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THE FEMALE SEMINAL RECEPTACLE AND ACCESSORY GLANDS IN *PYRRHOSOMA NYMPHULA* (SULZER) (ZYGOPTERA: COENAGRIONIDAE)

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Sperm, transmitted to the ♀ as individual filamentous cells suspended in a liquid medium, are discharged into a thick-walled pouch, the receptaculum seminis, on the dorsum of the vaginal canal. Spermatozoa soon appear concentrated in a single, smaller, pear-shaped accessory sac, the spermatheca, attached to the receptaculum-vagina junction. Particular cells in the wall of the accessory sac secrete a material that is thought to be added to the sperm concentrate. The purpose of the accessory sac is to serve as a store of spermatozoa for use in fertilization. A pair of posterior accessory glands has each an efferent duct that opens into the distal region of the vaginal canal; these ducts are provided with an elaborate muscular apparatus probably serving as a pump; in fresh material, efferently directed peristaltic waves have been observed. The glands are presumed to contribute to the investment of the eggs. The apical domains of the glandular epithelial cells contain intraplasmic assemblages of multiplicating bacteroids. They are likely to be transferred to the ooplasm and thereby transmitted to a new generation.

INTRODUCTION

Most zygopteran families possess a single spermatheca attached to the distal part of a bursa copulatrix (SIVA-JOTHY, 1987). Examination of representatives of the Coenagrionidae have not revealed any kind of aggregations (bundling or clustering) of their filamentous sperm cells either within the vasa deferentia of the male or when dispersed in insect saline (ÅBRO, 2000). The sperm bundles found in the male passageways of the anisopteran *Aeshna juncea* (L.) have been studied as to structural changes after transfer to the female genital tract (ÅBRO, 2004).

Based on female specimens of the coenagrionid *Pyrrhosoma nymphula* (Sulz.), captured at various times after mating, the present communication reports on structural features of the female genital tract and the storage of sperm; included is a description of the posterior accessory glands of the female reproductive system.

MATERIAL AND METHODS

Adult females, captured at a breeding site near Bergen, western Norway, had fixative injected into the abdomen with a fine hypodermic needle; the seminal receptacle, with additional structures, was excised in a larger volume of the same fixative. In addition, some specimens anaesthetized in carbon dioxide had fresh seminal receptacle and accessory glands dissected in insect Ringer's solution.

The fixative was made up of 3% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) with 0.17 M sucrose added; postfixation took place in a 1% solution of osmium tetroxide in the same buffer. Tissues dehydrated through a graded series of ethanol and cleared in propylene oxide were embedded in epoxy resin, and sections cut with diamond knives. Semithin plastic sections for light microscopy, from material processed. for electron microscopy, were stained in a 1 % solution of toluidine blue. Ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate.

