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STRUCTURE AND FUNCTION OF THE MALE SPERM DUCTS AND FEMALE SPERM-STORAGE ORGANS IN *AESHNA JUNCEA* (L.) (ANISOPTERA: AESHNIDAE)

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By the use of light and electron microscopy, the micro-anatomy of the ♂ genital ducts and the ♀ reproductive organs have been surveyed. Sperm bundles transmitted from the testis through the vas deferens become embedded in a carrier jelly and also have additional substances bound to them; obviously the sperm cells are undergoing maturation. In the ♀♀, sperm bundles in carrier jelly appear to be deposited in the vaginal canal and, particularly, in the receptaculum seminis, the latter serving for long-term conservation. It seems possible that agents emitted from the posterior accessory glands to the vaginal surface near the genital aperture diffuse forwards, reaching the receptacle entrance. Here they presumably induce a liquefaction of the jelly and break-down of sperm bundles, thus releasing individual sperm cells. Free sperm cells are expected to accumulate in the anterior accessory sacs which they leave during fertilization. The morphological changes taking place in the sperm after transfer to the ♀ genital tract appear ambiguous.

INTRODUCTION

In aeshnids, sperm cells are set free from the testes into the male genital tract as bundles comprising thousands of cells (ÅBRO, 1998). Sperm transfer to the female is accomplished by elaborate genital structures in both sexes resulting in precise species--specific devices for intromitting sperm into the female reproductive organs (PETERS, 1987). The mode of formation and the structural organization of the large sperm bundles in the dragonfly *Aeshna juncea* (L.) are given in ÅBRO (2003). The present report extends earlier examinations with more details concerning the male and female genital tracts and the break-down of sperm bundles after transfer to the female.

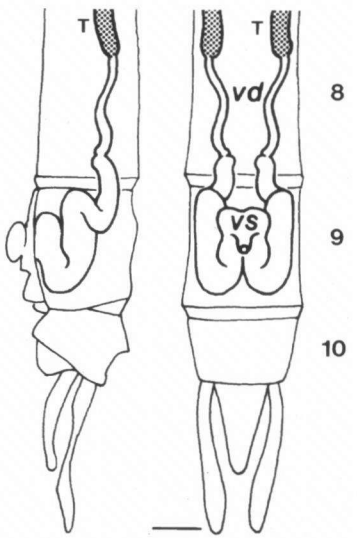


Fig. 1. The adult male genital tract in lateral (left) and ventral (right) views, based on dissections of several specimens. Surrounding musculature is omitted. – T: testis, rear portion; – vd: vas deferens, slender segment; – vs: vesicula seminalis; – 8-10: abdominal segments. – Scale bar, 2.0 mm.

MATERIAL AND METHODS

Adult males and females were captured at a breeding site near Bergen, western Norway. Females seized in copula were fixed immediately in the field to secure any sperm just deposited. Fixative was injected into the posterior abdomen with a fine hypodermic needle. The reproductive organs were subsequently dissected in fresh fixative.

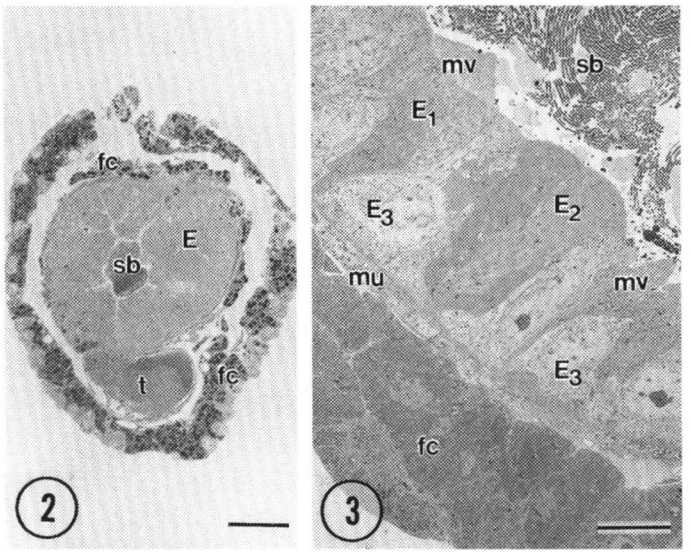
The fixative for electron microscopy was a conventional buffered solution of glutaraldehyde and osmium tetroxide. Embedding was in epoxy resin, and sections were cut with diamond knives and contrasted with uranyl and lead (ÅBRO, 2003). For scanning electron microscopy (SEM), fixed specimens were washed in buffer alone, dehydrated through a graded series of ethanol, and finally critical-point dried and sputter-coated with carbon and gold-palladium. For light microscopy, semithin sections from material processed for electron microscopy were stained with toluidine blue.

