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Morphology and ultrastructure of male accessory glands in *Lepinotus patruelis* Pearman and *Ectopsocus briggsi* Maclachlan (Order Psocoptera)

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**ABSTRACT**

Reproductive system in the male psocids, *Lepinotus patruelis* and *Ectopsocus briggsi* was described. Fine structure of the accessory glands of both species was compared. Several types of cells of both species are examined. Epithelial cells required absorbing materials from haemolymph. Molecules then passed through the basement membranes and interstices reaching secretory cells. Glycogen, lipoproteins and acid-mucopolysaccharides are the major components of cell secretions in both species presumably for maintaining the stored sperm in vesicular seminalis.

**INTRODUCTION**

The morphology of the male reproductive system and spermatogenesis in the Psocoptera have been described by Jentsch (1939), Finlyson (1949), Ahmed (1987, 1988 & 1991) and Ahmed & Bahgat (1991). The system consists of paired mono or tri-lobed testes, and paired vasa deferentia, which lead to paired glandular seminal vesicles connected by a short ejaculatory duct to the external genitalia. The glandular seminal vesicles in *Lepinous* sp. are large and each is divided into two parts, a dorsal tubular part and a ventral ovoid chamber (Finlyson, 1949; Wong and Thornton, 1968). Accessory glands have been described as tubular and unpaired simplex in the Lepidopteran *Ephestia kuhniella* Zell. (Norris, 1932 & Musgrave, 1937).

The ultrastructure of the vas deferens and the accessory glands has been described in *Drosophila melanogaster* Meig. (Bairati, 1968), *E. kuhniella* (Riemann and Thorson, 1967 & 1979), *Schistocerca gregaria* F. (Odhambo, 1971), *Locusta migratoria* (L.) (Cantacuzene, 1972) and *Calopodes ethlius* Stoll. (Lai-Fook, 1982).

One of the main functions of the male accessory glands, in some species, is the formation of spermatophore (Leopold, 1976 & Happ, 1984). Some accessory glands have other functions including a contribution to the seminal fluid, the formation of mating plugs, nourishment for sperm, regulation of female receptivity and to provide nutrients for use by the female (Leopold, 1970). They also have accelerating effect on oviposition (Lang and Loughton, 1985).

The males of many orders transfer semen contained in a sperm case or spermatophore (Davey, 1965 and Leopold, 1976).
It has been suggested that is a necessary adaptation by the insect ancestors for invading the terrestrial environment (Khalifa, 1949). Spermatophores are considered a primitive feature of insects (Hinton, 1964).

In the orthopteroid orders, the accessory glands are usually heterogenous, multi-lobed structure, which play a major role in the formation of spermatophores. Loher and Edson (1973), working on the cricket, *Teleogryllus commodus* L. and Leahy (1973) on *S. gregaria* showed that removal of the accessory glands complex prevented the formation of a spermatophore. Other workers used histological and histochemical methods to trace the origin of spermatophores in many species (Gerber *et al*., 1971).

The accessory gland secretions vary widely in their physical properties and physiological functions. They are either of low viscosity to bathe the sperm as in *Tribolium molitor* (Happ, 1984) or of higher viscosity and solidify to form the spermatofore as *S. gregaria* (Leahy, 1973). In *S. gregaria, A. kuhniella* and *T. molitor*, 5-10 different types of secretory cells produce their secretions either by holocrine, merocrine or apocrine (Happ, 1984).

The spermatophores in Psocoptera were described by Pearman (1928), Badonel (1934), Finlayson (1949) and Ahmed (1987). In Family Trogiidae, they are formed within the spermatheca after mating (Finlayson, 1949). Since no ultrastructural study of the male reproductive system in psocopteran species has been published, it was considered of interest to describe and to compare the male reproductive systems and associated accessory glands in *L. patruelis*, which produces spermatophores and in *E. briggsi* which does not.