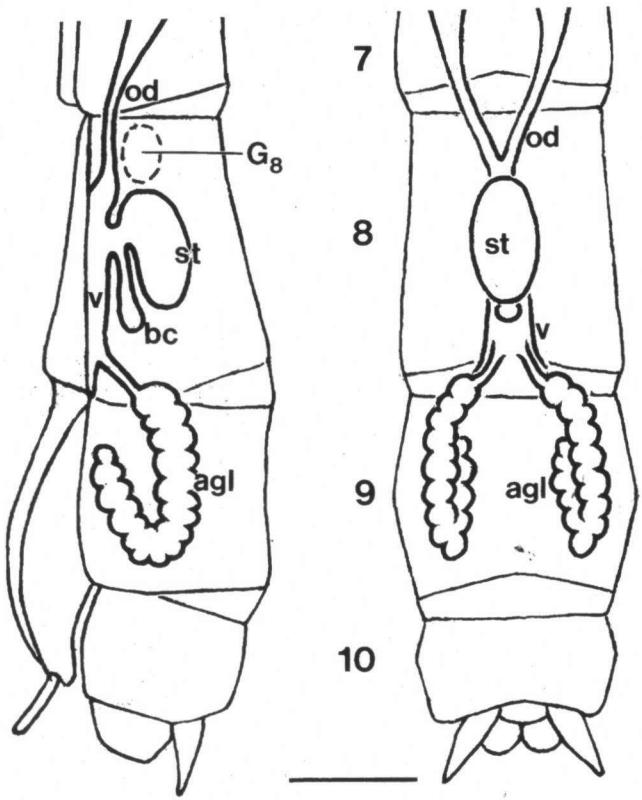
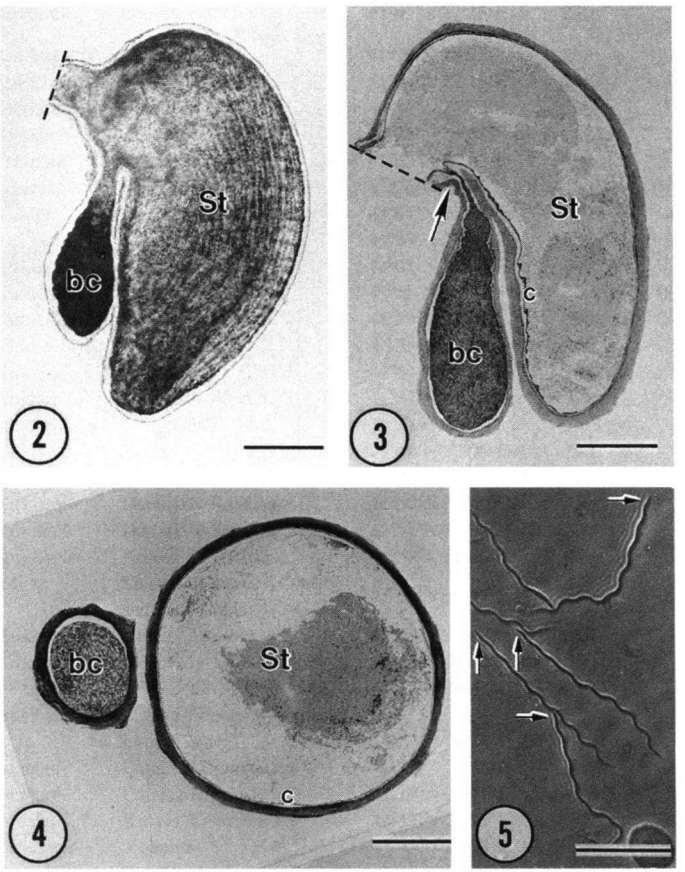


Fig. 1. Distal parts of the adult female genital tract: lateral (left) and dorsal (right) views, based on dissections of several specimens fixed and stored in buffered glutaraldehyde solution. Surrounding musculature is omitted. – agl: accessory gland; – bc: accessory sac; – G_8: position of the eighth abdominal ganglion; – od: oviduct; – st: receptaculum seminis; – v: vaginal canal; – 7-10: abdominal segments. [Scale bar 2.0 mm].

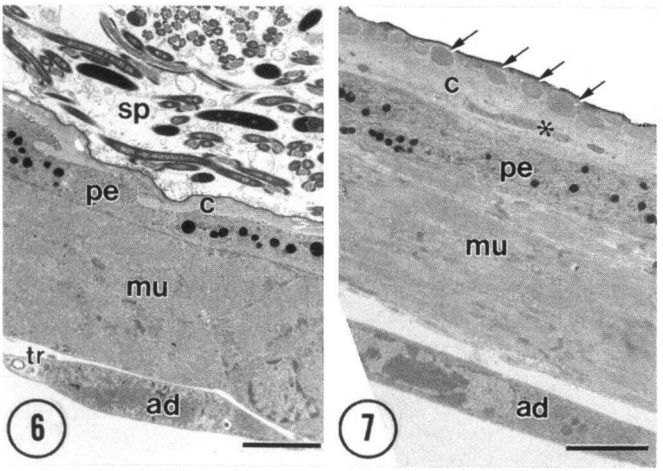
RESULTS

Connected to the vaginal canal by a short broad junction or neck, there is a rather thick-walled pouch, the receptaculum seminis, cuticle-lined and within its walls possessing muscle-fascicles, running lengthwise and crosswise (Figs 1, 2). Between the receptacle and the dorsal vaginal wall, a single oblong pear-shaped accessory sac is attached. Both