MALE GENITAL TRACT

The two vasa deferentia enter a short common duct which, in the mature male, becomes dilated dorsally, forming a vesicula seminalis filled with sperm bundles (Fig. 1). The slender proximal region of the vas deferens has a narrow lumen allowing the passage of only one sperm bundle at a time (Fig. 2). However, the distal ampulla-like region is more spacious and may become distended owing to accumulation of sperm bundles carried in a gelatinous substance secreted from the lining epithelium.

The pseudo-stratified columnar epithelium of both regions is provided with microvilli. The epithelium has small nests of undifferentiated replacement cells, as well as at least two types of slim columnar cells, one with numerous tall non-motile microvilli on the luminal surface (brush cells) and another with short irregular villi and with the apical cytoplasm containing dense granules or globules (Figs 3-5). The globules / granules have regular borders and are released into the lumen (Fig. 5). The tall microvilli have in their interior a weakly-staining, axially-arranged bundle of tiny filaments. In the proximal slender vas deferens, the epithelium is rather low (Fig. 3) compared to the epithelium of the distal ampullae that contains tall, slim columnar cells (Fig. 4).

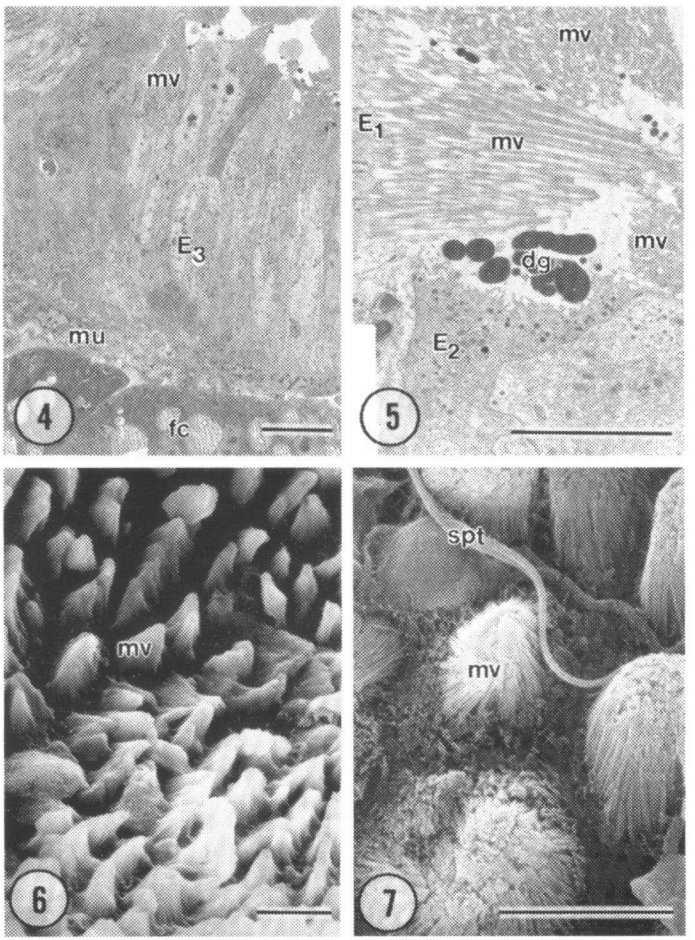
In the mature male, a gelatinous substance carrying sperm bundles is regularly found in the ampullae and the distal vesicula seminalis. The epithelium of the vas deferens rests on a basal lamina, underlying which there is a layer of circumferentially-oriented, thin, contractible muscle cells arranged spirally as fascicles around the tubule. Distal parts of the muscle layer become thicker. Outermost is a coat, partly discontinuous, of adhering fat cells (Figs 3, 4).



