Odonatologica 32(2): 153-157 *June 1, 2003*

SHORT COMMUNICATIONS

THE COMPOSITION OF SPERM BUNDLES IN *AESHNA JUNCEA* (L.) (ANISOPTERA: AESHNIDAE)

A. ÅBRO
Department of Anatomy, University of Bergen,
Årstadveien 19, N-5009 Bergen, Norway

Received February 12, 2002 / Revised and Accepted August 5, 2002

Using light and electron microscopy, sperm packing has been studied in the large sperm bundles of an aeshnid dragonfly. Each large bundle is built up of variously-sized smaller bundles which probably reflects the intracyst formation procedure. It is proposed that initially there is a gathering of immature sperm cells into small bundles at several sites within the testicular cyst, and secondly all sperm heads are bundled together. This construction of subunits may be of importance to bundle break-down and release of individual sperm cells after transfer to the female reproductive organs.

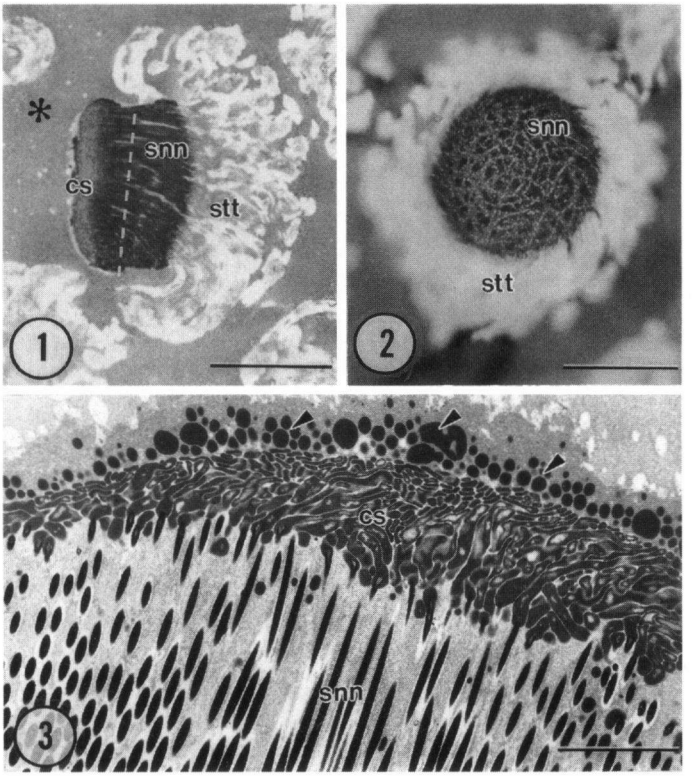
INTRODUCTION

The filamentous sperm cells of dragonflies are usually grouped into compact bundles or spermatodesmata; viewed in lateral aspect they appear like shuttlecocks. In aeshnids each testicular compartment, the spermatocyst, gives rise to one single spermatodesma comprising thousands of sperm cells (OMURA, 1957; ÅBRO, 1998, 1999). However, OMURA (1957) showed that species belonging to Gomphidae have several variously--sized bundles formed at a number of sites within the cyst and they remain apart. The purpose of the present study was to examine the aeshnid sperm bundles in more detail, and to suggest a relationship between the sperm bundles of aeshnids and gomphids.

MATERIAL AND METHODS

Adult male and female *Aeshna juncea* (L.) were captured at a breeding site near Bergen, western Norway. Dragonflies anaesthetized in carbon dioxide had vasa deferentia of the males and receptaculi seminii of the

females dissected free in insect Ringer's solution. For electron microscopic examination, specimens were transferred to a fixing fluid made up of 2.5% glutaraldehyde in a 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. Tissue specimens were dehydrated through a graded series of ethanol, cleared in propylene oxide, and embedded in epoxy resin. Ultra-thin sections cut with a diamond knife were contrasted with uranyl acetate and lead citrate. Semi-thin epoxy sections for light microsopy were stained in a 1% solution of toluidine blue. For demonstration of mucous substances, sections were stained by the periodic acid-Schiff reaction (PAS) without removing the resin (SNODGRASS et al., 1972). In addition, the present study drew on series of ultra-thin sections from testes.