### MATERIALS AND METHODS

Males of *Ectopsocus briggsi* were collected from Mango leaf litter in Kalubya Governorate, Egypt, while *Lepinotus patruelis* males were collected from tree barks in Alexandria, Egypt. They were dissected in ice-cooled 5% glutaraldehyde in 1.0M sodium cacodylate buffered with sucrose (pH 7.2-7.4). Then accessory glands were transferred to 1% osmium tetroxide and mounted in Epon-218 resin. Thin sections were then cut, mounted on grids and stained according to routine described by Reynolds (1963).

### OBSERVATIONS AND RESULTS

1) The male genital system in *L. patruelis*:

This consists of paired mono-lobed testes, vasa deferentia, accessory glands and a common ejaculatory duct.

The vasa deferentia: The distal parts pass towards the posterior of the insect, then anteriorly and then posteriorly again to join the tubular accessory glands (Fig. 1). In mature insects their lumens are packed with spermatozoa. The lining consists of a single layer of squamous epithelial cells which contain many mitochondria, polyribosomes, smooth and rough endoplasmic reticulum (ER) and numerous small vesicles. These vesicles coalesce to produce large ones close to the apical surface of the cell (Figs. 5A & B).

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The accessory glands are divided into an anterior tubular coiled region (1600 um long and 25 um in diameter) and a posterior heart-shaped region, “the heart-glands” (100 um long and 83 um in diameter) (Fig. 1). The latter is joined at the middle by muscle fibres and connective tissue cells (Fig. 6A). The “tubular glands”, which have thick walls and variable electron-dense secretory products, possess between one and three layers (Figs. 5C&7A).
Within the “tubular-glands”, the epithelium is one-cell thick and consists of three types of cells, each with a distinct secretory product. The first type “I” is located at the end of the tubules and contains few mitochondria though these have prominent cristae, few Golgi complexes and RER. Many vesicular structures are situated close to the RER and the mitochondria (Fig. 5C). Some lateral cell membranes are separated in places to produce intercellular spaces in which granular material is present. Others remain close together and are connected by separate desmosomes (Fig. 5C).

The second type cell “II” is similar to the first except that it is packed with RER, and has fewer and smaller mitochondria. These cells produce an electron –dense flocculent secretion which is released by exocytosis (Fig. 5D).

The third type “III” is situated at the end of the tubular part, close to the “heart-glands”. They surround a lumen packed with lipid globules, which are mixed with other electron-dense material (Fig. 5E). This material is probably formed from united Golgi vesicles (Fig. 5F). Histological tests showed that the material produced at the apex of the “tubular-glands” is protenaceous whilst that from the rest has a lipid content since it stains positively with Sudan Black B (Table 1).

In the secretory epithelium of the “heart-gland”, six types of cells are present each with different secretory products. Three types of cell, A,B&F occur throughout the outer wall of the glands whilst the other three C,D&E lie close to the junction zone at both sides (Figs. 2&6A). Histological tests showed that the secretions produced by cell types A, B&F consist of carbohydrate giving positive reactions with tests for protein and lipid stains (Table 1).

The epithelial cells of type “A” are positioned in the ventro-lateral area of the “heart-glands” (Fig.2). Their basal membranes are convoluted allowing an increased surface to come into contact with the haemolymph in the intercellular spaces (ICS) formed between membranes (Fig. 6B). The lateral membranes are bound together with separate desmosomes, holding the cells together (Fig. 6C). Their cytoplasm is packed with stacked RER and many Golgi complexes but few mitochondria. Material is passed to the apical area of the cells to be expelled into the lumen by “merocrine” secretion (Fig. 6C).

The cells of type “B” are arranged in a mono-layer of columnar-shaped cells, situated in a dorsal position on the “heart-glands” (Fig. 2). Their apical membranes possess numerous microvilli and their lateral membranes are convoluted (Fig. 6D). They have RER, Golgi complexes surrounded by numerous secretory vesicles, and mitochondria (Fig. 6E). Close to these mitochondria and RER lie para-crystalline inclusions, which are probably the remains of materials used within the cells (Fig. 6E). The secretion of cell type “B” is flocculent and mixed with vesicles possessing electron-dense peripheries and containing fibrous material. They are passed out, at the apical surface, by exocytosis. This surface may invaginate to form a “funnel” which apparently carries it to the lumen of the accessory glands (Figs. 6D&F).

