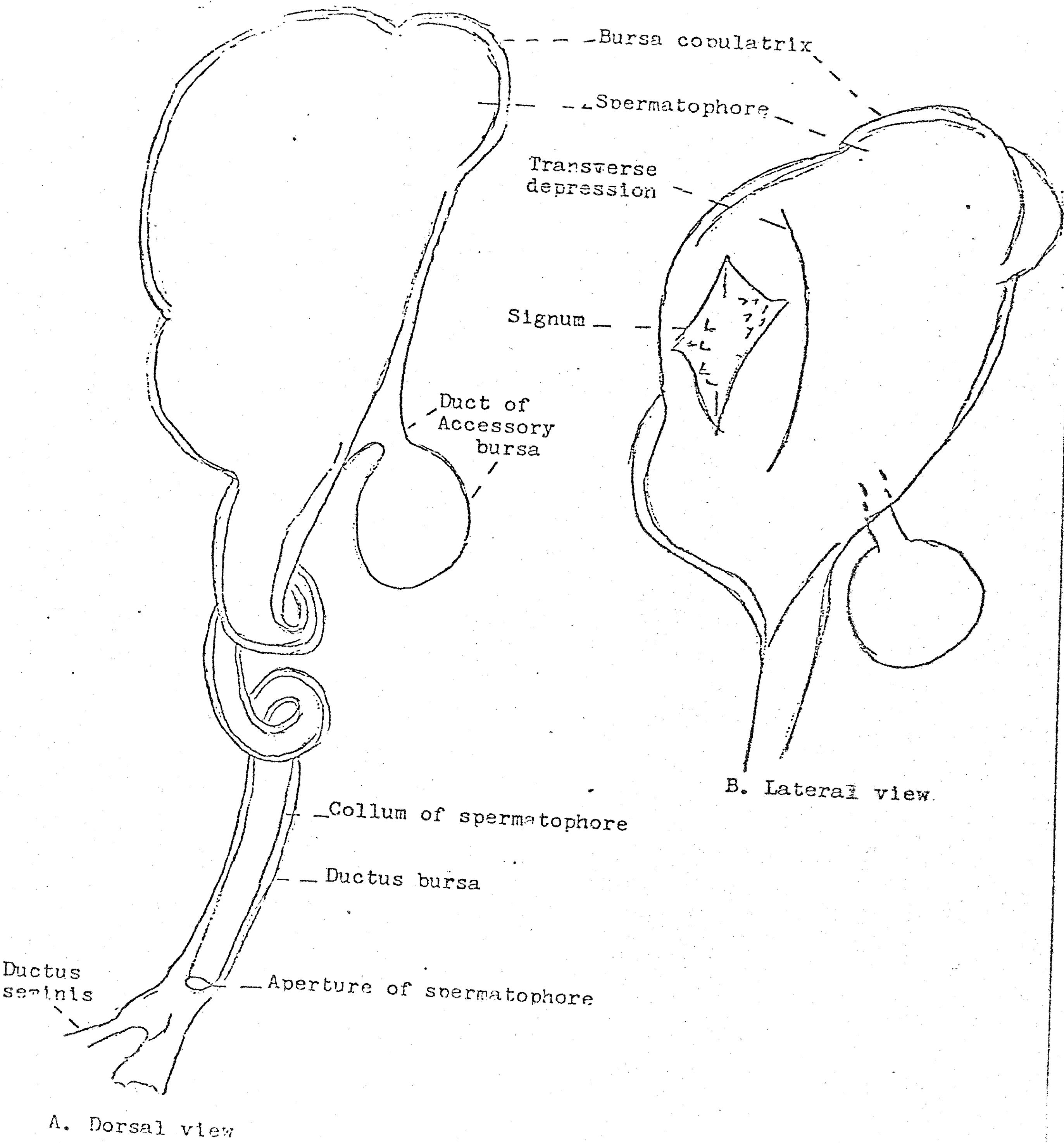


Figure 9 . Spermatophore in bursa copulatrix of female inseminated within 12 hour period.

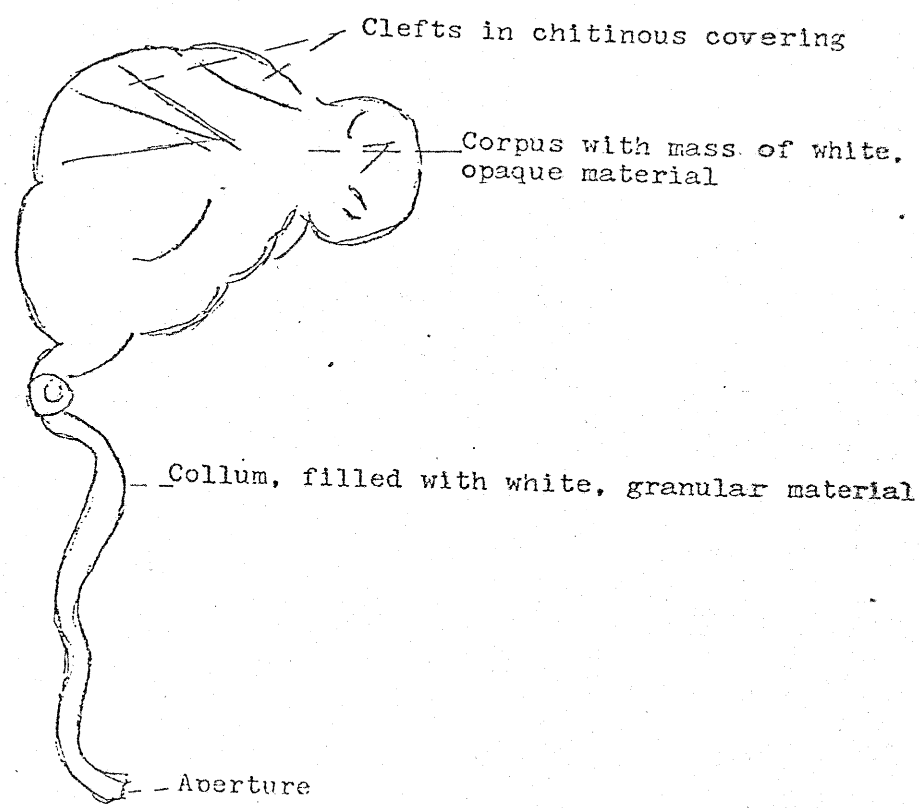


Figure 10. Spermatophore taken from female 2-3 days post-copulatory.

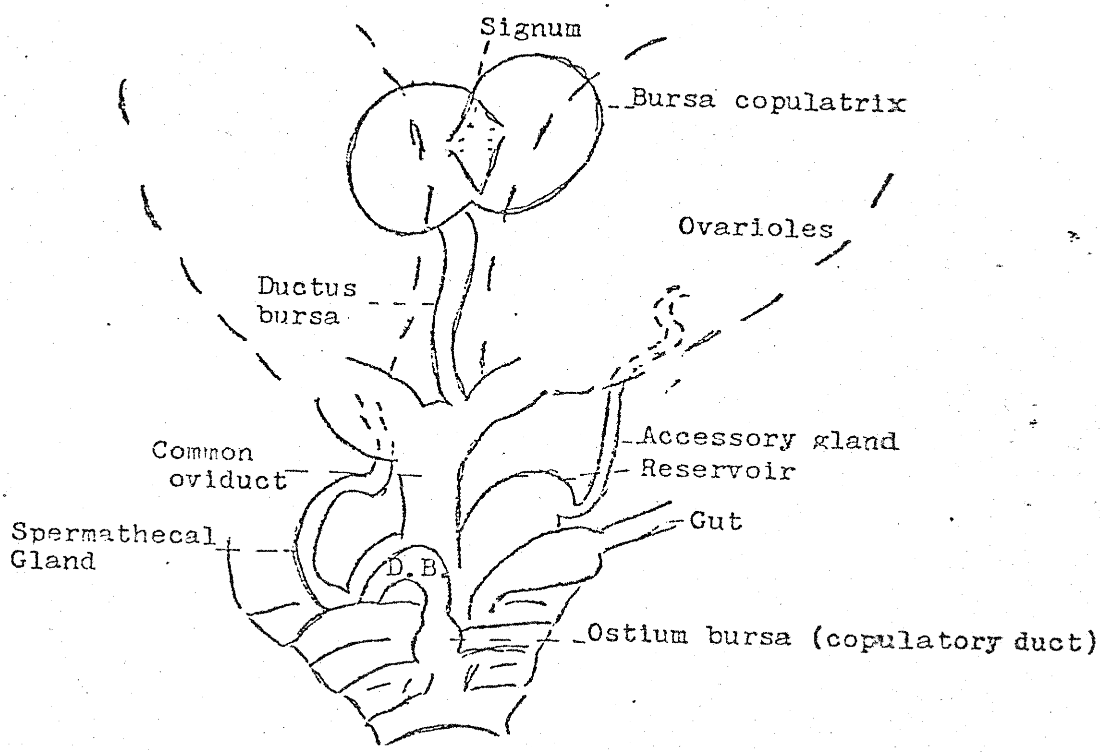


Figure 11. Ventral view. The ductus bursa (D.B.) comes down along the dorsal body wall and turns from posterio-dorsad to the ventral body wall in a half-circle. The upper spiral of the ductus bursa is not shown.