Figs 2-3. Transverse sections of the narrow, proximal region of the vas deferens: (2) a single sperm bundle (sb) in the narrow lumen [E: epithelial layer; – fc: fat cell; – t: posterior part of testis; – scale bar, 100 µm]. – (3) The wall showing the three types of epithelial cells: brush cells (E_1) with microvilli (mv), those with cytoplasmic dense granules (E_2) and undifferentiated "replacement" cells (E_3) [fc: fat cell; – mu: muscle cells; – sb: sperm bundle; – scale bar, 10 µm].

FEMALE GENITAL TRACT

In the following, the terminology used is in accordance with TILLYARD (1917). In the female the rather short vaginal canal (Fig. 8) is lined with cuticle. During mating, sperm appears to be discharged in clumps of jelly, either directly in a thick-walled pouch or bursa, the receptaculum seminis (= spermatheca), or on the vaginal wall. This is before entering the receptacle and hence is prior to fertilization. The morphology of freshly-deposited sperm, bundles in carrier substance on the vaginal lining, resembles that seen in the male vas deferens (Fig. 9). The seminal receptacle, also lined with cuticle, is continous with the vagina and opens like a diverticulum on its dorsum (Figs 8, 10). Opening dorsally into the receptacle by union of their two ducts is a pair of elongated sacs, the accessory sacs, whose blind ends terminate in a bulb (Fig. 8). They too possess a cuticular lining. In ovipositing females these sacs are usually tightly swollen and smooth. At other times they appear collapsed and wrinkled. One or both were sometimes found somewhat displaced, directed forwards. The walls of the receptacle and its accessory sacs exhibit no obvious structural differences; they possess a similar, rather uniform coat of epithelial cells. Their cuticular linings are continous with that of the vagina. Beneath the fibril-containing endocuticle of the receptacle and its accessory sacs, the apical membrane of the epithelial cells is tightly folded into a regular border



Figs 4-7. The distal region of the vas deferens: (4) the epithelium of tall, slender cells and "replacement" cells (E_3) [fc: fat cell; – mu: muscle cell; – mv: microvilli; – scale bar, 10 µm]. – (5) The lumen and apical parts of the two types of epithelial cells: those (E_1) with long microvilli (mv) and those (E_2) with small, apical dense granules (dg); – scale bar, 5 µm. – (6) The surface of the luminal epithelium showing tufts of long microvilli (mv) [the jelly-plug carrying the sperm bundles has been washed away in insect Ringer's solution to expose the epithelial lining; – scale bar, 10 µm. – (7) Epithelial microvilli (mv) that stick together with adherent mucous droplets forming tufts [spt: broken-off sperm tail; – scale bar, 5 µm].

of parallel leaflets (Figs 11, 12). No distinct glandular cells have been observed in these epithelia. In the majority of ovipositing females the seminal receptacle appears distended with stored sperm bundles in a jelly (Fig. 10) but in some specimens the pouch is collapsed. Occasionally small batches of individual sperm cells have been found within the lumina of the accessory sacs. The female genital tract is lined with cuticle, since its

hollow space develops from invaginations of ectodermal epidermis (TILLYARD, 1917).