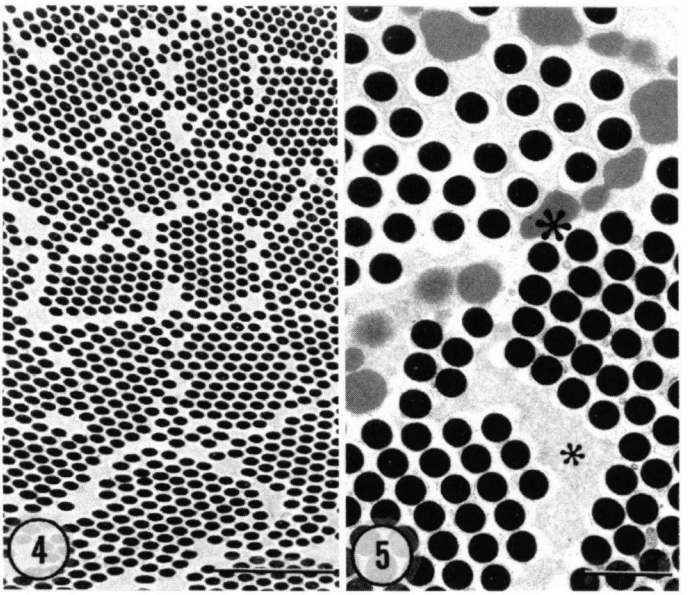
Figs 1-3. *Aeshna juncea*: (1) longitudinal section through a spermatodesma from the distal portion of vas deferens. Broken line indicates the level of the cross section shown in Fig. 2. Surrounding the spermatodesmata is a heavily stained gel-like substance (asterisk). Semi-thin section/toluidine blue. [Scale bar = 50 µm]; – (2) cross section of a spermatodesma at the level of sperm nuclei exhibiting a package of sperm cells in several subdivisions. Semi-thin section/toluidine blue. [Scale bar = 50 µm]; – (3) electron micrograph showing a near-longitudinal section of a spermatodesma from the distal vas deferens. note the layer of dense globules on the top of the cap (arrowheads). Uranyl acetate and lead citrate. [Scale bar = 5 µm] cs: cap of spermatodesma; snn: sperm nuclei; stt: sperm tailes.

RESULTS

Proximally the vas deferens of *Aeshna juncea* appears as a narrow tubule. The terminal portions of each tubule enlarge into an ampulla, which seems to serve as a sperm reservoir. The wall of the ampulla differs in the structure of its mucosa from that of the slender segment.

In the distal vas deferens, spermatodesmata are carried in a viscous gel-like substance that stains heavily with toluidine blue (Figs 1, 2); the stain exhibits trends towards metachromasia. Also, in the female receptaculum seminis, spermatodesmata are carried in a similar gelatinous substance. In fresh material from both vas deferens and receptaculum seminis, bunches of sperm tails exhibit slow, coordinated undulations. Bunches of sperm tails correspond to subdivisions of tightly packed sperm nuclei, and the sperm tails of various small bundles move independently (Fig. 6). Generally, the receptaculum seminis of females captured at the breeding site contain high numbers of spermatodesmata in a gelatinous substance. In different parts of the receptaculum seminis spermatodesmata display various degrees of break-down and release of individual sperm cells.

During spermiogenesis each elongated spermatid develops a slender cytoplasmic



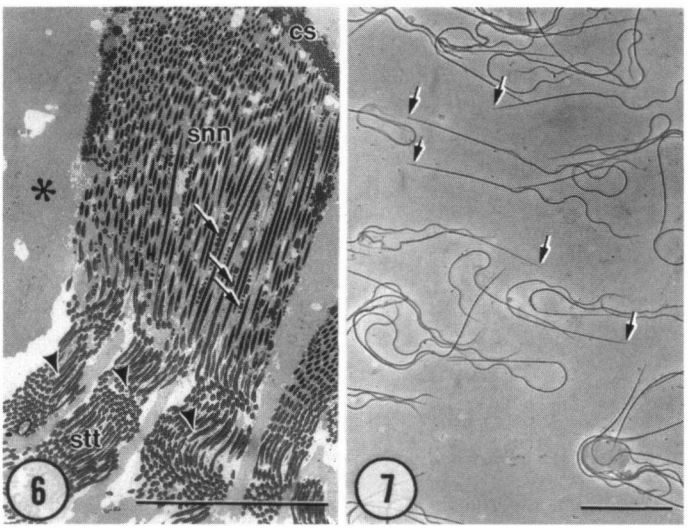
Figs 4-5. *Aeshna juncea*: (4) electron micrograph of sperm cell nuclei in a cross-sectioned spermatodesma demonstrating sperm nuclei packaged in subunits. Uranyl acetate and lead citrate. [Scale bar = 5 μm]; – (5) electron micrograph of a region corresponding to that of Fig. 4, enlarged to demonstrate segregation of sperm subunits by two substances, a mat of delicate filaments (small asterisk) and globules of a homogeneous dense one (large asterisk). [Scale bar = 1 μm].

protrusion in front of the acrosomal rodlet/nuclear head. Decomposing droplets of surplus cytoplasm from early spermatids tend to adhere to the caps of the spermatodesmata (ÅBRO, 1998). When spermatodesmata have arrived and accumulated in the sperm reservoirs, most of the debris has disappeared and instead globules made up of a dense homogeneous substance have been added (Fig. 3); the globules stain heavily with toluidine blue and consistently to the PAS reaction. In longitudinal sections, rows of similar globules could be seen between sperm nuclei (Fig. 6).