Figs 2-5. Receptaculum seminis (St) and its accessory sac (bc): (2) crowded with sperm, excised from the vaginal wall (at broken line). Fascicles of contractile musculature running lengthwise and crosswise in the receptaculum wall appear when viewed in transmitted light. Unstained whole-mount of glutaraldehyde-fixed tissues. [Scale bar 200 μm]; – (3) longitudinally cut (approximate mid-sagittal section), excised at broken line. Note narrow duct (arrow) to the sperm-repleted accessory sac and residual sperm in the receptacle lumen. c: cuticle. Semithin section/toluidine blue. [Scale bar 200 μm]; – (4) transversely cut. Note residual sperm in the receptacle lumen. Semithin section/toluidine blue. [Scale bar 200 μm]; – (5) fresh sperm cells from receptaculum seminis, here dispersed in insect Ringer's solution. Arrows indicate position of acrosome in front of the nuclear head. Unstained whole-mount/phase contrast illumination. [Scale bar 50 μm].

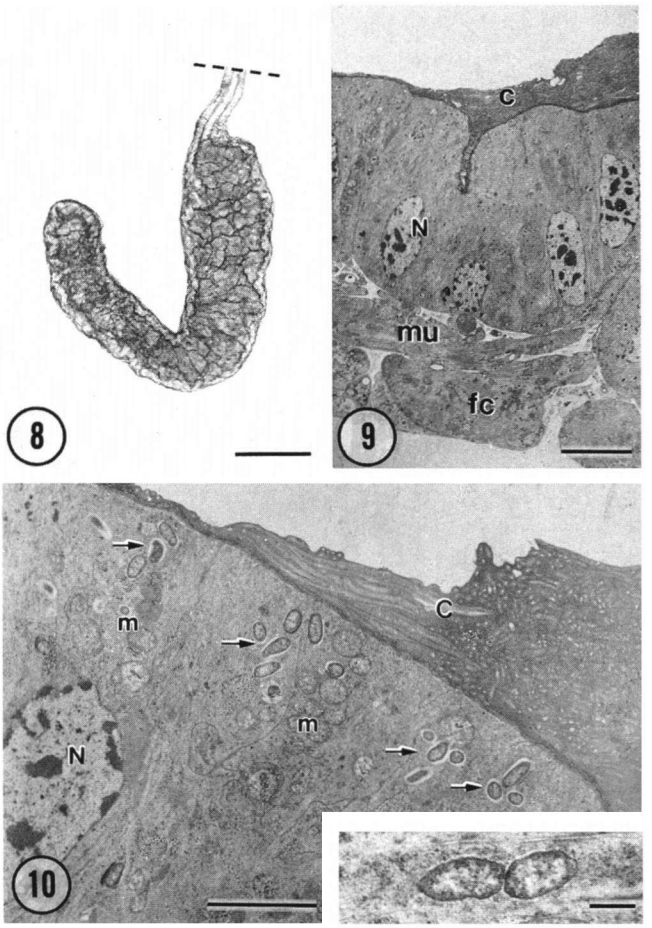
receptacle and accessory sac are cuticle-lined, and have beneath the cuticle a simple layer of low epitheloid cells containing prominent pigment granules (Figs 6, 7); their lateral borders are strongly interdigitated. There is also a broad muscle layer (Figs 6, 7). Outermost are investing, adventitious cells, some of which contain small tracheoles. In the accessory sac, just beneath the cuticular intima is a stratum of recesses within which a substance stains slightly positive according to the PAS reaction. This material appears to be secreted by flattened cells superficial to the pigmented cells (Fig. 7). The overall thickness of the wall is greater in the accessory sac.



Figs 6-7. Survey electron micrographs demonstrating the wall of (6) the seminal receptacle, and (7) the accessory sac. – ad: adventitious cell; – c: cuticle; – mu: layer of muscle fibres; – pe: pigmented cells. [Scale bars 10 μm]: (6) the receptacle wall from a female specimen just mated; in the cavity are numerous individual sperm cells (sp). – tr: tracheole; – (7) the accessory sac has a thicker wall. Beneath the cuticular intima is a layer of recesses (arrows) filled with a homogeneous substance that stains positively according to the PAS reaction. Within the cuticle, between the cuticular intima and the pigmented cells, are seen parts of flattened cells (asterisk) which may produce this substance.