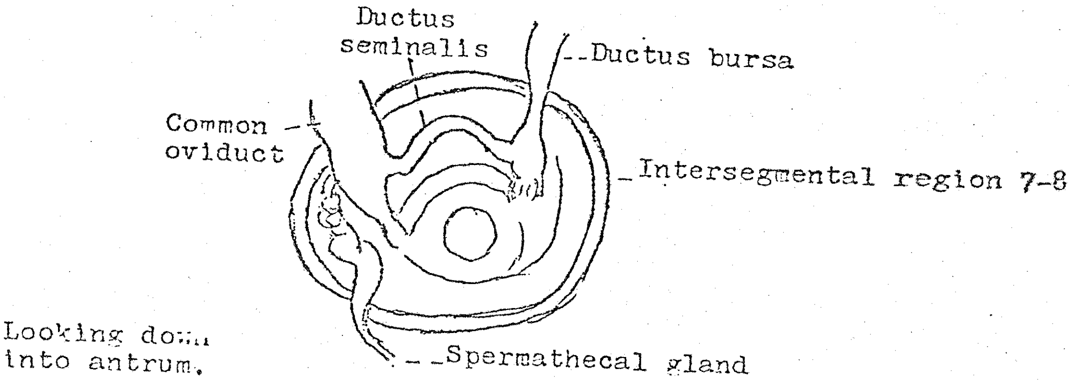


Figure 12. Young, un-mated female. The ductus seminalis is closely applied to 7-8 intersegmental margins.

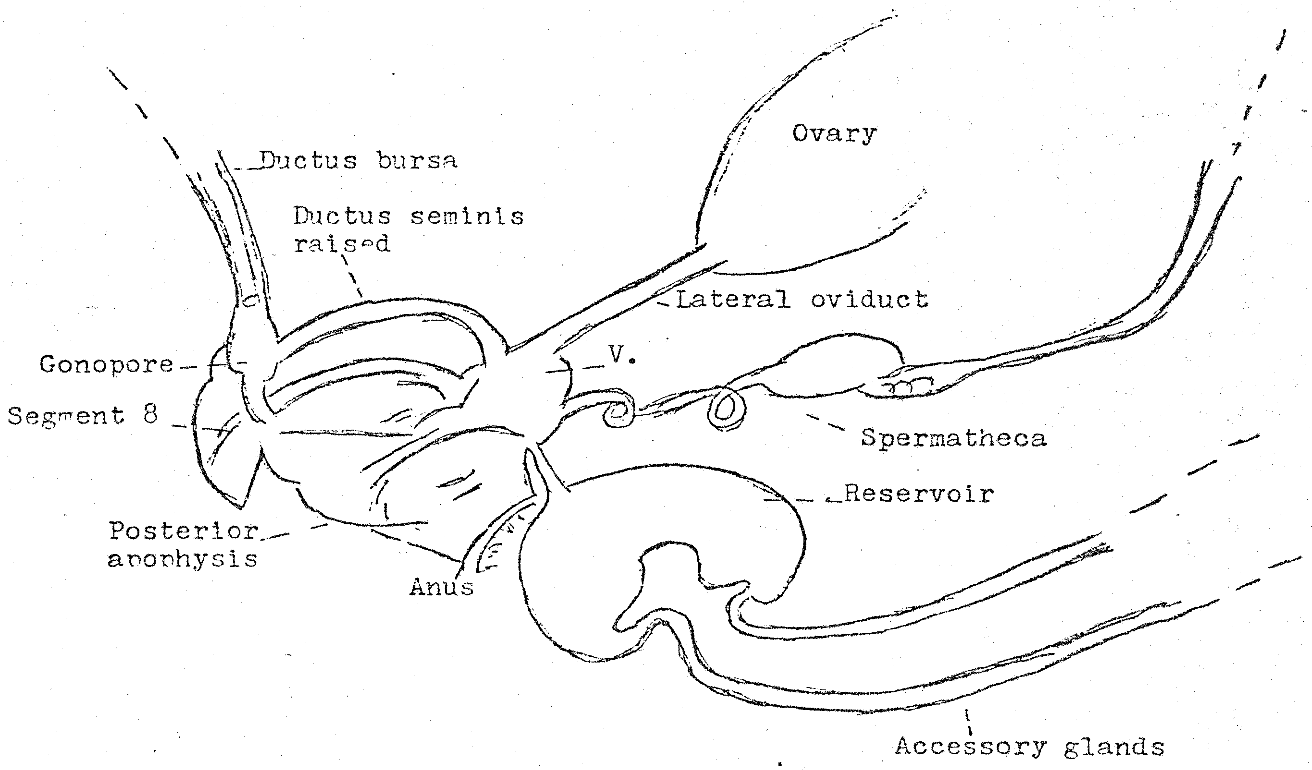


Figure 13. Ventro-dorsal view of lower abdominal segments of inseminated female. Ductus seminalis is now raised from intersegmental region.

Clearly visualized: spermathecal duct enters vestibulum (v.) almost directly opposite the entrance of the ductus seminis. The ductus seminis enters slightly dorsal into vestibulum of common oviduct, and the spermathecal duct enters anterio-dorsad.

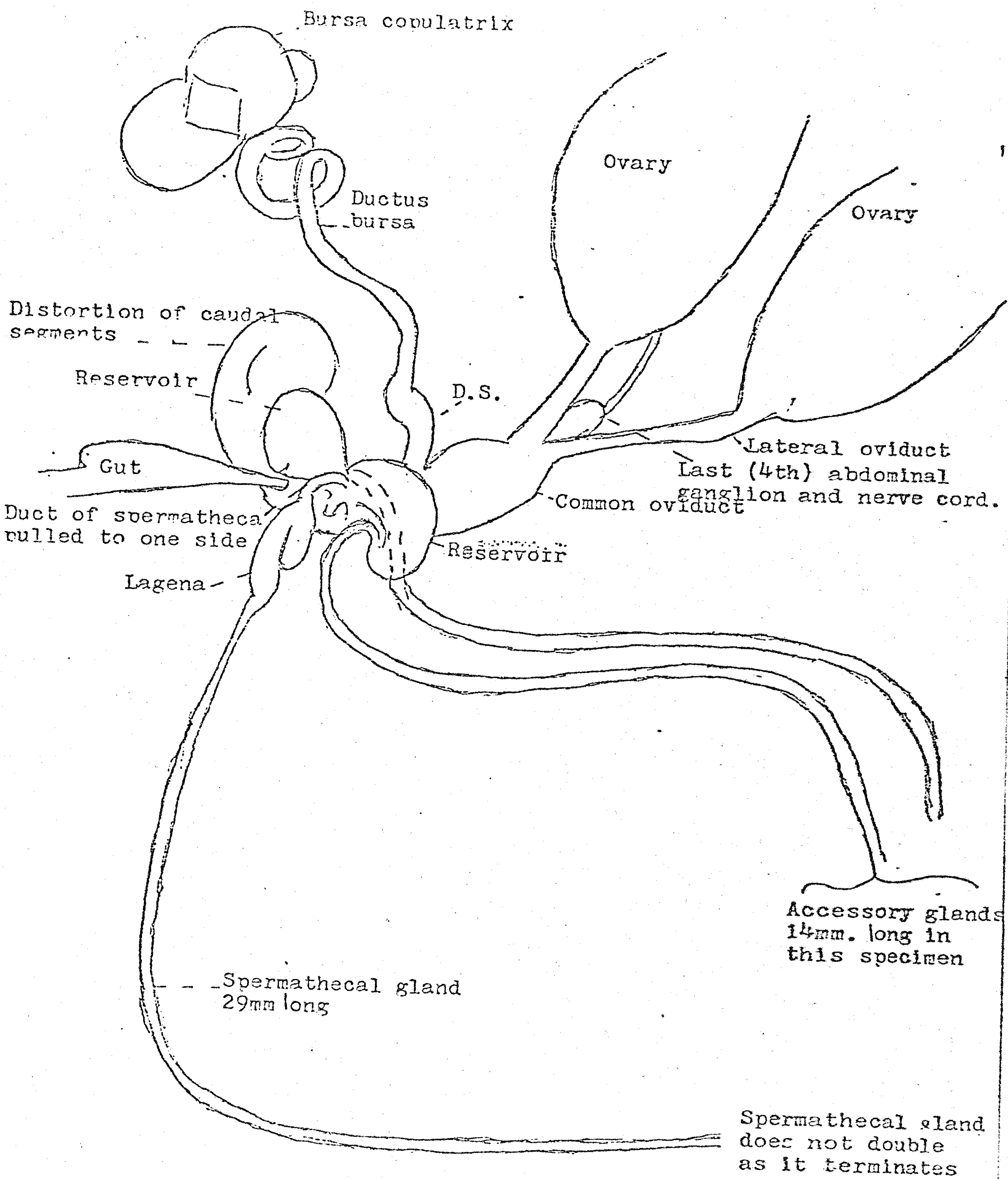


Figure 14. Dorsal dissection. Young, non-mated female. Organs distorted, ovaries pulled to one side and alimentary canal to the other side so that the spermathecal and accessory glands can be extricated and measured. The spermathecal duct enters common duct opposite the ductus seminalis (D.S.)--this entrance is hidden by the reservoir here.