Cells of type ”C”, lie close to the junction zone (Fig. 2). They are arranged in a double layer of cells, the outer of which is squamous and the inner cuboidal. They possess a large amount of RER which contains a moderately electron-dense granules packed in irregularly-shaped vesicles. The products are released at the ends of many microvilli and are arranged in whorls of concentric spheres (Fig. 7B). Their concentric pattern is gradually destroyed, producing a number of homogenous vesicles, packed together as they grow...
bigger and moving away from the apical surface of the cell (Fig. 7A). Secretions of cell types “C&B” are closely located unmixed within the lumen (Fig. 7C).

Cells of type “D” are located in a ventral position, close to the junction zone (Fig. 2). They produce small moderately electron-dense vesicles. These are initially aggregated in the basal area of the cells in large vesicular structures then later close to the apical surface (Fig. 7D).

Cells types “E” and “F” are situated in the ventral part of the glands (Fig. 2). Epithelium consisting of cells of type “E” is similar to type “A” except that the cells contain numerous short profiles of vesicular and multi-stacked RER, and numerous Golgi complexes which produce electron-dense granules (Figs. 8A-C). The secretory products within the cells are homogenous and electron-dense (Fig. 8A). They are expelled from the microvilli into the lumen and added to the vesicular secretions forming their peripheral grid fringes around moderately electron-dense homogenous inclusions (Fig. 8A).

Cells of type “F” are confined to a depression in the corner of the “heart-gland”, close to the junction zone (Fig. 2). Adjacent cells are separated towards their basal which are separated from the lumen by tight junctions possessing separate desmosomes (Figs. 8A&D). These cells contain many large vesicular inclusions which contain fine granular material. Their secretion is granular and is similar to the products, in vesicles, located within the cells (Figs. 8D & E). Before secretion, those vesicles present close to microvilli (Fig. 8F).

Sperm do not occur in the lumen of the accessory glands of *L. patruelis*. During mating, the male delivers the sperm and other secretions, produced by the accessory glands that form the “sermatophore”, to female.

2) The male genital system in *E. briggsi*:

This consists of paired tri-lobed testes, vasa deferentia which dilate near their ends forming ampullae, and muscular-walled vesicula seminalis/ accessory gland complex leading into a common ejaculatory duct (Figs. 3,10A&E). The complex and the ejaculatory duct are connected to the eighth sternum by two muscles on each side (Fig. 3).

The vasa deferentia: The epithelial cells contain numerous Golgi complexes, abundant RER, but few mitochondria (Figs. 4&9A). The lateral membranes of the epithelial cells are separated producing intercellular spaces. Numerous small vesicles are associated with the Golgi complexes. Other large moderately-electron-dense vesicles occur close to the apical surface before being extruded to the lumen in which sperm are interspersed within secretions (Figs. 4&9A-D).

Each ampulla is used for temporary storage of sperm and nutrients. It is a large (48um long and 36um wide) pear-shaped structure surrounded by a layer of muscles (Figs. 3& 9E). It has squamous epithelium surrounding a wider lumen (28um in inside diameter) than that of the vas deferens and contains secretions, similar to those present in the vas deferens (Fig. 9E).

The vesicula seminalis/ accessory gland complex: This consists of a large paired kidney-shaped structure joined by connective tissue (Fig. 3). The sperm pass from the vasa deferentia to the vesicula seminalis which leads to two similar lateral accessory glands which open into the ejaculatory duct.

The epithelial cells which surrounds the seminal vesicle contains stacked RER, mitochondria and microtubules which are located near the apical membrane (Figs. 10B&C). Many vesicles are present in the lumen at the apical surface which presumably released
from the cell. These vesicles unite to produce larger ones (Fig. 10C). Sperm are interspersed within these secretions (Fig. 10D).