In females taken during mating, sperm cells, not enclosed in bundles, were found deposited in the seminal receptacle (Fig. 6). Individual sperm cells were suspended in a flocculent, somewhat viscous, seminal fluid (Fig. 5). Quite soon after mating, filamentous sperm cells were concentrated within the accessory sac, which is connected to the seminal receptacle via an elongated narrow duct (Fig. 3). Cell debris and precipitates were left behind in the receptacle (Figs 3, 4). Apparently, the sperm are stored in the accessory sac until the eggs are ready for fertilization prior to oviposition.

In addition to the seminal receptacle and its accessory sac, a pair of flattened, curved lobulated posterior accessory glands, situated in the ninth abdominal segment lateral to the hindgut, are connected to the distal region of the vaginal canal (Fig. 1). Each of them is provided with a short efferent duct that opens close to the genital aperture (Fig. 8). The glandular cavity is lined with a cuticle maintained by a simple columnar epithelium on a basal lamina and forms acini. The cuticle, of variable thickness, is laminated and canalized (Fig. 10). Beneath the epithelium there are visceral muscle fibres arranged loosely in fascicles, often interwoven with surrounding somatic musculature

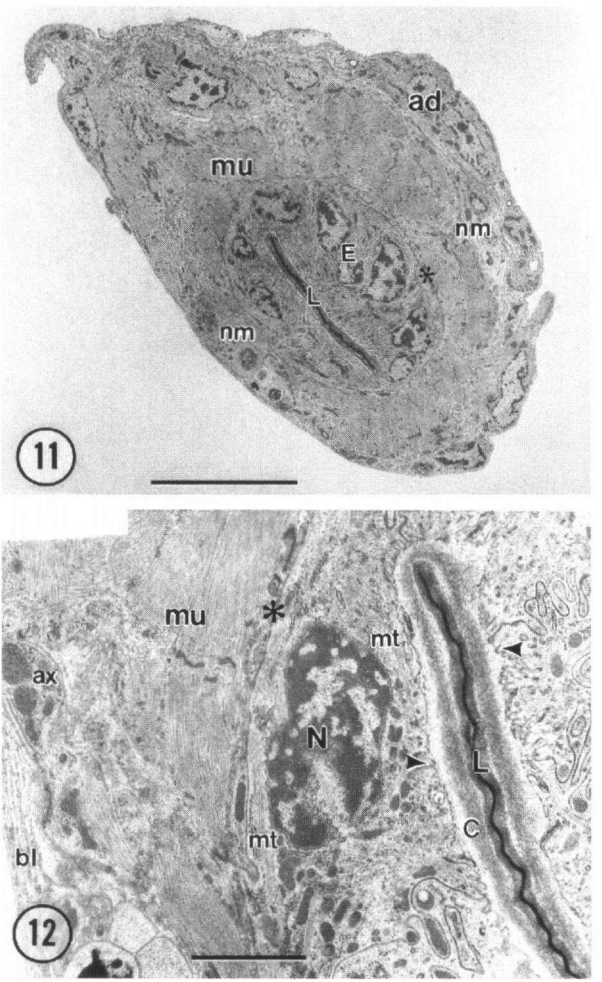


Figs 8-10. (8) The female accessory gland with efferent duct cut off near outlet to vagina (broken line). Tissues fixed in buffered glutaraldehyde solution, unstained whole-mount viewed in transmitted light. [Scale bar 400 µm]; – (9) electron micrograph of the epithelial stratum of a glandular acinus showing lining cuticle (c), laminated and canalized and with surface sockets. Beneath the epithelium are some adventitious cells (mu), possibly contractile, and associated fat cells (fc). – N: epithelial nucleus. [Scale bar 10 µm]; – (10) electron micrograph showing cuticle and apical domain of the glandular epithelium with intraplasmatic bacteroids (arrows) residing beneath the cuticle (c). – m: mitochondria; – N: epithelial nucleus. [Scale bar 5 µm]; – I n s e t: bacteroid rod, just divided in the host cell cytoplasm. [Scale bar 1 µm].

and adventitious fat cells (Fig. 9).