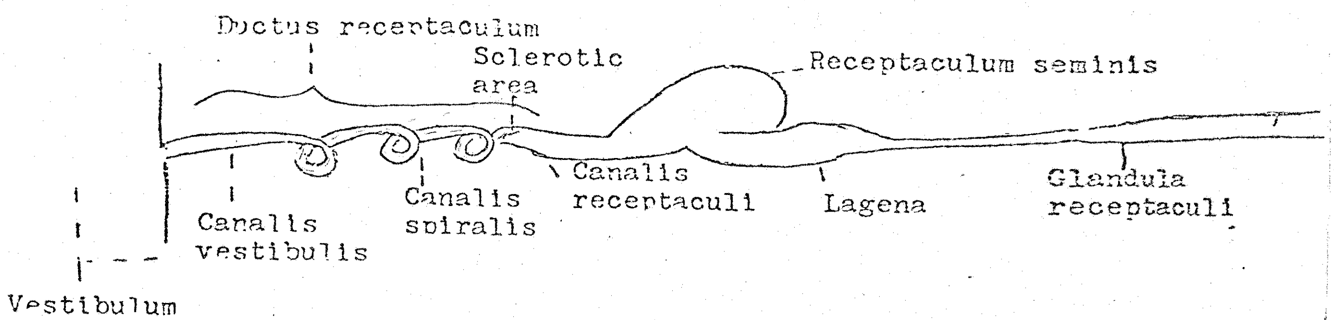


Figure 15. The spermatheca. The receptaculum and lagena do not appear to be completely separated. The sclerotic area in the spiral canal divides it into 2 ducts.

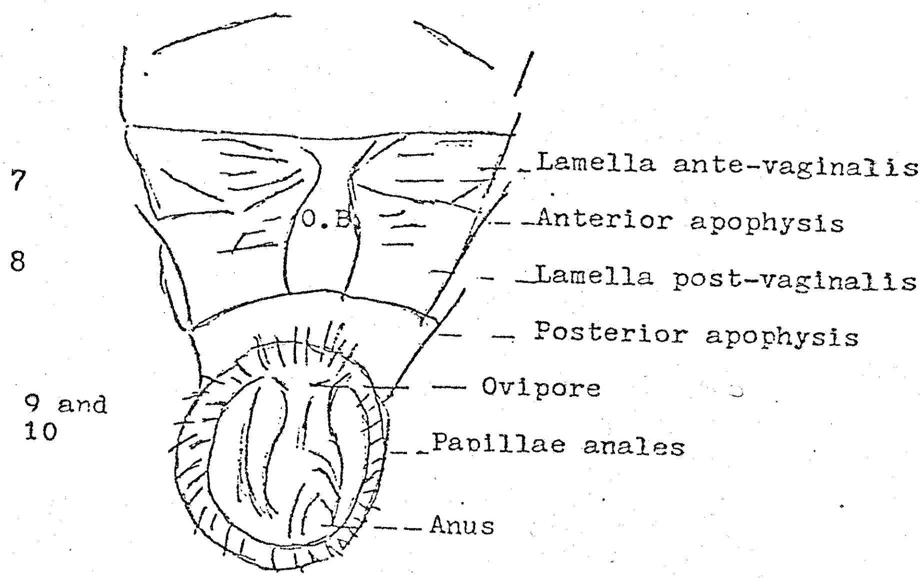


Figure 16 Ovipositor, lower ventral view. Papillae anales turned up for viewing. Abdominal segments 7-10, with 9 and 10 considered fused.
O.B. = ostium bursa

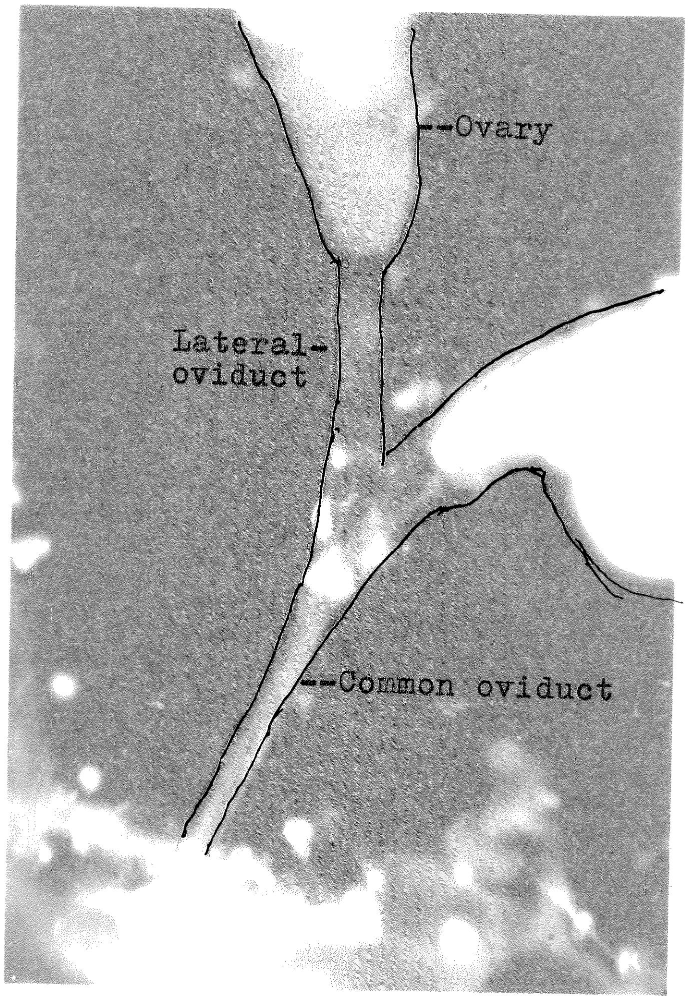
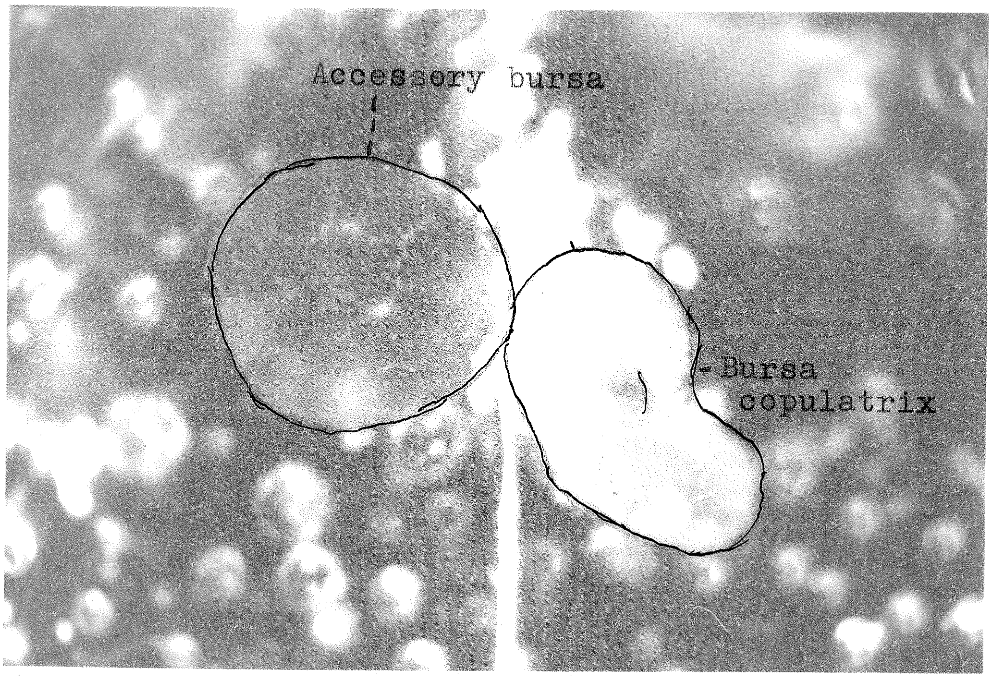


Photo. 17. Twelve hour female. Eggs beginning to mature. Lateral and common oviducts remain transparent.