A different type of epithelium surrounds the accessory glands. It contains numerous short profiles of vesicular RER, large mitochondria, and many Golgi complexes (Fig. 10F). The lateral membranes around intercellular spaces, in areas near to the basal side, are allowing the cell surfaces to come in direct contact with the haemolymph. Flocculent material is located within those extracellular spaces (Fig. 10F). Inflated cisternae of RER, filled with homogenous contents, are located in the apical area of the cells (Figs. 10E, F). Bristle-coated vesicles are located near the apical membrane of the cell among bundles of microtubules which are arranged normal to the apical surface of the cell (Fig. 10G). Some of these vesicles, which are connected to the cell surface, suggesting a pinocytotic activity is taking place (Figs. 10F, G). Secretory products which are produced by both types gave positive reaction with acid-muco-polysaccharide and protein testes and a weak reaction with Sudan Black B for lipids (Table 1). They may form an acid-mucopolysaccharide/protein complex.
Fig. 1: Reproductive system of male *L. patruelis* showing the genital duct and associated accessory glands. ej.d = ejaculatory duct, h-s. g. = heart-shaped accessory glands, M = muscles, ts = testis, t-s.g. = tubular-shaped accessory glands & v.d. = vas deferens.

Fig. 2: Diagrammatic representation of the 6 types of secretory cells (A-F), occur lining the heart-shaped glands of *L. patruelis*. G = Golgi complex, h-s. g. = heart-shaped accessory glands, ie = intercellular space, m = mitochondria, M = muscles, N = nucleus, RER = rough endoplasmic reticulum, sd = septate desmosomes, sv = vesicular secretion, Sg = granular secretion & VER = vesicular RER.
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Fig. 3: Dorsal aspect of the abdomen in male *E. briggsi*, showing the genital duct and vesicular seminalis/accessory gland complex.  
AG = accessory glands, am = ampulla, ej.d = ejaculatory duct, ts = testis. v.d. = vas deferens & VS = vesicular seminalis.

Fig. 4a: Diagrammatic representation of the secretory cells, of the vas deferens in *E. briggsi*, showing formation of secretory vesicles.  
Fig. 4b: Diagrammatic representation of a cell, from seminal vesicle in *E. briggsi*, showing the stacked RER in the cytoplasm and sperm in the lumen of the vesicle.  
Fig. 4c: Diagrammatic representation of a cell, from the accessory glands in *E. briggsi* showing formation of secretory products. Note the microtubules are arranged normal to the cell surface.  
GC = Golgi complex, M = mitochondria, mt = microtubules, ms = muscles, N = nucleus, RER = rough endoplasmic reticulum, S = secretion, sp = sperm & sv = secretory vesicles.
Figs. 5A-F. (*L. patruelis*)

A) Electron micrograph of the vas deferens of *L. patruelis* showing sperm in the lumen (1) which is surrounded by a layer of squamous epithelium (ep) and basement membrane (bm). Scale bar=0.5um.

B) Electron micrograph of part of a vas deferens epithelial cell, showing the presence of vesicular structures (vs) close to smooth ER (SER). Scale bar=0.5um.

C) Electron micrograph showing a cross section of the tip end of tubular glands (type I cells) showing electron dense secretion in the irregular lumen (L) and wide intercellular spaces (ics) and septate desmosomes (arrows) located between adjacent cells. Note the muscle fibres (m) surrounding the structure. Scale bar=1um.

D) Electron micrograph of a part of type II secretory cells which is rich in RER. Note the secretion is formed at cell surface associated with microvilli (mv). Scale bar=0.5um.

E) Electron micrograph showing part of type II epithelium. Note the vesicle (v) blebbing from the cell surface and the two types of secretory spheres, electron-dense (ed) and electron-translucent (et). Scale bar=0.5um.

