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SHORT COMMUNICATIONS

SPERM CLUSTERS IN ZYGOPTERA (COENAGRIONIDAE, LESTIDAE, CALOPTERYGIDAE)

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When within the testicular cyst, individual, immature sperm of *Lestes sponsa* acquire a cap of periacrosomal material. During passage through the spermiducts and vas deferens, the caps of individual sperm coalesce, producing clusters of sperm under a common cap. In *Calopteryx virgo*, entire sperm cells become embedded in an extracellular homogeneous substance. The joining substance in both species appear to be derived from decomposed surplus cytoplasm sloughed off from developing spermatids. The epithelial lining of the spermiducts adds secretions to this. Clustering of sperm cells was not demonstrated in species of the Coenagrionidae.

INTRODUCTION

Regular sperm bundles comprising up to several thousand filamentous spermatozoa are found to develop in some anisopteran dragonflies (BALLOWITZ, 1916; ÅBRO, 1998, 1999). To provide a sperm bundle, intracyst sperm cells become linked to one another by the slender cytoplasmic forepart of their nuclear heads. Sperm bundling has been reported also in zygopterans (OMURA, 1957). Furthermore, zygopteran spermatozoa are long filamentous flagellate cells. The present communication reports on observations concerning sperm aggregation in some zygopteran species.

MATERIAL AND METHODS

Adult zygopterans were collected near Bergen, western Norway. The following species have been examined: *Calopteryx virgo* (L.) (Calopterygidae), *Lestes sponsa* (Hans.) (Lestidae), and *Coenagrion hastulatum* (Charp.), *Enallagma cyathigerum* (Charp.) and *Pyrrhosoma nymphula* (Sulz.) (all Coenagrionidae).

Fresh vasa deferentia containing sperm cells were dissected out in insect Ringer's solution to dis-

perse the sperm cells. Samples were transferred to microscope slides and studied in transmitted light. Testes and spermiducts were also fixed (1-2 hours) and processed for transmission electron microscopy. The fixing fluid was made up of 3% 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. Tissues were dehydrated through a graded series of ethanol, cleared in propylene oxide, and embedded in epoxy resin. Ultrathin sections cut with diamond knives were stained with uranyl acetate and lead citrate.

RESULTS

All zygopteran species examined possessed long filamentous sperm cells. The Coenagrionidae did not exhibit aggregations of sperm cells either dispersed in insect saline (Fig. 1) or in sections of the spermiducts containing seminal fluid when viewed in transmission electron microscope. No sperm cluster at any developmental stage in the coenagrionids has been recognized. In contrast, the sperm cells in *Lestes sponsa* and *Calopteryx virgo* become aggregated (Figs 2, 4-6).

In *Lestes sponsa*, sperm cells when freshly dispersed in saline appear in small clusters (Fig. 2). These sperm associations seem readily disrupted. Individual sperm cells are seen to acquire a cap of periacrosomal material when within the testicular cysts (Fig. 3). After release into the vas deferens, the sperm cells appear to aggregate in clusters containing 10-25 cells. Electron micrographs of distal segments of the vas deferens reveal sperm clusters with periacrosomal material coalesced in a common cap (Fig. 4).

In *Calopteryx virgo*, the vas deferens is rather long and tortuous; small sperm aggregations occur in its somewhat distended, distal part. Dispersed in saline, the sperm cells of *C. virgo* appear aggregated in clusters that are liable to break up. In electron micrographs the sperm clusters appear as several cells (mostly 10-25) embedded in a homogeneous substance of fine-granular quality (Fig. 5). Complete cells, not only the sperm heads (=nuclei) or parts of them, are embedded and the sperm flagella appear spiralled (Fig. 6). Observations on developing testicular cysts indicate that cytoplasmic bodies, segregated during spermiogenesis, decompose and thus contribute to the substance that holds sperm cells together in clusters. The lining epithelium of the vas deferens exhibits deep invaginations from the luminal surface and the cells seem to have a secretory activity. In the invaginations as well as in the main lumen, free exocytosed bodies of a rather dense material can be seen in the seminal fluid (Figs 5, 6).

DISCUSSION

In a comparative study of sperm bundle formation in Japanese dragonflies, OMURA (1957), using light microscopy, summed up that aeshnids form only one large compact sperm bundle within each testicular cyst. Species belonging to the family Gomphidae form several various-sized bundles within the cyst, presumably

developed in a similar way to those of aeshnids (OMURA, 1957).