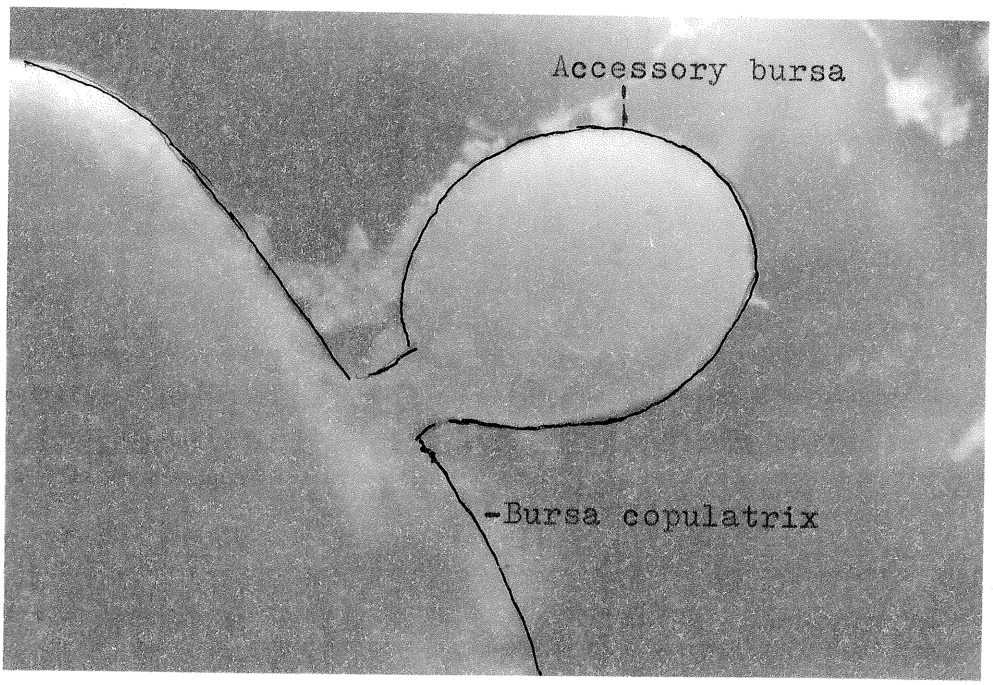


Photo. 18. Unmated female, 24 hours old. Saddle-shaped bursa copulatrix and accessory bursa which has enlarged due to duffusion of saline. Accessory bursa is usually without secretion until female is inseminated.

Photo 19. Inseminated female, 48 hours old. Accessory bursa filled with secretion.

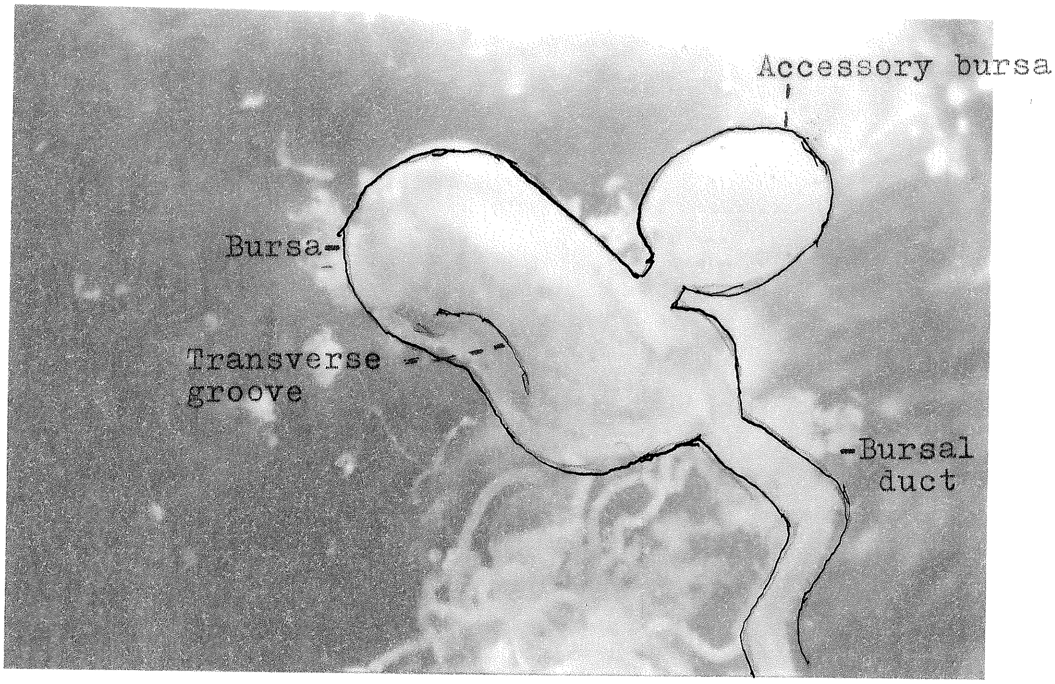


Photo. 20. Lateral appearance of the bursa copulatrix within 12 hours of insemination. A transverse depression is visible on the left near the signum.

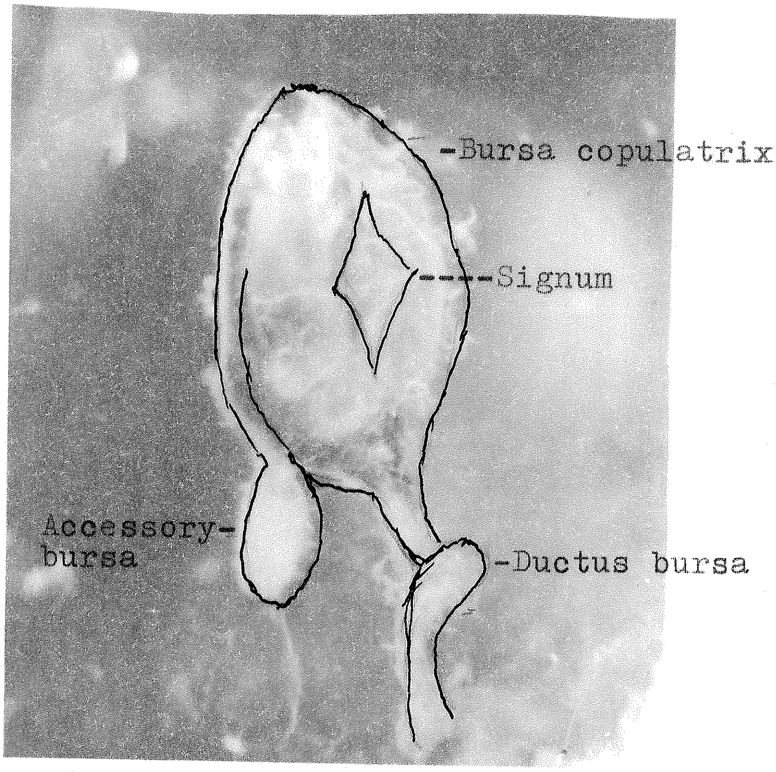
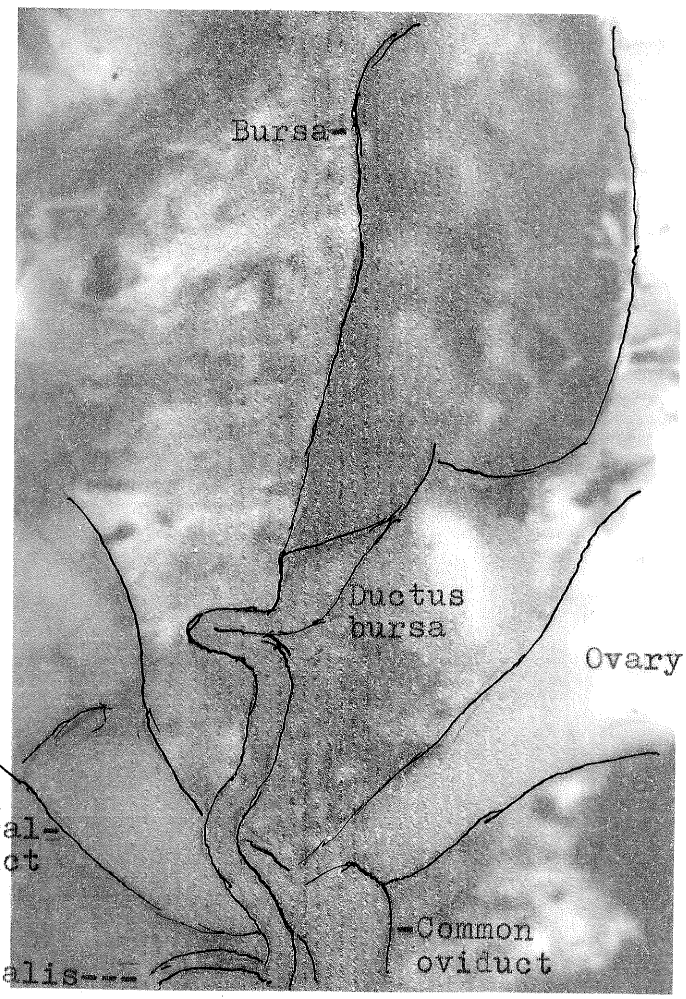


Photo. 21 and 22.
Females 3 and
5 days post-
copulatory.
Tanning process
of spermato-
phore secretions
as females age.