F) Electron micrograph of a part of an epithelial cell type III showing dense granules (dg) are formed by a Golgi complex (G). Scale bar=0.5um.
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Figs. 6A-F. (*L. patrulis*)

A) Electron micrograph showing part of the "heart-glands" showing, muscle layers (m) surrounding the zones containing different types of secretory epithelial cells (A-D), and both sides are connected by a junction zone (Jz) of muscles and connective tissue. S=secretory products. Scale bar=10um.

B) Electron micrograph showing type 'A' epithelial cells showing subspherical nucleus (N), stacked RER (RER) and Golgi complex (G), separated by lateral membrane which possesses septate desmosomes (arrow head) towards apical surface and intercellular spaces (ics) towards the basal side. Note the thick layer of muscle (m) at the basal side. Scale bar=1um.

C) Electron micrograph showing vesicles (v) formed in the cell and separated at the cell surface (v1, v2&v3), sd= septate desmosomes. Scale bar=0.5um.

D) Electron micrograph showing type 'B' epithelium arranged in one cell layer and surrounded by a layer of muscles (m). Note the funnel (f). Scale bar=0.025um.

E) Electron micrograph of part 'B' epithelium showing Golgi complex (G) and paracrystalline material (PC) formed close to RER. Scale bar=0.5um.

F) Electron micrograph showing the funnel (f). Scale bar=0.5um.
Figs. 7A-D. (*L. patruelis*)

A) Electron micrograph of type 'C' epithelium showing a muscle layer (m), a squamous epithelial layer followed by a cuboid epithelium containing rich RER, a Golgi complex (G) and vesicles (v). Scale bar=0.5um.

B) Electron micrograph of type 'C' epithelium showing the secretory vesicles (v) in process of formation at the periphery of the cell. Scale bar=0.5um.

C) Electron micrograph of secretions of cell types B&C, close to each other. Scale bar=0.5um.

D) Electron micrograph of type 'D' epithelium showing numerous vesicular structures (VS) within the cell. Scale bar=0.5um.
Morphology and ultrastructure of male accessory glands in *L. patrulis* Pearman and *E. briggsi*

Figs. 8A-F. (*L. patrulis*)

A) Electron micrograph of type 'E' epithelium showing Golgi complexes (G) and both stacked (RER) and vesicular (VRER) endoplasmic reticulum. Note the vesicular structures (vs) formed at the cell surface. Scale bar=1um.

B) Electron micrograph showing intercellular space (ics) containing electron-dense material (m) probably originating from the haemolymph. Scale bar=0.5um.

C) Electron micrograph showing Golgi complexes (G) producing small vesicles. Scale bar=0.5um.

D) Electron micrograph of type 'F' epithelium showing rich long sheets of RER (RER) and vesicular structures (vs). Scale bar=0.5um.

E) Electron micrograph of septate desmosomes (sd) formed by the joined lateral cell membranes (m). Scale bar=0.25um.

F) Electron micrograph showing vesicular structures (vs) close to the cell surface where many microvilli (mv) occur. Scale bar=0.5um.
Figs. 9A-E. (*E. briggsi*)

A) Electron micrograph of the vas deference of *E. briggsi* showing the surrounding muscle layer (m), epithelial cells rich in RER and Golgi complexes (G). Arrows = vesicles. Scale bar = 0.5 um.

B&C) Electron micrographs of the vas deference showing the formation of secretory vesicles (v). Scale bars “B&C” = 0.5 um.

D) Electron micrograph of the vas deference lumen showing contents (nebenkern-like structures, neb) similar to those located in the spermatheca. Scale bar = 0.5um.

E) Electron micrograph of the ampulla which is surrounded by a thin epithelial layer and muscle fibres (m). Note the secretion similar to that in the vas differences. Scale bar = 1 um.
Morphology and ultrastructure of male accessory glands in *L. patrulis* Pearman and *E. briggsi*

Figs. 10A-G. (*E. briggsi*)

A) Electron micrograph of a part of the vesicular seminalis in *E. briggsi*. m1, m2&m3=muscle layers, s=secretion. Scale bar=1um.