The intracyst formation of an aeshnid spermatodesma appears to take place in several steps:

(1) At the end of the elongation period the advanced spermatids are seen to approach each other by their nuclear heads at several sites within the cyst. Gradually, the immature sperm cells will aggregate abreast, constituting small tight bundles, usually containing in the region of 50-60 cells, each bundle with all nuclear heads and their straight cytoplasmic protrusions oriented in the same direction.

(2) The small bundles, which are at first variously oriented within the cyst, begin to gather in a parallel, tight alignment with their nuclear heads and slender protrusions pointing in the same direction. The sperm cells within a cyst then become entangled and linked to one another by their foreparts, thus forming a coherent cap that keeps all sperm cells together.



Figs 6-7. *Aeshna juncea*: (6) electron micrograph showing a near-longitudinal section of a spermatodesma carried in gelatinous substance (asterisk) in the distal vas deferens. Arrowheads indicate protruding small bunches of sperm tails. Between the straight sperm nuclei can be seen rows of dense globules (small arrows). [Scale bar = 25μm; abbreviations as in Figs 1-3]; – (7) free swimming filamentous sperm cells, released from the cap of the sperm bundle, taken from the female receptaculum seminis and freshly dispersed in insect Ringer's solution. Note the straightness of nuclear heads and lack of cytoplasmic foreparts. Arrows indicate position of the acrosomal region. Whole mount/phase contrast. [Scale bar = 50 μm].

Cross sections of the large sperm bundles from the distal vas deferens reveal that primary small bundles are still traceable in the final spermatodesma (Figs 2, 4). They appear segregated mostly by accumulations of a delicate, filamentous mat and also by some denser substance that stains heavily with toluidine blue and by the PAS reaction (Figs 4, 5), presumably the same substance as the globules on the cap. The globulous substance seems to dissolve and disappear after arrival of a spermatodesma in the female receptaculum, and at the same time the gelatinous carrier substance is converted into free fluid.

DISCUSSION

OMURA (1957), using light microscopy only, found an obvious difference between the pattern of sperm bundles in Aeshnidae and Gomphidae. In the present study an explanation for this difference is proposed. It appears that the incipient gathering of advanced spermatids at several places within the testicular cyst is similar in both aeshnids and gomphids; however, in aeshnids the gathering process goes further until all the sperm of a cyst form a single large bundle, within which the initial smaller bundles are recognizable subunits.

The occurrence of large bundles may constitute an efficient vehicle for transfering sperm to the female. In addition, the gelatinous carrier substance is likely to carry several bundles in every sperm parcel during the conveyance procedure. It may also retard flagellar movements of the sperm tails. Sperm seems to be stored in the female for a considerable time, presumably with the carrier substance as a supportive and nutritive medium. Preliminary observations suggest that the gel liquefies rather slowly.

As to their stainability properties, the globulous substance of spermatodesmata as well as the carrier substance, which seems to be contributed by epithelial lining cells of the vas deferens, are likely to be mucoproteins. They appear to be digested by mucolytic and/or proteolytic agents in the female genital tract.

REFERENCES

ÅBRO, A., 1998. Structure and development of sperm bundles in the dragonfly Aeshna juncea L. (Odonata). *J. Morphol.* 235: 239-247.

ÅBRO, A., 1999. The size range of sperm bundles in aeshnid dragonflies (Anisoptera: Aeshnidae). *Odonatologica* 28(3): 273-277.

OMURA, T., 1957. A comparative study of the spermatogenesis in the Japanese dragonflies. 2. Families Aeschnidae, Gomphidae, and Calopterygidae. *Biol. J. Okayama Univ.* 3: 1-86.

SNODGRASS, M.A., C.H. DORSEY, W.H. GEORGE & L.G. DICKSON, 1972. Conventional histopathologic staining methods compatible with Epon-embedded osmicated tissue. *Lab. Invest.* 26: 329-337.