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FINE STRUCTURE AND SECRETORY ACTIVITY OF THE SPERMATHECA OF THE FRESHWATER CRAB *PARATELPHUSA HYDRODROMOUS* (CRUSTACEA : DECAPODA)

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The multifibrilar musculature that encircles the spermathecal epithelium of *Paratelphusa hydrodromous* is composed of slow acting fibres characterized by the abundance of sarcoplasm, scarcity of myofibrils, lack of division of myofibrils into sacrcomeres, absence of T-system, poorly developed sarcoplasmic reticulum and sparseness in mitochondria. The secretory epithelium is composed of a single type of columnar cell with basally located multinucleolated nuclei. October-November (the pre-breeding season) is the period of synthesis of intrinsic secretion of the spermathecal epithelial cells. The mode of release of secretion is apocrine and occurs towards the end of the pre-breeding season.

Key words : Crab, *Paratelphusa hydrodromous*, ultrastructure, spermatheca, secretory activity, pre-breeding season.

INTRODUCTION

Many crustaceans store spermatozoa received during copulation in the spermatheca, a sac-like evagination of the female reproductive tract, for fairly long periods without losing sperm viability. In some brachyurans, sperms received during copulation are stored in spermatheca for several months preceding ovulation and sometimes even beyond one ecdysial cycle. Transmoult retention of spermatheca has been reported in *Portunus sanguinolentus* (Ryan, 1967), *Menippe mercenaria* (Cheung, 1968) and *P. hydrodromous* (Anilkumar, 1980).

Information on the fine structure and secretory activity of the crustacean spermatheca is limited, although a number of studies have been made on the ultrastructure and physiology of spermathecae and/ or their associated glands in insects (Gupta & Smith, 1969; Odhiambo, 1969; Jones & Fischman, 1970; Happ & Happ, 1970; Bhatnagar & Musgrave, 1971; Dallai, 1975; Grodner, 1979; Ahmed & Gillott, 1982; Gillott; 1988; Kressin *et al.*, 1996; Martin *et al.*, 1999) and some polychaetes (Carol & Greg, 2005). Earlier studies have shown that the spermathecal epithelium of *P. hydrodromous* shows two peaks of activity-one related to annual ecdysis and mating (June-July), and the second during the pre-breeding season (October-November) (Krishnakumar, 1985). Biochemical analyses reveal a substantial build up of spermathecal protein content related to annual ecdysis and pre-breeding season (Krishnakumar, 1985). We report below the ultrastructural details and secretory activity of the spermatheca of *P. hydrodromous* during the pre-breeding season.

MATERIALS AND METHODS

Sexually mature females (carapace width 3.2-3.8 cm) were collected from the paddy fields near Calicut University campus during October-November. Spermathecae were dissected out and quickly transferred to 3% Gluteraldehyde and kept overnight, washed in phosphate buffer, post-fixed in 1% Osmium tetroxide, dehydrated in graded alcohol series. Tissues then transferred to Propylene oxide and Araldite (1:1), kept over night in rotator at 48^0C and embedded in it. Semithin (1 μm thick for light microscopic observations) and ultrathin (80 nm thick for electron microscopic observations) sections were cut with a Reichert-Jung ultra microtome. Ultrathin sections were stained with Uranyl acetate and Lead citrate and examined under JEOL 100 C X II Transmission electron microscope (TEM) operating at 80 KV.

RESULTS

Light Microsocpy (LM)

Under LM the walls of the spermatheca is composed of an outer muscular layer and an inner layer of columnar epithelial cells lining the lumen (Fig. 1). The muscle fibres are arranged longitudinally. The muscle layer has a thickness varying from 35-45 μm. Each muscle cell possesses an elongate spindle shaped nucleus having a length of about 15 μm and a width of about 5 μm. Secretory epithelium is single layered. Each secretory epithelial cell has a single, basally located, intensely basophilic multinucleolated oval or irregular-shaped nucleus. Small patches of condensed heterochromatin can be seen scattered in the nucleoplasm and also attached to the inner nuclear membrane. The middle and apical cytoplasm is filled with small, highly basophilic secretory granules.