The cytoplasm of the glandular epithelial cells contains numerous mitochondria and Golgi bodies and, in its apical region, several conspicuous intraplasmatic bacteroids, which appear to undergo multiplication (Fig. 10, inset). Clusters of bacteroids are often seen in close proximity to Golgi membranes, glycogen deposits and lipid inclusions.

The glandular efferent duct exhibits a cuticle-lined, folded-up lumen surrounded by a simple layer of epitheloidal cells (Fig. 11). These have interdigitated lateral borders and are furnished with intraplasmatic arrays of multiple microtubules in varying orientations (Fig. 12). The apical border of these cells adjoining the cuticle is regularly pleated with membranous patches resembling hemidesmosomes. Outside the epitheloidal cells is a sheath of tightly-packed, striated muscle cells arranged around the duct, constituting a tunica muscularis. Muscle and epitheloid cells are closely attached without a basal lamina between. Outermost is an investment of adventitious cells, some with a clear tracheolar



Figs 11-12. Electron micrographs of the efferent duct of the female accessory gland. The borderline between the epitheloid (E) and the muscle layer (mu) is indicated by an asterisk. – L: lumen: (11) cross section, showing tissue coats surrounding the cuticle--lined lumen; – ad: adventitious cells; – E: epitheloid layer; – nm: neuromuscular junction. [Scale bar 20 μm]; – (12) tissue investments. Arrowheads point to membraneous patches at the top of pleats apically on epitheloid cells. – ax: nerve axon; – bl: basal lamina; – C: cuticle; – mt: microtubules; – N: nucleus of epitheloid cell. [Scale bar 5 μm].

supply, on a basal lamina. Between this layer and the muscle sheath are seen several nerve axons and neuromuscular junctions (Figs 11, 12). In fresh material dissected out in insect saline, the ducts display tiny waves of efferently directed peristalsis.

DISCUSSION

The female genital ducts and accessory structures of insects exhibit great diversity in arrangement of details, and the situation becomes complicated by development of various out-pocketings (CHAPMAN, 1974). The receptaculum seminis and accessory sac and accessory glands are all ectodermal structures, derived by invagination from the surface integument, and hence are lined with cuticle. Libellulids have paired accessory

sacs attached to the single receptacle dorsal to the vagina. A corresponding situation has been described from the aeshnid *Aeshna juncea* (ABRO, 2004). However, among libellulid genera there appears to be considerable variation in the structure of the female seminal receptacle and its accessory sacs (SIVA-JOTHY, 1987). The seminal receptacle in *Pyrrhosoma nymphula* is considered homologous with the receptacle in *A. juncea* and, likewise, the single accessory sac of Zygoptera might be homologous with the pair of accessory sacs in Anisoptera.

In *Pyrrhosoma*, the pigmented epithelia of the receptacle and the accessory sac are thought to promote the accumulation of heat from the sun, and the pigmentation is also presumed to protect against harmful radiation. How the sperm cells discharged into the seminal receptacle are subsequently transferred to the accessory sac and concentrated within it is not known. The motility of the spermatozoa alone cannot explain the normal, rather fast filling of the accessory sac; the organs themselves are presumed to play an important role. Rightly the accessory sac of *Pyrrhosoma nymphula* could be termed a spermatheca. Sperm deposited in the female receptaculum of the mosquito rapidly locomote and assemble in the spermathecae (JONES & WHEELER, 1965).

In the accessory sac/spermatheca, the cells situated between the pigmented epithelium and the cuticular intima produce a PAS positive secretion which is likely to be supplied to the stored sperm. These secretory cells probably function as unicellular glands rather than a secretory epithelium. Spermatozoa may be stored in the accessory sac in viable condition for long periods and expelled from it following ovulation. In *Pyrrhosoma* it has not been possible, on morphological characters, to decide the composition of the ejaculate and the female fluids. Nourishment to sperm cells could be supplied by the male in the seminal fluid.