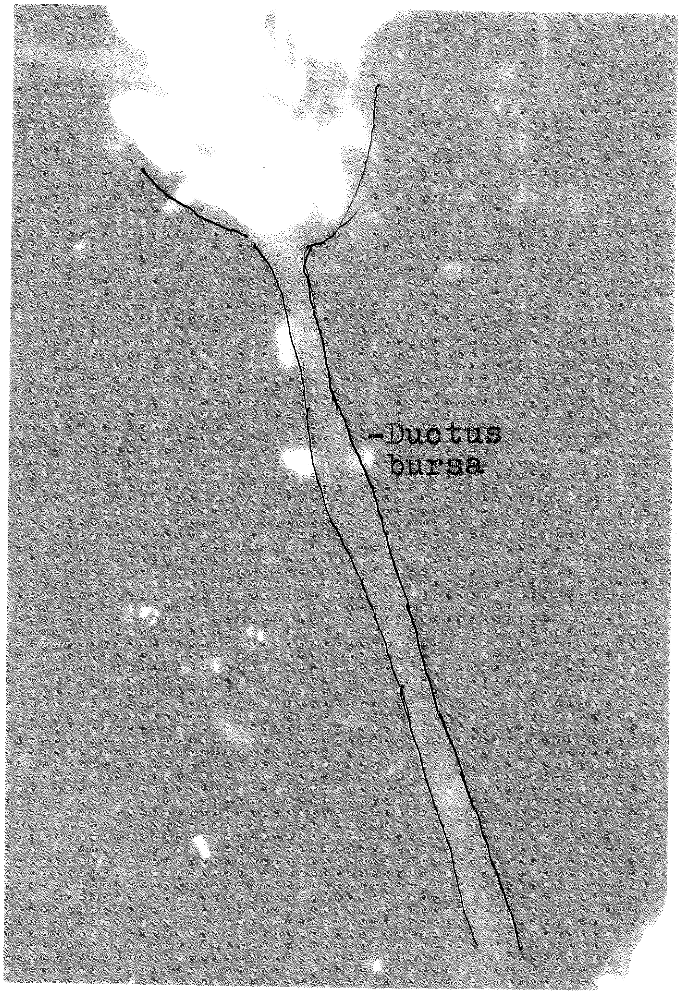


Photo. 23. Ventral view. The transparent ductus bursa in a newly emerged female imago.

The ductus becomes more transparent when the female is inseminated.

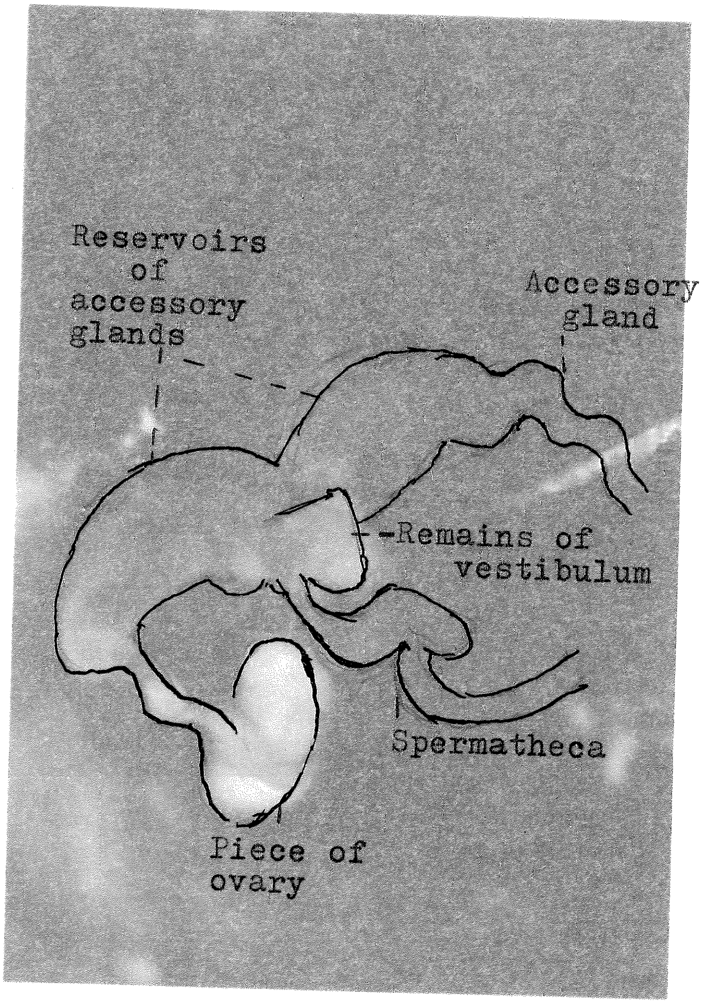
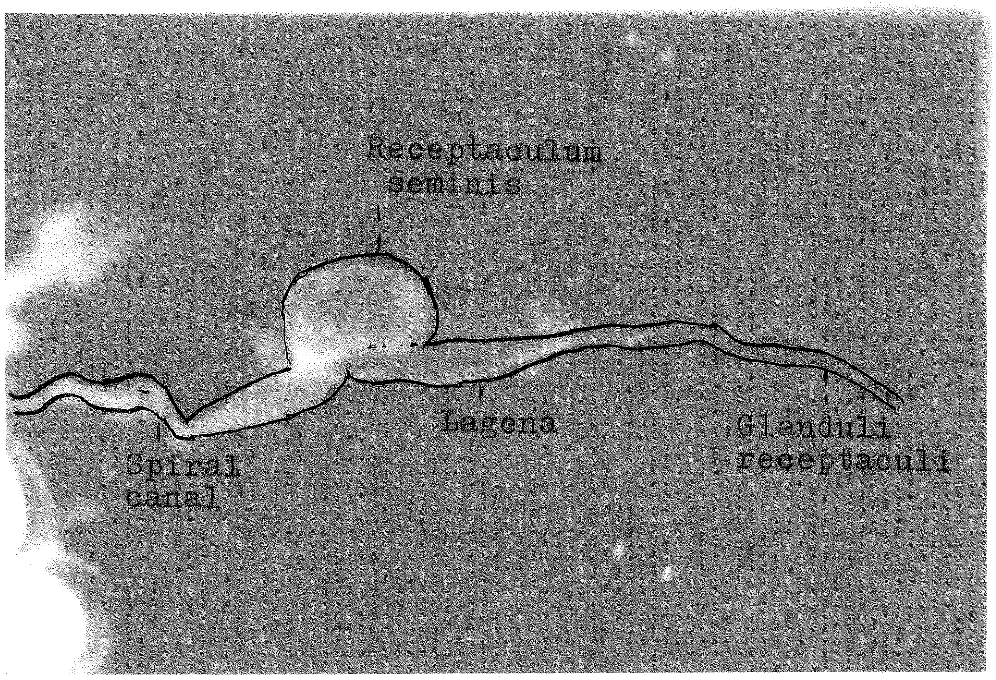


Photo. 24. Non-mated female, 24 hours old. The reservoirs are deflated due to injury. They are filled with a golden yellow fluid in both inseminated and non-inseminated. The view is lateral, somewhat dorsal, except for the ductus bursal part which has twisted ventro-laterally.

The transparent spermatheca is caudal with the remains of the vestibulum in central position.



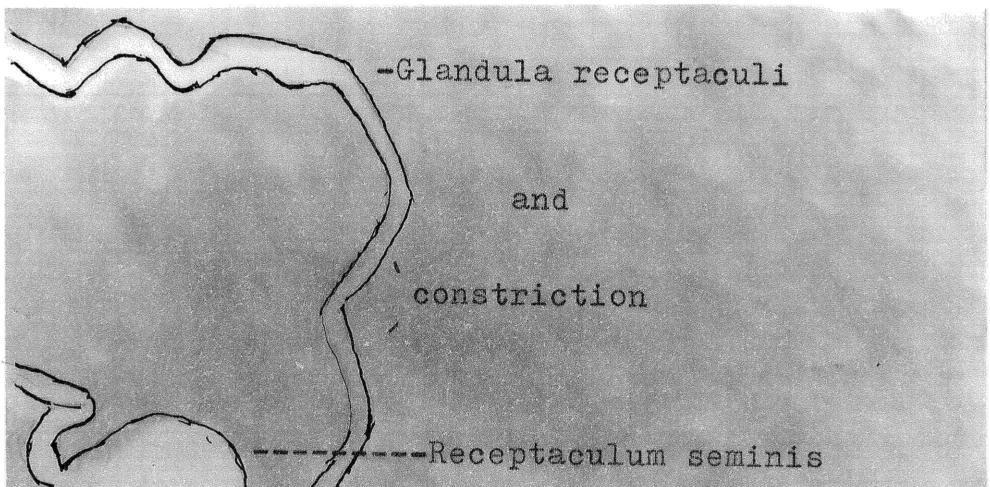


Photo 25. The transparent spermatheca in the unmated female. Part of the glandula receptaculi has broken off.

Photo 26. Inseminated female. The spermatheca and its ducts have a whitish secretion. About 2-3mm of the glandula receptaculi has become highly constricted and plastic-like in appearance.

Photo 27. Squashed slide of spermatheca from a female inseminated 12-24 hours previously. Spermatozoa present and possibly remains of bundle walls.