Ultra structure of the spermatheca

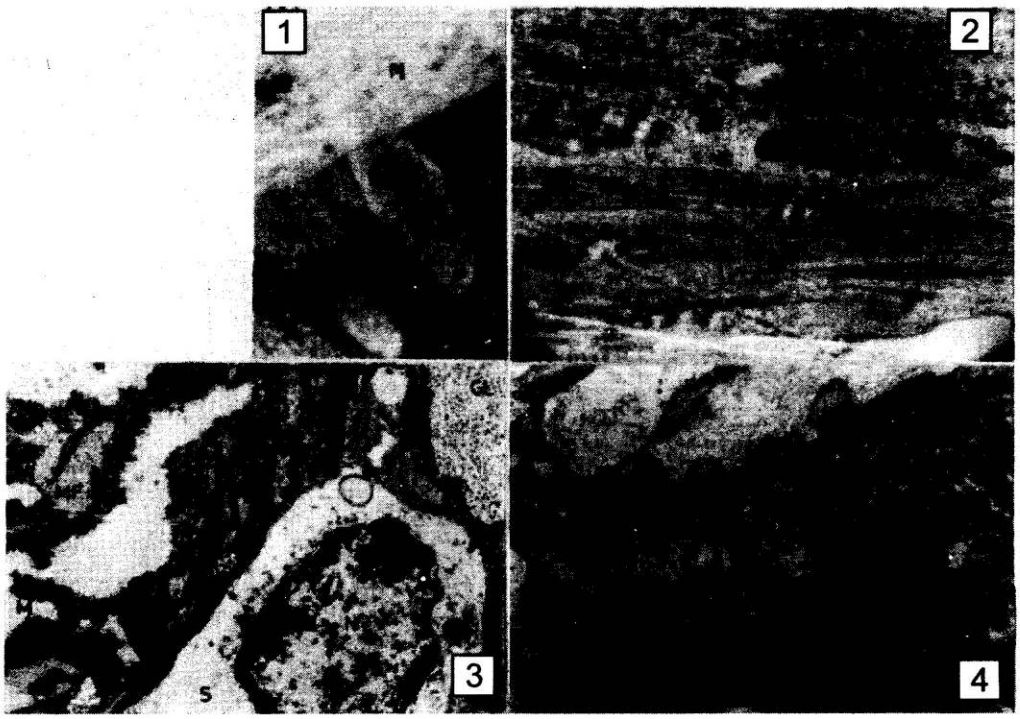
Muscle layer (Fig. 2) : A multilayered and longitudinally arranged, non-striated, muscular sheath is present external to the epithelium. Each muscle fibre has a thickness of about 3.7 μm. The oval (4.3 μm long and 3.2 μm wide) or narrow elongated (3.9 μm long and 0.5 μm wide) nuclei of these fibres are located either peripherally or slightly internally. Nucleus is delimited by a clearly defined double nuclear envelope and dense patches of heterochromatin are seen attached to the inner nuclear membrane. The nucleus did not occupy a position in close contact with the myofibrils.

The spermathecal muscle fibres generally appear to have a relatively sparse contractile apparatus (Figs. 2 & 3). The sarcoplasm seems to occupy more than 50% of the cell volume. Each muscle fibre contains longitudinally or obliquely arranged myofibrils having a thickness of 0.4-0.9 μm, generally lacking the sarcomere pattern. The component myofilaments of these myofibrils appear to be of uniform thickness. In some myofibrils dense Z-band like zones are observed (Fig. 2). In addition to the regularly organized myofibrils, regions outside the perinuclear area contain loosely arranged microfilaments (24 mm in thickness) which appear throughout the sarcoplasm. Aggregates of filaments (about 30 nm in diameter) are seen attached to the inner surface of the sarcolemma (Fig. 4). The

sarcoplasm forms a prominent electron-lucent annulus surrounded by nucleus (Fig. 3). This area is generally devoid of filaments but contain a few scattered elements of short cisternae (about 96 nm across) of sarcoplamic reticulum (SR), a few small spherical mitochondria and free ribosomes.