In the vagina, close to the genital pore, a pair of accessory glands opens with short ducts (Fig. 8). These lobulated alveolar glands, situated ventrally in the ninth and tenth abdominal segments, are provided with a narrow, branching luminal space lined with a cuticle. Secretory epithelial cells, lying on a thin basal lamina and facing inwards toward the lumen, exhibit a rather complex structure with an intricate endoplasmatic reticulum and numerous cytoplasmic granules and vacuoles.

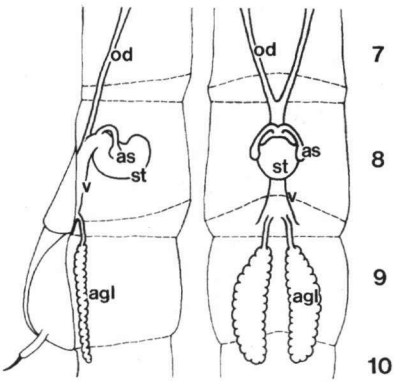


Fig. 8. Distal region of the adult female genital tract, lateral (left) and dorsal (right) views, based on dissections of several specimens captured during oviposition. Surrounding musculature is omitted. The lateral oviducts (od) run into a short unpaired common oviduct which continues into the vaginal canal (v). The mid-ventral genital pore opens between the eighth and ninth abdominal segments; – agl: accessory gland; – as: accessory sac; – st: receptaculum seminis; – 7-10: abdominal segments; – scale bar, 2.0 mm.

Sperm bundles in the receptacle seem to undergo slow morphological alterations: liquefaction of the gelatinous carrier substance takes place near the passage to the vagina (Fig. 10). At this site pronounced changes in the sperm bundle appear. In the sperm store, those bundles situated most anteriorly, i.e. farthest away from the communication to the vaginal canal, exhibit little or no morphological alterations. In several bundles near the vaginal canal end the condensed substance of the nuclear heads is expanded somewhat, and clearings or vacuoles of varying size (and not membrane-bound) appear, in which can be seen tiny specks (Figs 14, 15). In some sperm cells fine granular deposits become visible along the nuclear membrane (Fig. 15). Some released individual sperm cells are provided with a reorganized plasma membrane in front of the acrosome (Fig. 15, inset), apparently without acquiring clear areas in the nuclear substance. Single free sperm cells are found here and, in particular, accumulated in the lumen of the anterior accessory sacs. In each released sperm cell a loss of the cytoplasmic, elongated acrosomal region has occurred.

The electron-dense granules supplied to the sperm bundle from the sperm duct epithelium exhibit clear areas and crowded filaments in parallel alignment radiate from their interior into the surrounding area (Fig. 16).

DISCUSSION

During the conveyance of sperm bundles through the intratesticular spermiduct and the slender, proximal portion of the vas deferens towards final accumulation in the distal ampullar reservoir further maturation of the sperm cells probably occurs. This presumably results from exposure to the epithelial lining, in a manner similar to the sperm