B&C) Electron micrographs of parts of a cell from the vesicular seminalis showing secretory vasicules (v) formed close to RER and introduced into lumen by exocytosis. Scale bar 'B'=0.5um. Scale bar 'C'=0.25um

D) Electron micrograph showing sperm (sp) in the lumen of the vesicula seminalis. Scale bar=1um.

E) Electron micrograph showing the three-layered epithelium (e1,e2,e3) of the accessory glands and muscle fibres (m) around it. Scale bar=1um.

F) Electron micrograph of an accessory gland cell showing the rich vesicular RER, Golgi complex (G) and a material is produced at cell surface. Scale bar=0.5um.

G) Electron micrograph showing apical surface of an epithelial cell containing pinocytotic-like vesicles (pv) and microtubules (mt) arranged normal to the cell surface, probably arranging the passage of these vesicles (Locke, 1964). Scale bar=0.1um.
DISCUSSION

The male reproductive system of *L. patruelis* is similar to that in *L. inquilinus* with separate accessory glands (Wong and Thornton, 1968) whilst that of *E. briggsi* which is similar to *Stenopsocus stigmaticus* (I. & L.) (Jentsch, 1939), and *Caecilius* sp. (Wong and Thornton, 1968), the accessory glands and vesicular seminalis are fused. Wong and Thornton (1968) suggested that trogiomorphan species (i.e. *Lepinotus*) are different from the psocomorphans (i.e. *Ectopsocus*) in having mono-lobed testes and unfused seminal vesicles, which are considered to be primitive characters.

In *E. briggsi*, the spermatozoa are stored in the vesicula seminalis whilst in *L. patruelis*, they are stored in the vasa deferentia since there is no vesicular seminalis.

The ultrastructure of the vasa deferentia in *E. briggsi* is similar to that described in other insect species such as *D. melanogaster* (Eairati, 1968) and *C. ethlius* (Lai-Fook, 1982a). The vasa deferentia contain nutrient in addition to the sperm. Their secretions appear, in the epithelial cell cytoplasm membrane. Their function is to maintain the sperm before they reach the vesicular seminalis.

The glands are surrounded by layers of muscle fibres which provide pressure during ejaculation (Figs. 10A&E) (Odhiambho, 1970; DeLoof and Lagasse, 1972; Happ, 1984). Tight junctions with septate desmomes link the secretory cells and thus maintain the integrity of the whole epithelium during the pressure dures which accompany sperm pumpig and transfer. The tight junctions between cells also cut off communication between the haemolymph and the lumen of the glands, but may allow ionic and molecular communication between adjacent cells (Lowenstein and Kanno, 1964). Within the epithelial cells the bundles of microtubules probably act as "Scaffolding" (Odhiambho, 1969b) to support and maintain the shape of the cells.

The functions of the accessory gland secretions vary between insects. They are concerned mainly with maintenance of the sperm and aiding their transfer to the female. In *L. patruelis*, in which "spermatophores" form, nine types of secretory epithelial cells are present, each has particular secretory product. In *E. briggsi*, in which there is no spermatophore, only two types of epithelial cells with different secretions occur. This difference in the number of cell types may be related to "spermatophore" formation in *L. patruelis*. This suggestion is supported by the fact that in *D. melanogaster*, an insect with no spermatophores, two different types of secretory cells, each producing a different secretion are present (Bairati, 1968) but in *C. ethlius* (Lai-Fook, 1982b) and *T. molitor* (Happ, 1984), 6 or 8 secretory products are combined to form the spermatophore during mating. In *L. patruelis* and *E. briggsi* each epithelial cell, like those of other accessory glands, acts as a gland and releases its secretion into the lumen of the accessory gland.

The epithelium of accessory glands, in the species observed in the present study, is rich in RER and Golgi complexes which may reflect the secretory function of those glands (Odhiambho, 1969c; Happ, 1984). Similar secretory cells to types "C" and "F" in the "heart-glands" of *L. patruelis*, were reported in *S. gregaria* in which the cisternae of the RER are dilated (Odhiambho, 1969c). In those cells the secretory activity is mainly a product of RER.