Immediately external to the sarcolemma is a distinct basal lamina (about 0.4 μm thick) closely applied to each cell, surrounding it and separating it from its neighbour. The basal lamina of adjacent muscle cells are separated by a connective tissue space (0.2-0.4 μm thick). Numerous golgi vesicles (76-153 nm diameter) are found in clusters beneath the basal lamina and also seen scattered in the rest of the sarcoplasm. The sarcoplasmic reticulum is not well developed in muscle cells. It appears in the form of numerous small vesicles (about 11.3 nm in diameter) (Figs. 2 & 3). Many clusters of large dense glycogen granules are seen in the muscle layer (Fig. 3).

Secretory epithelium : The secretory epithelium (Fig. 4) is composed of a single type of columnar cell. Each cell possesses a single large nucleus, oval or irregular in outline (13



Figs. 1-4 : *P. hydrodromous*. **1**. Spermathecal wall (Light micrograph) x1200; **2**. Muscular layer of Spermatheca (EM) x9300; **3**. Myofiber (EM) x15000; **4**. Muscle layer & spermathecal epithelium (EM) x5000.
(E : Epithelium (secretory); F : Myofilaments (short, unconnected); G : Granules (basophilic); GL : Granules (glycogen); L : Lumen; M : Mitochondrion (spherical); M' : Muscle layer; MF : Myofibrils; MF' : Myofibrils (parallel); N : Nucleus; RER : Endoplasmic reticulum (rough); S : Sarcoplasm forming annulus encircling nucleus; V : Vesicles (small); W : Z-zone)

μm long and 9 μm wide) that occupies a central or basal position. Prominent nuclei (1 or 2 in number) are located peripherally attached to the inner nuclear membrane. Nucleoli are composed of highly dense granular and fibrillar components. Small masses of chromatin are seen condensed along the inner face of the nuclear membrane and also scattered in the nucleoplasm. The rest of the neucleoplasm is filled moderately dense and loosely dispersed granular and or fibrillar material. The outer surface of the outer nuclear membrane is richly studded with ribosomes.

Basal region (Fig. 5) : The plasma membrane is the basal region of the spermathecal epithelial cell is bent into extensive involtions which extend a length of 0.6-3.4 μm. Many electron-lucent and electron-dense vesicles (100-350 nm across) are seen associated with these involutions. Oval and elongate mitochondria with transverse or oblique cristae and dense matrix were abundant in this region. A large number of vesicles were seen adorning the basal region adjacent to the basal lamina. Rough endoplasmic reticulum (rER) is extensively developed and appeared mostly as arrays of cristernae lying parallel to the longitudinal axis of the cell. Some of these cristernae appear swollen and filled with a flocculent material.

Perinuclear region : The perinuclear region is rich in rER arranged as arrays of flattened cisternae (60-192 nm across), free ribosomes and polyribosomes. Golgi complexes are also observed in this region.

Middle region (Fig. 6) : Certain changes are noticeable in ultra structure of this area during October and November. In October, the middle cytoplasmic region is rich in vesicles of varying sizes ranging from 0.2-1.6 μm in diameter. These vesicles are loosely filled with electron-lucent or moderately dense coarse granules or parallel stacks of electron-dense membranous lamellae. Sometimes the limiting membranes of these vesicles appear incomplete. Golgi vesicles (71-285 μm across) are found scattered in the vicinity of these large vesicles. The middle region is also rich in free ribosomes and polyribosomes. A few dense elongate mitochondria with transverse cristae are present amidst these vesicles.