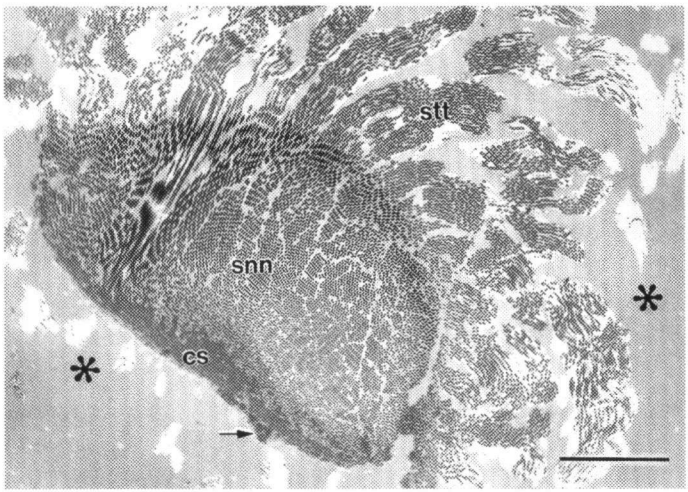


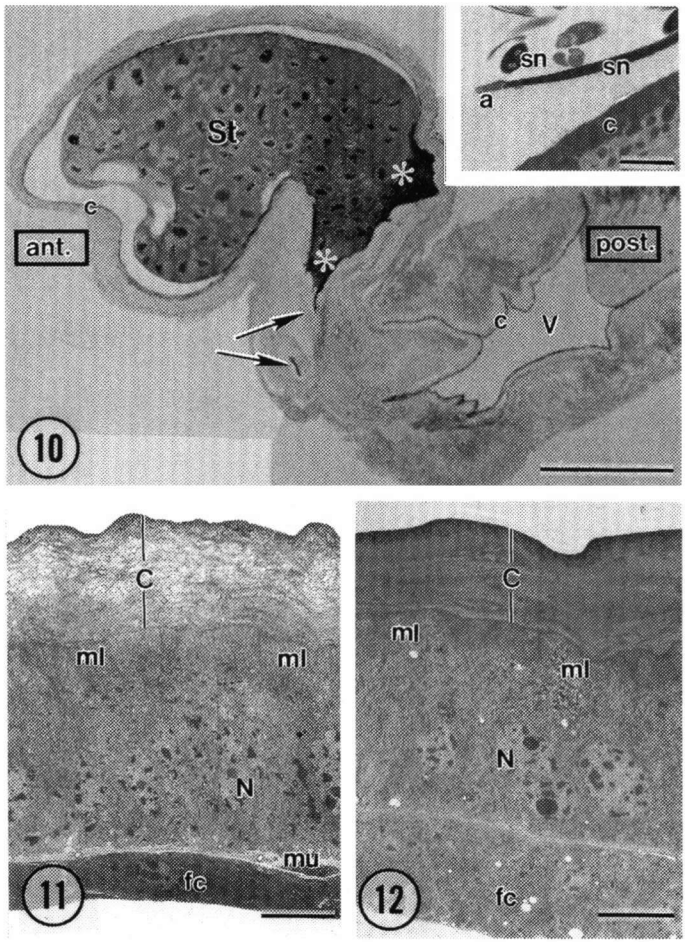
Fig. 9. A freshly deposited sperm bundle in carrier substance (asterisks) on the vaginal lining. Dense granules (arrow) adhere to the cap (cs) of the bundle. Note package of sperm nuclei (snn) in subunits – [stt: sperm tails; – scale bar, 10 μm].

maturation known from the mammalian ductus epididymis, which is provided with non-motile stereocilia representing a considerable enlargement of the luminal cell surface. Thus it has been shown in mammals that glycoproteins from the epididymal epithelium are adsorbed onto and possibly incorporated into the membranes of the spermatozoa during their passage through the duct (FLICKINGER, 1983). In the aeshnid vas deferens, the products secreted into the lumen, in addition to functioning as a carrier substance, are probably also necessary for maturation to take place during transit through the male genital tract, and finally for the liberation of individual sperm cells capable of fertilization. MIDTTUN (1974), using light microscopy only, stated that the secretory epithelium of the vas deferens in *Somatochlora arctica* is ciliated (i.e. motile kinocilia). However, this seems to be a misconception due to the use of obtrusive, strongly-acid fixatives.

