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SHORT COMMUNICATIONS

THE SIZE RANGE OF SPERM BUNDLES IN AESHNID DRAGONFLIES (ANISOPTERA: AESHNIDAE)

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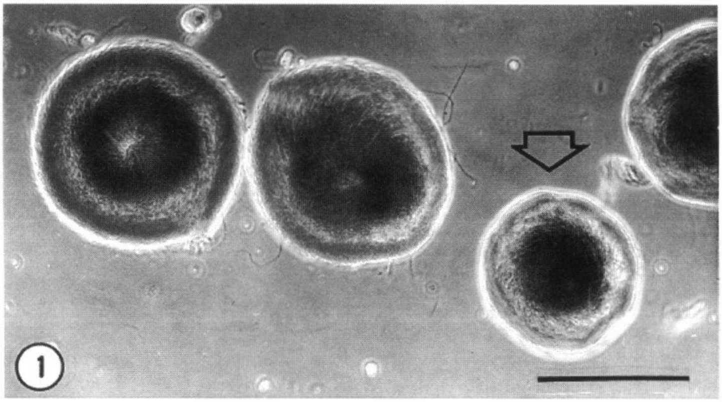
Sperm bundles of alternative size and number of sperm cells are regularly found in the spermiducts of aeshnid dragonflies. This arrangement can be traced to earlier stages of spermatogenesis, where the number of generations of spermatogonia does not appear strictly determined. This is considered an archaic feature of the order Odonata. Dragonfly spermatogenesis is discussed.

INTRODUCTION

Filamentous sperm cells of aeshnid dragonflies are emitted as bundles, each comprising thousands of cells. Spermatogenic cells while enclosed within testicular compartments, the spermatocysts, undergo near-synchronous development. In *Aeshna juncea* there are in all 11 or 12 successive secondary spermatogonial mitoses; in the last premeiotic mitosis the testicular cysts come to contain about 1,000 (theoretically 1,024) or 2,000 (theoretically 2,048) cells (OKSALA, 1944). When the cell line has gone through the meiotic divisions, and resulting spermatids have entered the morphological alteration phase called spermiogenesis, the number of cells has multiplied by the factor 4 to 4,000 and 8,000 respectively. All elongated spermatids within a cyst aggregate and stand abreast to become linked to one another by the slender cytoplasmic forepart of the nuclear heads, thus constituting a bundle that is difficult to disentangle, the spermatodesma (ÅBRO, 1998). The sperm tails protrude outward and move freely. The present communication reports on how to distinguish among aeshnid spermatodesmata consisting of alternative numbers of sperm cells.

MATERIAL AND METHODS

Adult male *Aeshna cyanea* (Müll.), *A. grandis* (L.) and *A. juncea* (L.) were collected near Bergen, western Norway. Captured specimens were placed in cardboard boxes in the dark and kept at 10°C. In the laboratory they were anaesthetized in carbon dioxide, and testes and spermiducts dissected free and isolated in insect Ringer's solution. By teasing apart segments of the vas deferens, bare sperm bundles were allowed to disperse in the saline. Bundles were transferred to microscope slides and gently squashed beneath a coverslip. In addition to observations of fresh material the study drew on observations of material processed for scanning and transmission electron microscopy.