The production of secretion, by the epithelial cells, in both species requires absorption of material from the haemolymph. Molecules may pass through the basement membranes and the interstices to reach the secretory cells. In
the columnar cells of the "heart-glands" in *L. patruelis*, there are zones above the tight junctions, where the membranes of adjacent cells are separated, forming intercellular spaces in which material, may pass from the haemolymph. A similar process was reported in *Periplaneta americana* L. (Adiyodi and Adiyodi, 1974), *C. ethlius* (Lai-Fook, 1982b), and in *T. molitor* (Happ, 1984) in which labeled amino acids were detected in the lumen of the accessory glands after being injected into the haemolymph. Pinocytosis occurs at the apical membrane of cell type I in *E. briggsi*, probably reflects uptake or recover of material via vesicles for reprocessing (Odhiambo, 1969c; Lai-Fook, 1982a; Oliver, 1982).

Secretion of the products in both species is similar to that described in *C. ethlius* (Lai-Fook, 1982a) and *T. molitor* in which termed "merocrine" (Happ, 1984). In type I cell of *E. briggsi*, the apical microvilli are shed off (apocrine, Happ, 1984) to export materials into the lumen.

In *E. briggsi*, the secretory products are mainly acid-mucopolysaccharides. Histochemical examinations of secretions in the "heart-glands" of *L. patruelis* showed that they are glycogen and acid-mucopolysharides in one region (close to cells type B, A&F) and lipoproteins on the other (close to cells type C, D&E), whilst those of the "tubular-glands" are protein and lipid. The contents of the accessory glands in *E. kuhniella* are heterogenic consisting of protein and glycogen (Riemann and Thorson, 1979). The difference in the chemical structures of the secretions in *L. patruelis* and *E. briggsi* leads to the suggestion that the "spermatophores" in the former species, are produced by secretions of the "tubular-glands" and those produced by the cells type B, C&D of the "heart-glands" (mainly protein and lipid).

In *L. patruelis*, the insemination of the female is accomplished as the male external genitalia grab the spermathecal aperture (Finlayson, 1949) and by using the pumping action of the muscles; in the accessory glands and the ejaculatory duct, forces the sperm and "spermatophore" precursors into the spermatheca.

During ejaculation the muscles, surrounding the "heart glands", may contract thus squeezing and hence introducing the secretory products into the female. The sperm, in the vasa deferentia, are sucked into their place in the heart-gland lumen. Then the heart-gland muscles contract, causing the passage of the sperm into the female followed by the tubular-gland secretion. So the inner container of the "spermatophore" is probably formed by the secretions of the heart-glands whilst the outer wall of the "spermatophore" is formed from material produced by the "tubular-glands" and introduced last thing into the spermatheca. Similarly, the spermatophore is laid down layer after layer and the last outermost layer of the spermatophore is produced by cells located on the anterior tip of the accessory glands (Gerber et al., 1971; Riemann and Thorson, 1979).

Ejaculation and hence emptying the contents of the accessory glands lumens may trigger the activity of the gland cells which will soon refill the lumen before the next ejaculation (Happ, 1984).

The accessory gland secretions in *E. briggsi* affect the female receptivity. In *E. briggsi*, the males mate only when they meet virgin females for 2-4 times within 35 to 45 minutes. The mating period, each time, lasts between 5 to 15 minutes. In the 2 or 4 successive times of mating the female receptivity was increasing as the copulation time increased (unpublished observations). In *Aedes aegypti* (L.), a peptide named "matron" produced by the male accessory glands introduced into the females during mating causes the female to become
refractory to further mating (Craig, 1967; Gwadz et al., 1971).

In both *E. briggsi* and *L. patruelis* the sperms are active within the vasa deferentia and the seminal vesicles, when dissected in an insect saline or in distilled water. That may also prove that they have not kept immotile until mixed with the gland secretion which appears to be for maintenance rather than initiation of their motility.

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