In Calopterygidae, OMURA (1957) found immature sperm cells gathering together at several places within each testicular cyst, thus forming a number of small fairly compact bundles. This mode of sperm association appeared different from that seen in anisopterans. In contrast to findings in *Calopteryx* and *Mnais* of intracyst sperm gathering at severeal places (OMURA, 1957), a

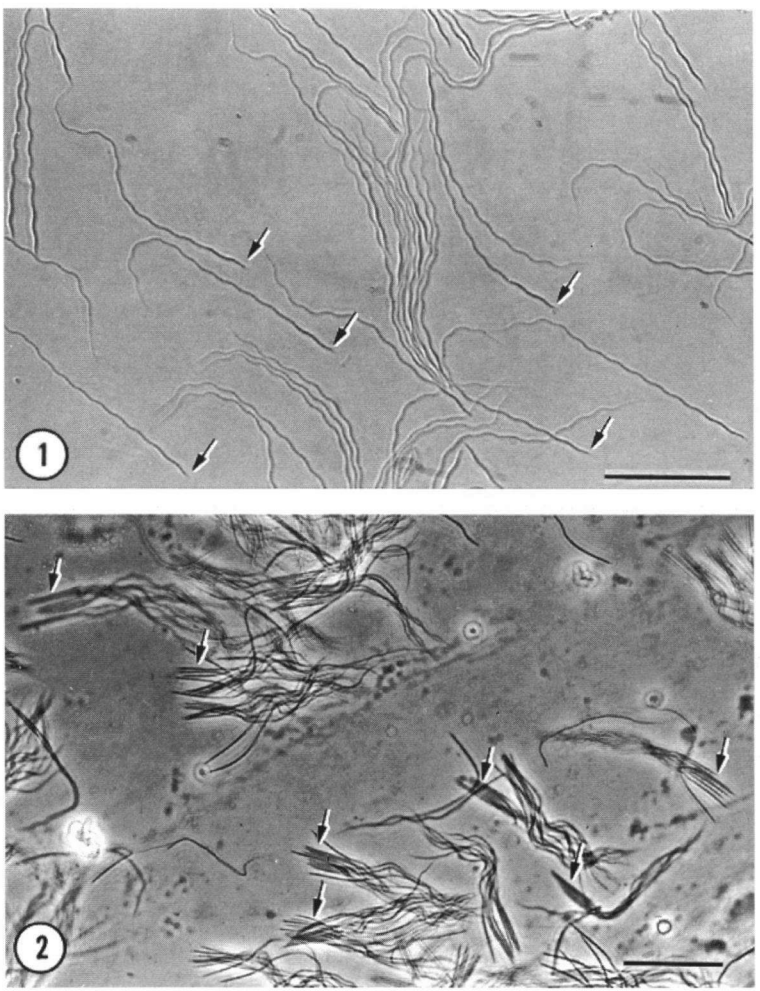
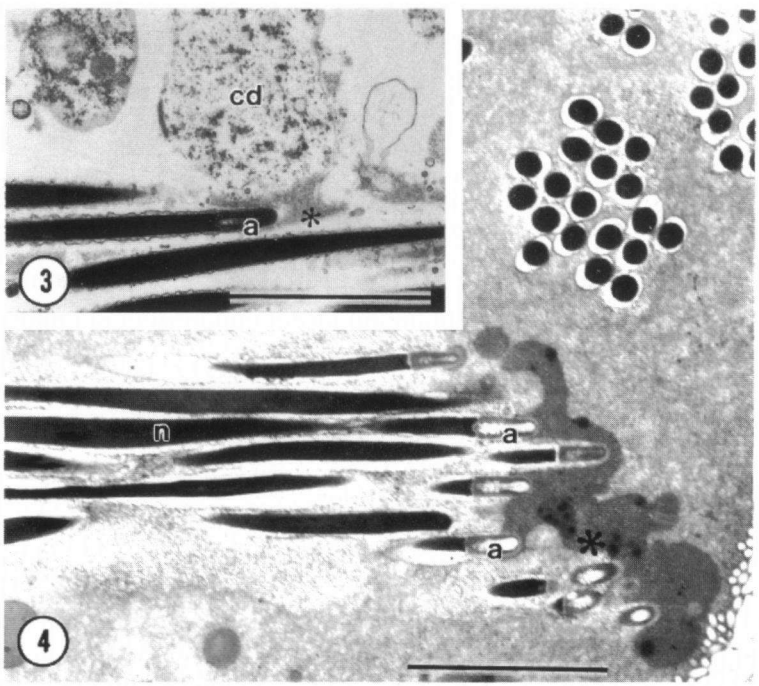