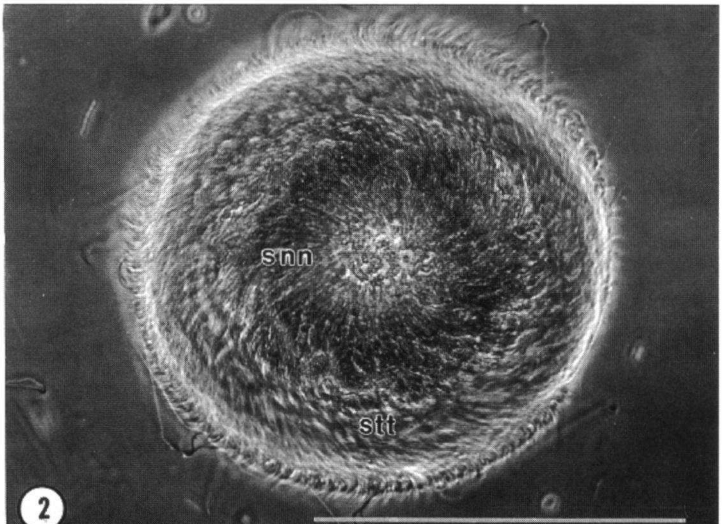


Fig. 1. Apical aspects of a small (arrow) and three large regular spermatodesmata from the vas deferens of *Aeshna juncea*, freshly dispersed in insect Ringer's solution. Whole mount with phase-contrast illumination [scale bar = 100 μm]. – Fig. 2. A large spermatodesma in apical aspect from *Aeshna cyanea*. Note undulation of the sperm tails. snn = sperm nuclei; stt = sperm tails. Whole mount in saline with phase-contrast illumination [scale bar = 100 μm].

RESULTS

Counting filamentous sperm directly in sperm bundles has proved a difficult task. A more convenient method is to count, towards the end of the elongation period, the parallel oriented and tightly packed advanced spermatids or immature sperm cells in cross sections of fusiform testicular cysts, using low magnification electron micrographs. However, this too can become troublesome because of the high numbers of spermatids within each cyst.

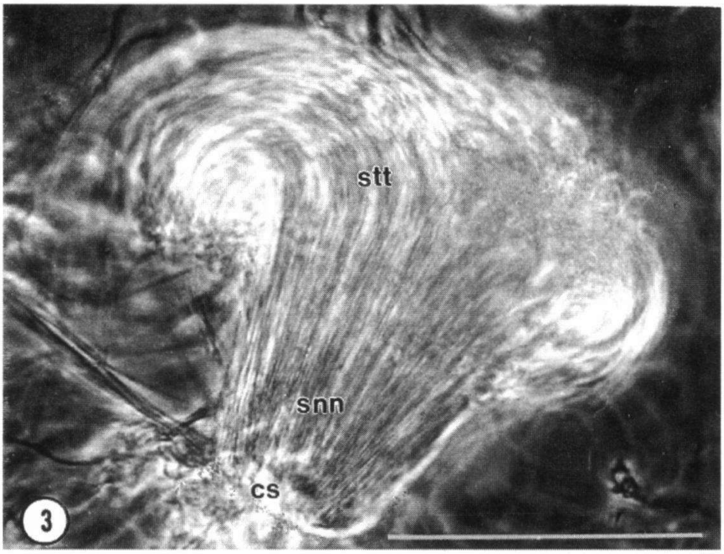
When looking at sperm bundles in the present material, in order to classify them by size, they were usually seen to be made up of two regular classes of which the larger clearly outnumbered the smaller one. In apical aspect the bundles appear like balls of different diameters (Figs 1, 2). When viewed in lateral aspects they appear shuttlecock-like (Figs 3, 4) and it could be even more ardous to assess numerical differences. The distribution of the two types of testicular spermatid cysts corresponds to the distribution of final sperm bundles in the spermiducts. The size of a cyst is roughly proportional to the total number of cells in it. In *A. juncea* the larger testicular cysts contained approximately 8,000 spermatids and the smaller ones approximately 4,000 (theoretical numbers are 8,192 and 4,096, respectively). These figures are extrapolated to apply to the final number of sperm cells in bundles of the two sizes. Sperm bundles of similar form, size and incidence have been recorded also from the dragonfly species *A. cyanea* and *A. grandis*.

In one specimen of *A. grandis* three size classes of bundles were seen, with a few megabundles in addition to the two classes regularly recognized in all three aeshnid species. It was not possible to determine directly the number of sperm cells in these. However, based on the extended diameter of the megabundles, when viewed in apical aspect, they were estimated to contain in the region of 16,000 cells (theoretical sum 16,384).

In the sperm passageway of *A. juncea* and *A. cyanea* the frequency of small bundles (about 4,000 sperms) seemed to rise with increasing adult age. The dragonfly age was estimated in relation to the point of time in the flight season and on the number of emptied testicular cysts, together with accumulated, unsuccessful, abortive cysts containing abnormal spermatids/sperm.