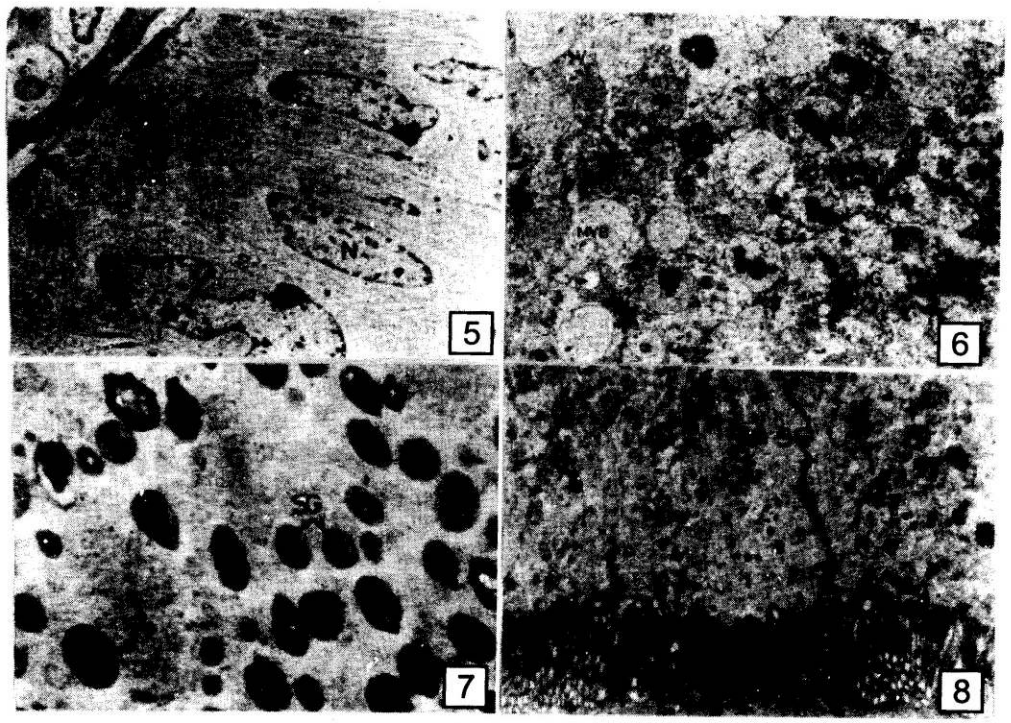
By November, the middle cytoplasm changes to become particularly prominent in small and large, dense, oval or spherical membrane-bound secretory granules (156-375 nm across) (Fig. 7). Sometimes these secretory granules enclose several smaller vesicles. Large number of golgi complexes are perceptible amidst these granules. Each golgi complex consists of 7-9 parallel cisternae and numerous associated vesicles. Both cistenae and vesicles are filled with dense granular substances. As with the previous month scattered elements of short cisternae of rER, free ribosomes, polyribosomes and slender mitochondria with transverse cristae are prominent in the middle region during November.

Apical region (Fig. 8) : The plasmalemma facing the lumen of the spermatheca is extended into numerous, long unbranched, tightly packed microvilli. The interior of each microvillus is packed with microfilaments. The cytoplasm immediately above the microvilli contain several small electron-dense and moderately electron-dense vesicles about 150-270 μm in diameter. A few of these dense vesicles are seen very close to the luminal plasma-

lemma and sometimes even fused with it. Electron-lucent, membrane-bound vesicles enclosing smaller vesicles or concentric layers of membrane-bound lamellae are found in the terminal web region. The apical cytoplasm is characterized by an abundance of oval mitochondria with transverse cristae.

Lateral plasma membrane : The lateral plasma membrane of adjacent spermathecal epithelial cells are more or less straight towards the middle and apical regions of cells, but convoluted towards the basal regions. The lateral plasma membranes of adjacent cells form gap junctions, septate junctions and desmosomes.