The intracellular bacteroids detected in the present material appear similar to the endoplasmic bacteroids found in haemocytes of larval *Enallagma cyathigerum* in connection with wound healing (ÅBRO, 1999). Judged by morphological features alone, the bacteroid bodies resemble rickettsia. They appear to comprise a bacterial endosymbiosis and have been observed in other zygopteran cells as well as within the deutomerite plasma of eugregarine trophozoites, parasites in the zygopteran alimentary tract. In *Pyrrhosoma* females, it appears that the bacteroid rods are released into the cavity of the posterior accessory glands and eventually reach the vaginal canal where they in due course can enter the ooplasm. This might represent a mode of transmission by means of which the micro-organisms are passed from one generation to the next. The present bacteroids resemble corresponding endoplasmic rods observed in fleas (ROTHSCHILD et al., 1986). In cockroaches numbers of bacteroids exist between ovarian follicle cells and the surface of developing oocytes; they are believed to enter the eggs (MILBURN, 1966; SMITH, 1968). Intracellular symbiotic bacteria found in termites and cockroaches are acquired via transovarial transmission (SACCHI et al., 2000).

Bacteroids found in zygopterans may belong to the genus *Wolbachia*, cytoplasmically inherited rickettsia-like bacteria, widespread and common in insects, often seen in reproduction tissues (WERREN, 1997; WERREN & WINDSOR, 2000).

The female accessory glands in insects probably serve several functions. Sometimes they are called colleterial glands, as they add something to the egg-shell (WIGGLESWORTH, 1972). Since there is a rather poor visceral muscular supply close to the glandular acini in *Pyrrhosoma*, the complex muscular apparatus arranged round the efferent duct, and the occurrence of peristaltic movements along this tubule, suggest a pumping function for the gland. The abundance of microtubules in the epitheloid cells likely meets cytoskeletal requirements of changes in cell shape.

REFERENCES

ÅBRO, A., 1999. Reactions to leg excision in larvae of Enallagma cyathigerum (Charpentier) (Zygoptera: Coenagrionidae). *Odonatologica* 28: 117-125.

ÅBRO, A., 2000. Sperm clusters in Zygoptera (Coenagrionidae, Lestidae, Calopterygidae). *Odonatologica* 29: 51-56.

ÅBRO, A., 2004. Structure and function of the male sperm ducts and female sperm-storage organs in Aeshna juncea (L.) (Anisoptera: Aeshnidae). *Odonatologica* 33: 1-10.

CHAPMAN, R.F., 1974. *The insects: structure and function*. [2nd edn]. English Univ. Press, London.

JONES, J.C. & R.E.WHEELER, 1965. Studies on spermathecal filling in Aedes aegypti (Linnaeus). 1. Description. *Biol.Bull.* 129: 134-150.

MILBURN, N.S., 1966. Fine structure of the pleomorphic bacteroids in mycetocytes and ovaries of several genera of cockroaches. *J. Insect Physiol.* 12: 1245-1254.

ROTHSCHILD, M., Y. SCHLEIN & S. ITO, 1986. *A color atlas of insect tissues via the flea*. Wolfe, London.

SACCHI, L., C.A. NALEPA, M. LENZ, C. BANDI, S. CORONA, A. GRIGOLO & E. BIGLIARDI, 2000. Transovarial transmission of symbiotic bacteria in Mastotermes darwiniensis (Isoptera: Mastotermitidae) ultrastructural aspects and phylogenetic implications. *Ann. ent. Soc. Am.* 93: 1308-1313.

SIVA-JOTHY, M.T., 1987. The structure and function of the female sperm storage organs in libellulid dragonflies. *J. Insect Physiol.* 33: 559-567.

SMITH, D.S., 1968. *Insect cells: their structure and function*. Oliver & Boyd, Edinburgh.

WERREN, J.H., 1997. Biology of Wolbachia. *A. Rev. Ent.* 42: 587-609.

WERREN, J.H. & D.M. WINDSOR, 2000. Wolbachia infection frequencies in insects: evidence of a global equilibrium? *Proc. R. Soc. Lond.* (B) 267: 1277-1285.

WIGGLESWORTH, V.B., 1972. *The principles of insect physiology*. [7th edn]. Chapman & Hall, London.