DISCUSSION

In insect spermatogenesis, the complete sequence of events encompasses (a) production of unencysted spermatogenic cells, the primary spermatogonia, by stem-cell division, (b) sequential intracyst differentiation of secondary spermatogonia culminating in the primary spermatocyte, (c) prophase maturation and the ensuing meiotic divisions, and (d) the transformation of the spermatid, spermiogenesis, by which the mature sperm cell is formed (DUMSER, 1980). In the present material a hypothetical cell line that eventually produces a sperm bundle is thought to start as



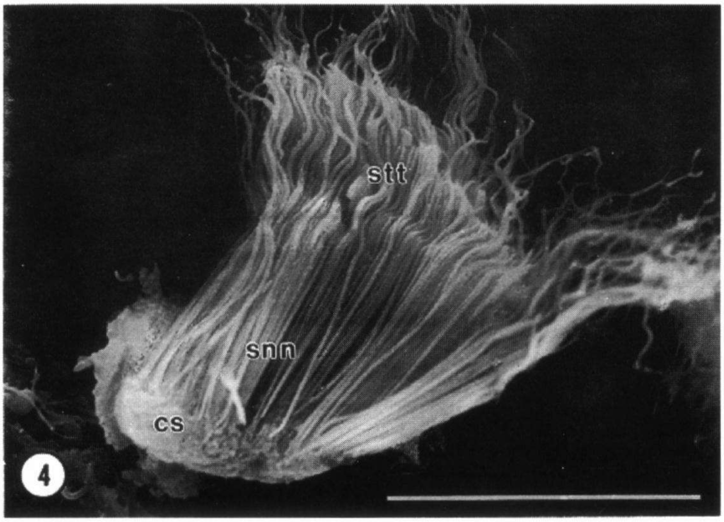


Fig. 3. A spermatodesma in lateral aspect from *Aeshna cyanea*. cs = cap of spermatodesma made up of cytoplasmic foreparts of the sperm cells; snn = sperm nuclei; stt = sperm tails. Whole mount in saline with phase-contrast illumination [scale bar = 50 μm]. – Fig. 4. Scanning electron micrograph of a spermatodesma (from *Aeshna cyanea*) in lateral aspect resting on the epithelial lining of the vas deferens. cs = cap of spermatodesma made up of cytoplasmic foreparts of the sperm cells; snn = sperm nuclei; stt = sperm tails. Critical point dried and sputter-coated with carbon and gold-palladium [scale bar = 50 μm].

somatic cell(s) developing into the testicular cyst, enveloping at least one spermatogonium. Propagation of encysted spermatogonia occurs by near-synchronous mitotic divisions; thus the number of spermatogonia per cyst increases according to a geometric series 2^n (n = 0, 1, 2, 3, 4, 5 ...). This is true also during and after the meiotic divisions, because both divisions occur near-synchronously within a cyst. In Odonata, the number of generations of spermatogonia is not strictly determined (OKSALA, 1944; OMURA, 1955, 1957); accordingly alternative numbers of sperm cells per bundle could be encountered even in one and the same dragonfly specimen. This is presumably why sperm bundles of different sizes are found in the vas deferens.

In the present aeshnid material, sperm bundles in the region of 8,000 and 4,000 sperm seem to be a regular occurrence, while finding megabundles (about 16,000 sperm) seems to be a rare event and is considered quite irregular. As the number of intracyst spermatogonia increases, the cyst wall becomes thin and membraneous. Megabundles might result from a fusion of neighbouring cysts of advanced spermatogonial generations.

In the Indian gomphid, *Ictinogomphus rapax* (Ramb.), 16,000 spermatid/sperm cells have been recorded in a cyst (OMURA, 1955, 1957). It has been maintained that up to 6 different numbers of sperm cells per bundle could be found in dragonflies (OMURA, 1955, 1957). According to VIRKKI (1969), the number of sperm cells per bundle in Odonata, even in one individual, could be as follows: 2,048 (2^{11}), 4,096 (2^{12}), 8,192 (2^{13}), 16,348 (2^{14}), 32,765 (2^{15}), 65,636 (2^{16}).

Archaic insect orders have more sperm cells per bundle than more modern ones and, within an order, the most modern or most specialized groups tend to have the least (VIRKKI, 1969). Huge numbers of sperm cells per bundle (up to 65,536) are said to be typical of an ancient primitive insect order (VIRKKI, 1969). The Odonata is considered closely related to the Protodonata (Meganeuridae), which are only known as palaeozoic fossils (WOOTTON, 1981).

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