Lumen of the spermatheca : The area immediately beneath the luminal plasmalemma of the secretory epithelial cells is filled with an electron-dense substance which appears to be the basophilic intrinsic secretion of the spermatheca. Electron-dense granles are seen embedded in this substance. Towards the interior of the lumen is found a flocculent material in which free spermatozoa (not enclosed in spermatophores) distributed in small or large groups, are visible (Fig. 8).



Figs. 5-8 : *P. hydrodromous*, spermathecal epithelial cell (EM). **5**. Basal region x 16500; **6**. Middle region in October x 18200; **7**. Middle region in November x 16000; **8**. Apical region & lumen x 19200.
(BL : Basal lamina; G : Golgi complex; I : Plamalemma (involution); L : Lumen; M : Mitochondrion; MVB : Multivasicular body; R : Ribosomes; S : Septal junction; SG : Secretory granules; V : Vesicles)

DISCUSSION

The presence of a multilayered, nonstriated muscle coat in *P. hydrodromous* suggests that the luminal contents may be ejected by muscular contraction during oviposition. Many insects have muscles associated with their spermathecae (Gupta& Smith, 1969; Jones & Fischman, 1970; Taber, 1977) and the thickness of the muscle coat seems to be related to the consistency of the product of the gland. For example in the beetle, *Tenebrio molitor* the tubular spermathecal gland producing fluid secretion is surrounded by two or three layers of muscles whereas the bean-shaped gland producing a semisolid plastic plug was surrounded by six to eight layers of muscle cells (Happ & Happ, 1970).

Unlike skeletal muscle fibres, the spermathecal muscle fibres in P. hydrodromous spermatheca appear to have relatively sparse contractile apparatus indicated by the abundance of sarcoplasm, scarcity of myofibrils, indistinct boundaries of myofibrils, lack of division of myofibrils into sarcomeres, absence of the T-system, poorly developed SR, irregular Z-lines and sparseness in mitochondria. This is the striking contrast to the prominent T-system, regular occurrence of dyads, numerous large mitochondria and well developed SR characteristic of fast acting muscles (Mill & Lowe, 1971; Mill & Riley, 1972).

The short unconnected filaments throughout the sarcoplasm could supply a degree of rigidity and anchorage to the muscle cells. Similar structures produce a moderate contractile force and tend to preserve the shape of early mammalian heart cells during contraction of the better developed myofibrils (Challice & Viragh, 1974). The aggregates of filaments seen attached to the inner surface of the sarcolemma possibly represent areas where the contractile apparatus exerts its pull. Similar areas were reported in the ductal muscle cells of the spermatheca of the mosquito *Aedes aegypti* (Jones & Fischman, 1970).

Glycogen deposits found in the muscle cells probably serve as a source of energy. Glycogen was reported to be stored in sizeable amounts in the seminal receptacle of insects (Bhatnagar & Musgrave, 1971), bats (Racey, 1975), crabs (Anilkumar & Adiyodi, 1977) and snakes (Andrew *et al.*, 1982). Presence of a basement membrane surrounding the muscle like layer and also the basal cells of the secretory epithelium is thought to function as a molecular sieve preventing large molecules from blocking the basal channels.

Unlike insect spermathecal secretory epithelial cells, those of *P. hydrodromous* are not associated with an end apparatus formed by the invagination of the apical plasmalemma. In *P. hydrodromous* microvilli adorn the apical plasmalemma of the spermathecal secretory epithelial cells. These microvilli have uniform diameters are closely packed during the pre-breeding season and act to increase the surface area. A similar situation was found in *Periplaneta americana*, during the early phases of its secretory cycle (Gupta & Smith, 1969). Microfilaments noticed in the microvilli of *P. hydrodromous* spermathecal secretory epithelial cells may serve as a cytoskeleton and provides a structural basis for very dynamic cytoplasmic movements as previously reported in *T. molitor* (Happ & Happ, 1970). The presence of similar microvilli has been reported in the spermathecae of other insects

(Dallai, 1975; Grodner, 1979; Ahmed & Gillott, 1982).