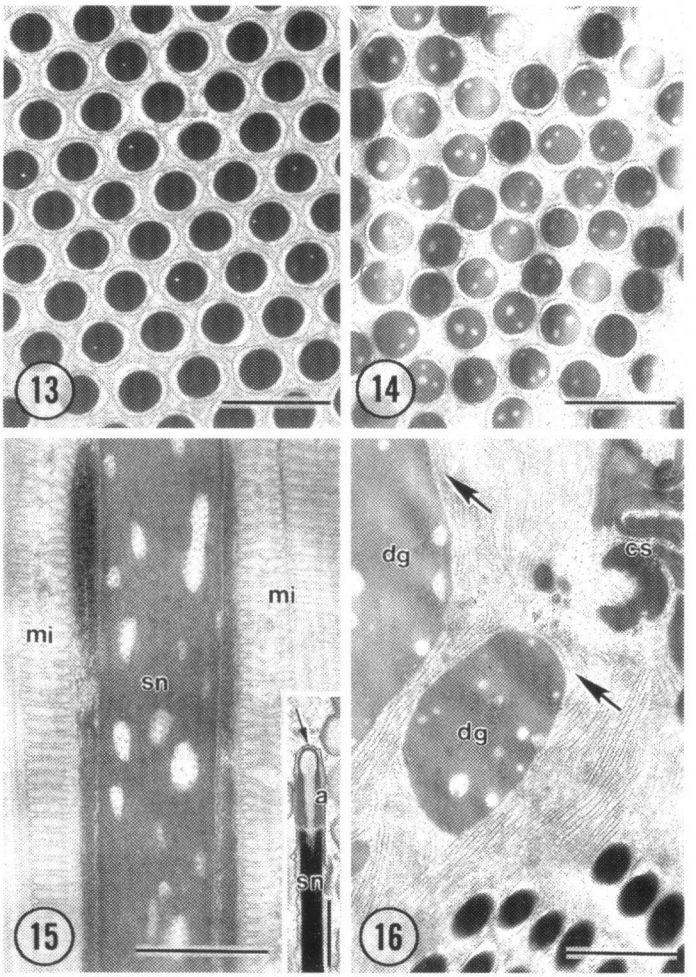
The presence of basally-situated "replacement" cells in the epithelium of the vas deferens of *Aeshna juncea* indicates a high rate of cell renewal as well as high secretory activity. In previous work (ÅBRO, 1998, 1999), fresh sperm bundles from the sperm ducts have been washed in insect Ringer's solution to remove adherent mucous substances. Freshly dispersed in the saline, bundles demonstrate exceedingly rapid flagellar movements of the sperm tails. However, this is considered to be an artificial condition not met with in nature. Observations hitherto have not confirmed the occurrence of similar rapid flagellar movements under natural conditions. In the present study, sperm bundles in carrier substance deposited on the vaginal cuticle demonstrated only slow flagellar movements. Embedded in jelly, subunits of sperm tails exhibit slow coordinated undulations. The gradual natural liquefaction of the carrier jelly taking place in

Aeshna females during release of individual sperm cells (ÅBRO, 2003) apparently does not accelerate flagellar movements.

It has not been possible to decide whether most sperm is transferred directly into the



Figs 10-12. The seminal receptacle and its accessory sacs: (10) montage of seminal receptacle (St) and vagina (V) in sagittal section. Arrows point to section profiles of the duct of an accessory sac. Near the passage to the vagina, liquefied jelly (asterisks), containing released individual sperm cells, stains heavily with toluidine blue – [c: cuticle; – ant./post.: anterior / posterior; – scale bar, 200 µm. – Inset: electron micrograph of released individual sperm cells from the site of liquefied carrier substance – [a: acrosome rodlet; – c: cuticle; – sn: sperm nucleus; – scale bar, 2 µm]. – (11) The seminal receptacle wall with fibrous cuticular lining (C), a homogenous epithelial layer, and a thin middle-layer of muscle cells (mu) – [fc: fat cell; – ml: border of membranous leaflets; – N: epithelial cell nucleus; – scale bar, 10 µm]. – (12) An accessory sac wall showing a structure similar to that of the seminal receptacle – [C: fibrous cuticular lining; – fc: fat cell; – ml: border of membranous leaflets; – N: epithelial cell nucleus; – scale bar, 10 µm].