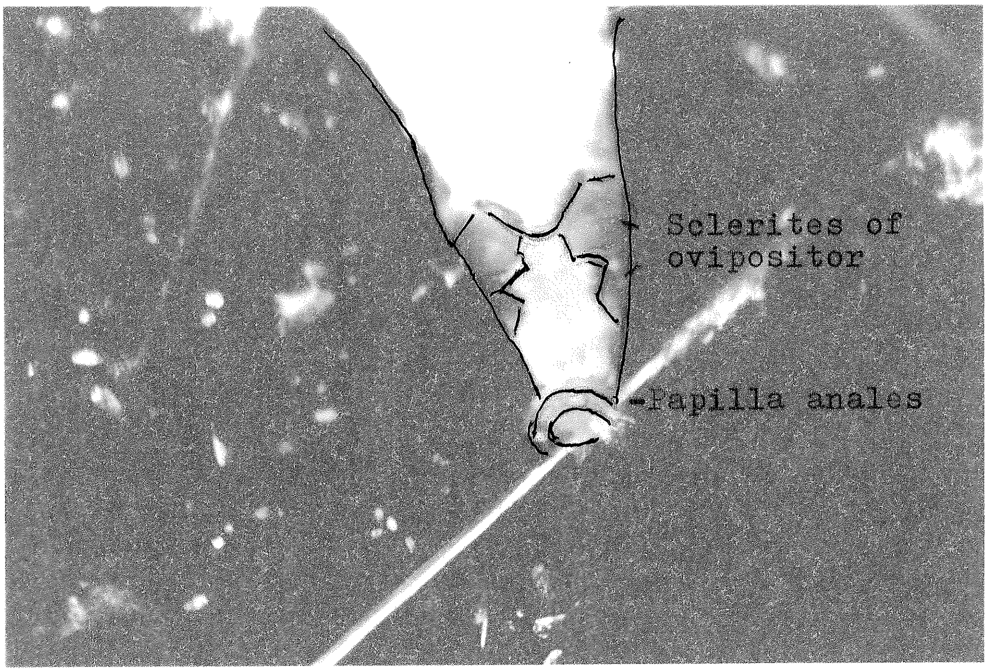
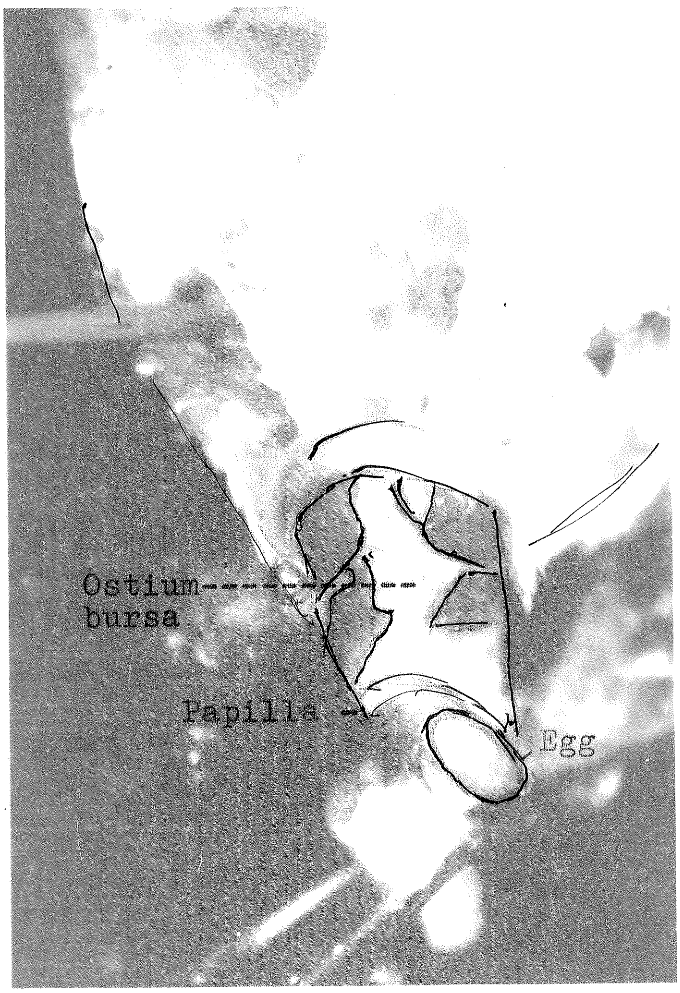


Photo 28. Ventral view. Caudal segments 7,8, and fused 9-10, in young female. The ovipositor.

Photo 29. Ventral view. Inseminated female 48 hours old. Ova mature and being extruded. Ostium bursa can be seen.



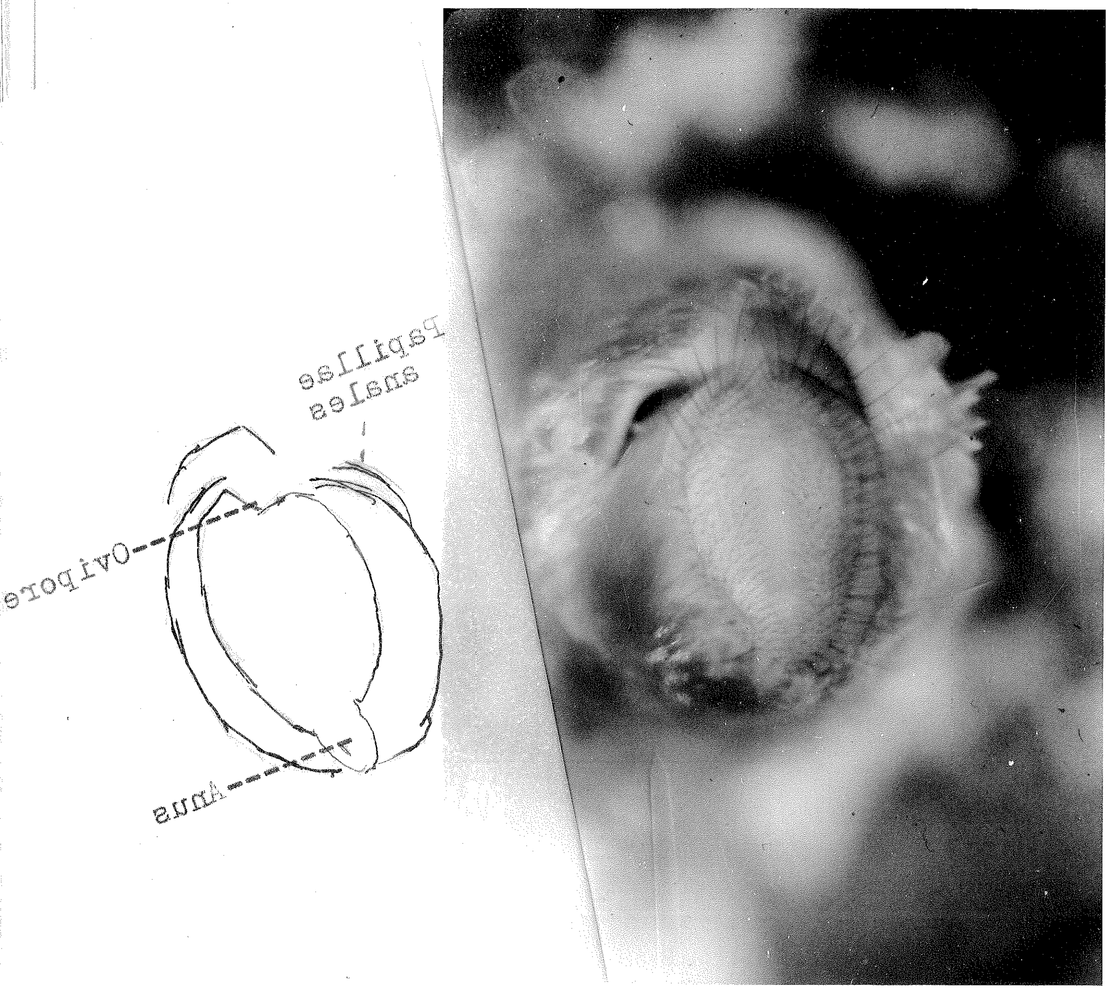


Photo 30. The fused 9th-10th segments turned up for viewing. The segments form a ring covered with hairs, the papillae anales.

The ovipore and anus are barely visible.

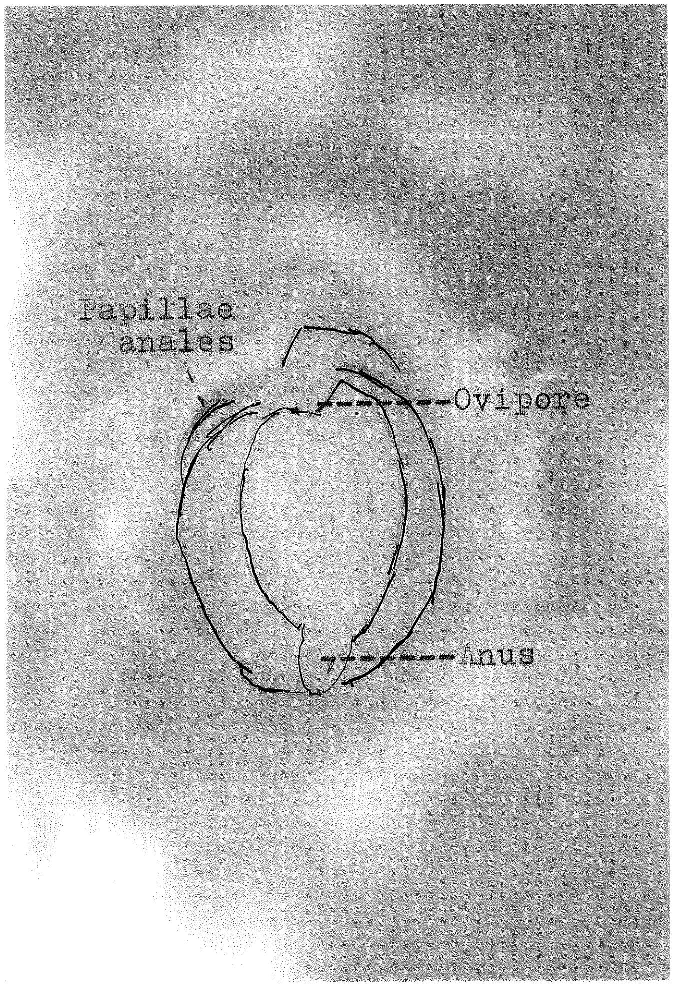


Photo 30. The fused 9th-10th segments turned up for viewing. The segments form a ring covered with hairs, the papillae anales.