The assumed pinocytotic activity at the basal borders of spermathecal epithelial cells is supported by the presence of indentations at the bases of the microvilli and vesicles observed associated with the basal plasmalemma. Pinocytosis has been reported in the basal region of the spermathecal epithelial cells of *T. molitor* (Happ & Happ, 1970). The abundance of mitochondria associated with numerous vesicles and infoldings in the basal region indicate that they have a role in the active transport of substances into the cell. The apical concentration of mitochondria in association with the electron-dense and lucent vesicles indicate their involvement in the release of secretory substances from secretory vesicles.

In the spermathecal epithelial cells, the nuclei appear to be very active during October-November as indicated by the presence of prominent nucleoli and very low heterochromatin content. A highly active nucleus is characteristic of metabolically active cells.

In all secretory cells, the secretory apparatus is modified according to the type of secretion produced. Thus in the spermathecal epithelial cells of *P. hydrodromous* both rER and golgi complexes are well developed, a phenomenon commonly found in the cells that synthesize proteins and polysaccharides. A comparable pattern exists in the spermathecal gland cells of *P. americana* (Gupta & Smith, 1969). The presence of well developed golgi complexes (in the vicinity of secretory vesicle) and the patches of coarse granules similar to those seen in secretory vesicles enclosed within golgi vesicles suggest that mucopolysaccharide material is synthesized within golgi bodies. The clusters of isolated golgi vesicles observed some distance away from golgi units may serve as shuttle carriers between rER and golgi complexes as has been reported in gland cells of the locust *Schistocerca gregaria* (Odhiambo, 1969).

The microtubules present in the region containing the secretory granules provide mechanical support. This is comparable to the pattern observed in the spermathecal epithelial cells of *Melanoplus sanguinipes* (Ahmed & Gillott, 1982) and the ductal epithelial cells of the spermathecal complex of virgin mosquitoes *Aedes aegypti* (Jones & Fischman, 1970). The presence of loosely packed vesicles with moderately dense secretory granules with distinct limiting membranes in November suggests that the first type is converted into second type. This view is supported by the observation that the spermathecal lumen immediately underlying the apical plasmalemma shows the presence of an electron-dense secretion (the basophilic intrinsic secretion reported by Krishnakumarm 1985) having the same consistency as the contents of th dense vesicles observed in the apical cytoplasm in November. The perceptible rise in the number of mitochondria and golgi complexes in the vicinity of the secretory vesicles by November is indicative of their role in transforming the first type into the second type.

The intrinsic secretion of spermathecae seems to play a significant role in the long term storage (8-9 months) of spermatozoa in a viable state (Krishnakumar, 1985) and provides a

lubricant coat to the oviducal lumen facilitating the smooth transport of eggs during oviposition (Anilkumar, 1980). The luminal contents have a cytoplasmic consistency observed near the microvilli leading us to suspect the pinching off of cytoplasmic blebs characteristic of an apocrine mode of release. This is contrary to the holocrine mode of release reported in the spermathecal epithelium of the crab, *P. sanguinolentus* (Ryan, 1967) and *Ocypode platytarsis* (Krishnakumar, 1985).

The present study clearly indicates that the spermathecal epithelium undergoes a period of synthesis during October and the synthesized product undergoes a phase of maturation by November (pre-breeding season). Earlier studies indicate that in *P. hydrodromous* the synthesized substances are released by the beginning of the breeding season (Krishnakumar, 1985).

The separate junctions (Fig. 8) formed by the lateral plasma membranes of adjacent cells may provide a permeability barrier as well as a means of communication between neighbouring cells. Septate desmosomes seem to be of great importance in ensuring synchrony of secretory function in glands arranged in the form of a thin epithelium. Septate junctions and desmosomes have been reported in the accessory sex glands of the locust *S. gregaria* (Odhiambo, 1969).

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