Figs 13-16. (13) Transverse section through regularly packed nuclei in a sperm bundle delivered on the vaginal lining. Note condensed nuclear substance and distinct plasma membrane; – scale bar, 1 μm. – (14) Transverse section of sperm nuclei from a bundle stored in the seminal receptacle. The nuclear diameters are somewhat expanded and clearings in the nuclear substance are apparent; – scale bar, 1 μm. – (15) Longitudinal section through a sperm nucleus from the seminal receptacle. The nuclear material includes clear areas containing tiny electron dense speckles – [mi: midpiece mitochondrial sheath belonging to adjacent sperm cells; – scale bar, 0.5μm]. – Inset: A released sperm cell with reorganized plasma membrane (arrow) in front of the acrosome rodlet (a) – [sn: sperm nucleus; – scale bar, 1 μm]. – (16) Part of a sperm bundle in the seminal receptacle near its outlet. Note dense granules (dg) with clear areas and filaments in parallel alignment radiating from the interior of the granules into the surrounding area (arrows). At the bottom right are obliquely sectioned sperm nuclei – [cs: residual forepart cytoplasm from the cap of bundle; – scale bar, 1 μm].

seminal receptacle or remains in the vaginal canal or both. It may be that sperm will normally be deposited in the seminal receptacle and that the findings of sperm mass on the vaginal lining represent displacement in interrupted-copula dragonflies during seizure and subsequent handling. It was found expedient to follow the generalized description of the female genital tract in an aeshnid by TILLYARD (1917). However, the receptaculum seminis of the present work is termed bursa copulatrix by MIDTTUN (1976) and SIVA-JOTHY (1987), who also called the accessory sacs of the seminal receptacle, spermathecae. The confusing terminology regarding bursa copulatrix, spermathecae and accessory sacs used by dragonfly workers has been discussed by MIDTTUN (1976).

In the present material, since no typical glandular tissues have been recognized in the walls of the seminal receptacle and its accessory sacs, it might be secretions from the posterior accessory glands that bring about liquefaction of the stored clump of carrier jelly and a release of individual sperm cells from the bundles. This is in accordance with the observation that liquefaction and sperm release become visible in a zone close to the opening of the receptacle into the vaginal canal. Obviously, these conspicuous glands have multiple functions in the reproductive process. From the ducts of corresponding anterior accessory sacs in *Somatochlora arctica* large secretory cells have been found located among the epithelial cells (MIDTTUN, 1976).

The seminal receptacle appears to serve for long-term conservation of transmitted sperm bundles. The present work has not revealed any other purpose of the anterior accessory sacs than to serve as a storage of free sperm cells capable of fertilizing. In the present *Aeshna* material both the receptacle and its accessory sacs possess fascicles of contractile muscle cells in their walls, so it is likely they are able to squeeze out their contents.

As to the sperm mass after transfer to the female genital tract, the morphological changes appear ambiguous so far. The electron-dense globules / granules sticking to the sperm bundles stain positively according to the periodic acid-Schiff reaction (PAS), indicating a content of mucoproteins (ÅBRO, 2003). These substances, together with secretions from the posterior accessory glands, seem to play a role during release of free sperm cells. In some bundles the nuclear substance of sperm cells shows clear areas, while other released sperm cells keep their original compact nuclear substance. Probably both categories of sperm cells are capable of fertilization.

SIVA-JOTHY (1987) has described campaniform sensilla from the vaginal wall of libellulids, which suggests that mechanical stimulation is important in this area. In the present investigation corresponding sensilla have not been searched for.

In a series of reports, VISCUSO et al. (1998, 2002) have recognized several features in locusts which appear similar to those of *Aeshna juncea*, in the succession of events such as formation of sperm bundles, secretory material set free from epithelial cells in the passageways of the male, transit of sperm from male to female, the seminal receptacle and sperm conservation inside the female.

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