The ovipore and anus are barely visible.

Photo 31. Mating position, dorsal side up.

DISCUSSION: THE MALE REPRODUCTIVE SYSTEM

The Testis

1. As in most other Lepidoptera, the testes have fused into a single organ, and in this species is enclosed by a very fragile capsular membrane.

2. The 8 follicles within the testis are closely packed without any trace of fusion, which follows Cholodkovsky's (1884) description of the final phase (4th stage) in the development of imaginal testes types.

3. Since the follicles do not appear to be twisted in the fused testis and no plane of fusion can be seen, the testis can be designated as Type C in Roepke's (1909) plan of different types of testicular structures, and not Type D as has been suggested (Drecktrah and Brindley, 1967).

4. On microscopic studies of compressed slides, the testis in the newly emerged male is filled with open sperm bundles, some still retaining portions of the bundle sheath. Yet, ejected spermatophore secretions in the abdominal cavity of the male B corn borers of all ages show closed or sheathed sperm bundles.

Either squash preparations rupture the bundle sheaths within the testis, or a rebuilding of the bundle sheath may occur during passage from the testis, as described for one species of Lepidoptera (Reiman, 1970).

The Vasa Deferentia and Vesiculae Seminales

1. The vasa deferentia show 2 major structural variations, a singly expanded portion on exit from the testis or a double expansion (2 vesiculae seminales).

2. Vasa deferentia with a series of constrictions and expansions appeared in both living and preserved specimens. These are possibly incipient cases.

It is not known whether the new male imago has a single expansion of the vasa which become double shortly after emergence due to the passage of sperm bundles, or if some of the developing males have the single expansion.

Should the second expansion of the vasa form, as open sperm bundles pass down the ducts, then it must be assumed that these widenings become a permanent morphological feature for the rest of the male adult life cycle.

4. Terminology of the expansion of the vasa deferentia presented a problem as authors differ in distinguishing the vesiculae seminalis from the rest of the vasa (kuznetsov, 1015; Schroeder, 1928; Musgrave, 1937; Callahan and Cascio, 1963; Tedders and Calcote, 1967; Drecktrah and Brindley, 1967; Outram, 1970; Fatzinger, 1971).

The expansions have been found to serve as storage for the spermatozoa (Reiman, 1970), but no literature has been seen on Lepidoptera that reports the expansions also function by contributing a reproductive sugar to the seminal secretions, as in other animals, primarily mammals.

5. A difference in pH value is noted in the expanded and narrow portions of the vasa, registering 6.0 for the expanded regions and 4.5 for the narrower parts in the one day old male, which may indicate different secretions added to the seminal fluid by local cells.

The Male Accessory Glands

1. The male accessory glands in the B corn borer are very short but have large terminal bodies which are constricted off from the rest of the glands.

These flask-shaped bodies have an unusually bright, gleaming appearance when seen on dissection in living and preserved specimens and are considered a distinguishing characteristic of Ostrinia nubilalis.

They have not previously been described as such (Drecktrah and Brindley, 1967) nor do they resemble the terminalia of the male accessory glands in other species (Outram, 1970; Fatzinger, 1971).

2. In recently emerged imagos, the glands and end bodies have a pH value of 4.5. Such acidity would negate the activity

of alkaline phosphatase enzymes reported by Day (1948) in the accessory glands of several insects.

The Male Ejaculatory Ducts

The male ejaculatory ducts have been divided into the ductus ejaculatorius duplex (Cholodkovsky, 1884; Roepke, 1909) and the ductus ejaculatorius simplex (Roepke, 1909). The simplex duct has been further subdivided into the primary segment or secretory portion and the cuticular segment in which the spermatophore is moulded (Callahan, 1958; Callahan and Cascio, 1963).

The Duplex

1. The paired ducts appear to be highly secretory in males of all ages, displaying what seems to be 2 different secretory regions in the proximal and distal portions of the ducts.

2. On gross observation, the structural changes that occur in the ducts such as bulging of the ends and adherence of portions of the ducts, are associated with increased secretory activity.

The Simplex: Primary Segment

1. In the zero hour male, the duct is transparent and 3 constrictions can be counted along the duct, though only one constriction was previously reported (Drecktrah and Brindley, 1967).

2. The number of constructions increase in the copulatory male. Nine constrictions have been counted which appear grossly to equal 9 secretory areas.

In other Lepidoptera, 3-9 constrictions and secretory areas have been reported (Norris, 1932; Musgrave, 1937; Khalifa, 1950; Srivastava, 1957; Outram, 1970). However, Callahan (1958) has shown that there are only 2 physiological divisions of the simplex duct. The differences observed in the duct are due to the differences in the secreting substances.

The Simplex: Cuticular Segment and Aedeagus (Penis)

1. The cuticular segment has a thick muscular portion looped around the lower primary segment and separated from it by a strong constriction

2. The cuticular segment flattens, going caudally, and loops again prior to joining the endophallus.

3. Spermatophores, containers for the spermatozoa, have been found in the cuticular segment in males just prior to copula and in males disturbed during early copulation.

The spermatophores retrieved from the males have the same structural features as those spermatophores found in the female, though smaller in size and volume. As such, spermatophore formation in Ostrinia does not resemble any of the 4 methods described by Gerber (1970) but is apparently a combination of 2 of the methods, being both male-determined and female-determined, particularly in the length of the neck of the spermatophore equalling the length of the bursal duct. The shaping of the spermatophore in the corn borer also fits into types described by Petersen (1901), Norris (1932) and Williams (1941).

4. The spermatophore consists of a corpus (head) and a collum (neck). No frenum or cap can be found at the terminal aperture of the neck.

The external appearance of the spermatophore is white (from the secretions) and plastic-like.

5. The aedeagus or penis is quite long for the small B strain. A sclerotic spoon-shaped piece called the cornutus is located at the point where the inner penis (vesica or endophallus) will evert.

6. A sclerotic wish-bone shaped structure is present in the dorsal wall of the aedeagus which may possibly serve as a strengthening feature to hold the aedeagus in the female duct.

DISCUSSION: THE FEMALE REPRODUCTIVE SYSTEM

Ostrinia nubilalis represents the ditrysian type of development in possessing 2 genital apertures, the copulatory pore (ostium bursa, vulva) and the egg-pore (ovipore), and in the relationship of the female reproductive ducts and rectum (Snodgrass, 1935; Imms, 1960; Wigglesworth, 1967).

The Ovaries and Oviducts

1. The ovaries are polytrophic, as in other Lepidoptera, having oocytes and nurse cells alternating.

2. The ovaries are the most prominent organs in the female abdomen, reaching from the second to the seventh abdominal segments.

3. The female imago emerges with unripe eggs but can be inseminated after wing-drying.

Two other species have been seen to emerge with unripe eggs (Norris, 1932; Callahan, 1958).

4. The lateral and common oviducts are transparent on emergence.

This has not been reported in the literature. It is possible that insemination stimulates secretory activity in this species.

5. The common oviduct in the B female does not possess lateral pouches as previously described (Drecktrah and Brindley, 1967).

6. Terminal filaments extending from the germaria were thought to be seen in some of the females although no documentary evidence for this in Ostrinia and most Lepidoptera.

All Lepidoptera have been said to lack terminal filaments (Norris, 1932). Others have reported finding a suspensary ligament (Davis, 1968) and/or terminal filaments (Fatzinger, 1971).

7. As the eggs ripen, the ovaries become bulky masses obliterating other organs. The ovarian membranes become more fragile. The common oviduct also becomes more prominent and

asymmetrically positioned, while the lateral oviducts foreshorten and the calyx and pedicel cannot be distinguished.

The Bursae Copulatrix

The bursae copulatrix in Lepidoptera is inclusive of the ostium bursae or copulatory aperture, the ductus bursae and the corpus bursa which is the pouch or main body of the bursa (Petersen, 1900; Kuznetsov, 1915; Weidner, 1934). In recent years the term seem to be more restricted to the main body.

1. The bursae copulatrix is the second most prominent organ in the female system.

2. On emergence the bursal pouch is opaque, but a large, reddish brown rhomboid signum is visisble, located medially.

When the female is inseminated, the signum is pushed against the wall as the pouch becomes greatly enlarged. No function of the signum has been determined in Ostrinia.

3. An accessory or appendix bursa extends from the larger bursa by a short duct. The accessory bursa is empty in unmated females and may be found lying folded against the large bursa in the recently emerged female. Upon insemination, the accessory bursa becomes filled with a white secretion.

The accessory bursa is not a new organ in the corn borer as reported (Drecktrah and Brindley, 1967). It was seen by Heinrich (1919) and was described and named earlier in other species (Petersen, 1901; Kuznetsov, 1915).

4. The spermatophores in females 3-5 days post copulatory appear to undergo a tanning process within the bursa.

No previous description of this phenomenon exists in the literature surveyed. Tanning suggests the presence of phenol oxidases secreted by the bursa or possibly by the spermatophore itself. The exoskeleton of insects undergo tanning when the phenol oxidases (tyrosinases) catalyse the oxidation of phenol derivatives (i.e. tyrosine) to quinones. In higher vertebrates, such as man, they catalyse reactions producing melanin.

The Ductus Bursae

1. The ductus bursa in the corn borer is fairly long, approximating the length of the male cuticular segment.

2. In the emerging and non-mated females, the duct is transparent. After insemination, the transparency is somewhat greater, indicating some change in the refractive index.

The transparency of the bursal duct has only been reported in inseminated females (Callahan, 1958).

3. No distinct morphological cervix bursa of the duct was observed.

4. Close to the copulatory aperture the duct has a sclerotized area, cuff-like in shape.

This sclerotization may serve as a guide for the penis (Busck, 1931).

5. The bursal duct is connected to the common oviduct by a short ductus seminis which lengthens somewhat in mated females.

The seminal duct may not be visible in non-mated females as it lies closely against the intersegmental margins of the seventh and eighth abdominal segments. The duct becomes elevated after copulation.

No bulla seminalis (expanded sac) is present in the seminal duct, but an enlargement of the duct occurs near its entry into the ductus bursae.

The Female Accessory Glands

The accessory glands in _Ostrinia_ consist of paired reservoirs joined to glandular, tube-like extensions by odd finger-like projections. The reservoirs have a common duct which connect them to the caudal part of the common oviduct.

1. The paired reservoirs are large and contain a golden, yellow fluid in females of all ages, mated and non-mated.

It is not known whether this fluid aids in digesting the spermatophore, or serves as a medium in which sperm live, or produce substances useful in sticking the eggs to the plant host.

2. The glandular extensions follow a torturous course through or around the left ovary. They consistently terminate dorsally on the bursal pouch and not in the lower abdominal segments once described (Drecktrah and Brindley, 1967).

The Spermatheca

The parts of the spermatheca were difficult to designate because of the variations in terminology and shifts that occurred in the use of terms, especially as differences in spermathecal structures were better visualized.

The spermatheca observed in the corn borer consists of a spiral canal which enters the vestibulum of the common oviduct opposite the ductus seminis. At the other end, the canal extends into a bilobed structure, the larger lobe being designated as the receptaculum seminis and the smaller lobe as the lagena. From the lagena a single, long glandula receptaculi winds torturously through the right ovary and positions terminally with the accessory glands to the dorsal bursae copulatrix.

1. The spiral canal has a sclerotic region which divides it into 2 ducts for part of its length.

2. The spermatheca is fairly transparent in non-mated females, and this has not been described in other reports.

3. After insemination, the spermatheca becomes opaque.

4. The receptaculum seminis and the lagena do not appear to be completely separated in this species.

5. The glandula receptaculi in unmated females is moderately narrow for 2-3mm as it extends from the lagena. When insemination occurs, this portion becomes highly constricted and plastic-like in appearance.

This change in the glandula receptaculi was not described in the literature.

6. The glandula receptaculi is single throughout its length and does not double before terminating as reported (Drecktrah and Brindley, 1967).

7. Squashed slides of the spermathecal lobes from mated females show spermatozoa and open sperm bundles.

BIBLIOGRAPHY

INDEX OF AUTHORS ALPHABETICALLY ARRANGED

Busck, August, On the Female Genitalia of The Microlepidoptera and Their Importance in the Classification and Determination of These Moths. Bull. Brooklyn Entom. Soc. 26 No. 5 pp. 199-216 1931

Callahan, P.S., Serial Morphology as a Technique for the Determination of the Reproductive Patterns in the Corn Earworm, Heliothus zea. Annals Entom. Soc. Amer. 51 pp. 413-480, 1958

Callahan, P.S. and Cascio, T., Histology of the Reproductive Tracts and Transmission of the Sperm in the Corn Earworm, Heliothus zea. Annals Entom. Soc. Amer. 56 pp. 535-556, 1963

Cholodkovsky, N., Uber die Hoden der Lepidopteren Zool. Anzeiger 7 pp. 564-569, 1884

Davis, F., Morphology of the Reproductive Systems of the Southwestern Corn Borer, Diatraea grandiosella (Lepidoptera: Crambidae) Annals Entom. Soc. Amer. 61 (2) pp. 1143-1147, 1968

Day, M.F., Distribution of Alkaline Phosphatase in Insects Austr. J. Sc. Res., Series B 2 pp. 31-41, 1948

Drecktrah, H.W. and Brindley, T.A., Morphology of the Internal Reproductive Systems of the European Corn Borer. Iowa State Jr. of Sci. 41 No. 4 pp. 467-480, 1967

Fatzinger, C., Morphology of the Reproductive Organs of Dioryctria abeitella (Lepidoptera: Pyralidae: Phycitinae) Annals Entom. Soc. Amer. 63(2) pp. 1256-1261, 1970

Gerber, G.H., Evolution of the Methods of Spermatophore Formation in Pterygote Insects. Can. Entom. 102(1) pp. 358-362, 1970

Guthrie, W.D., Chromosome Studies of European Corn Borer, Smartweed Borer and Lotus Borer (Pyralidae). Annals Entom. Soc. Amer. 58 pp. 100-105, 1965

Imms, A.D., Textbook of Entomology, 9th Edition Methuen and Co. Ltd., 1960

Khalifa, A., Spermatophore Production in Galleria mellonella. Proc. Roy. Entom. Soc. London (A), 25 pp. 33-43, 1950

Kuwayama, S., I. Studies on the Corn Borer, Pyrausta nubilalis Hubner, in Japan. II. Notes on the Japanese Pyraustinae. Japan-Hokkaido Agric. Exp. Sta. Sapporo. Vol. 21-25 Report #25 Kotoni, Japan 1930

Kuznetsov, N., Fauna of Russia and Adjacent Countries. Lepidoptera Vol. I Trans. by Israel Program for Scientific Translations for the U.S. Dept. of Agric. and Nat'l Sci. Foundation, Washington. 1915, Trans. 1967

Loughner, G.E. and Brindley, T.A., Mating Success of the European Corn Borer as Influenced by Environmental Factors. Annals Entom. Soc. Amer. 64 pp. 1091-1094, 1971

MacArthur, J.W. and Baillie, W., Sex Difference in Mortality Abraxes-Type Species. Quart. Rev. Biol. 7 pp. 313-320, 1932

Musgrave, A.J., Histology of the Male and Female Reproductive Organs of Ephestia kuhniella Zeller (Lepidoptera) I. The Young Imagines. Proc. Zoo. Soc. 107 Series B pp. 337-364, 1937

Mutuura, A. and Munroe, E., Taxonomy and Distribution of the European Corn Borer and Allied Species. Genus Ostrinia (Lepidoptera: Pyralidae). Memoirs Entom. Soc. of Canada 71-74 pp. 1-112, 1970

Norris, M.J., Contributions Toward the Study of Insect Fertility. I. Structure and Operation of the Reproductive Organs of the Genera Ephestia and Plodia (Lepidoptera; Phycitidae). Proc. Zoo. Soc. London 3 pp. 395-611, 1932

Outram, I., Morphology and Histology of the Reproductive System of the Male Spruce Budworm, Choristoneura fumiferana, Can. Entom. 102 pp. 404-413, 1970

Petersen, W., Zur Morphogenese der Doppelten Bursa Copulatrix bei Schmetterlingen. Allg. Zeitschr. Ent. VI pp. 323-325, 1901

Raros, R.S. and Chiang, H., Maternal Age as an Ecological Factor in Populations Dynamics of the European Corn Borer. Entom. Exp. et Appl. 12 pp. 74-80, 1969

Reiman, in Comparative Spermatology, Int'l Symposium; Ed. B. Baccio. pp. 321-332, Academic Press; 1970

Roepke, W., Ergebnisse Anatomischer Untersuchungen an Standfussschen Lepidopteren I. Zeitschr. Naturw. XLVI pp. 1-122, 1909

Schroeder, C., Handbuch Der Entomologie Band 2. Jena, Von Gustav Fischer 1928

Snodgrass, R.E., Principles of Insect Morphology McGraw Hill 1935

Srivastava, U.S. and Srivastava, B.P., Notes on the Spermatophore Formation and Transference of Sperms in the Female Reproductive Organs of Leucinodes orbonalis Guen (Lepidoptera: Pyraustidae) Zool Anzeiger 158 pp. 158-266, 1957

Tedders, J.W., and Calcote, W., Male and Female Reproductive Systems of Laspeyresia caryana, the Hickory Shuckworm Moth (Lepidoptera: Olethreutidae) Annals Entom. Soc. Amer. 60 pp. 280-281 1967

Weidner, H., Beitrage Zur Morphologie und Physiologie Des Genital Apparates. Zeitschr. For Angew. Entom. Germany 21 pp. 239-290 1935

Wigglesworth, V.B., Principles of Insect Physiology 6th Ed. Methuen and Co., Ltd. 1967

Williams, J.L., Relations of the Spermatophore to the Female Reproductive Tract in Lepidoptera. Ent. News 52 pp. 61-65 1941