Fig 1. Sperm cells from the vas deferens of *Enallagma cyathigerum* dispersed in insect Ringer's solution. No sperm clustering. Arrows indicate position of the acrosome in front of the nuclear head on individual sperms. Whole mount/bright field. [Scale bar = 50 µm]. – Fig. 2. Sperm cells from the vas deferens of *Lestes sponsa*. Arrows indicate clusters of sperm cells bound together by a hyaline substance. Whole mount in saline/phase contrast illumination. [Scale bar = 50 µm].

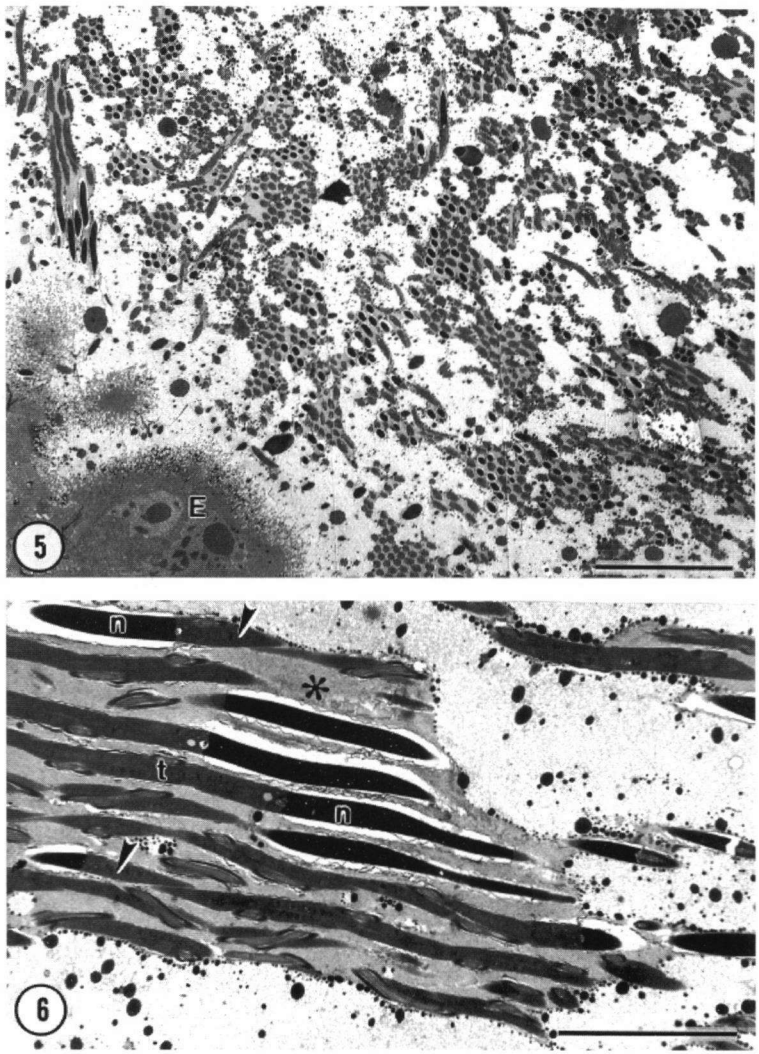
similar developmental pattern could not be demonstrated in the present material from *Calopteryx virgo*. With no intracyst clustering, sperm association is likely to take place during passage through the rather long vas deferens.

It should be emphasized that, in *C. virgo*, complete sperm cells are embedded in an extracellular substance. In contrast, the sperm heads of *Lestes sponsa* are attached by a periacrosomal material forming a common cap that holds the sperm together, much like the extracellular substance found accumulating around the acrosomes of locust sperm (SZÖLLÖSI, 1974). Moreover, this minimal attachment might be the reason why individual sperm so readily liberate themselves from the *Lestes* clusters.

The adhering extracellular substance of both *C. virgo* and *L. sponsa* could be a complex mucoprotein which has a trophic function. This substance seems to be derived from segregated droplets of excess cytoplasm in the lumen of advanced testicular cysts. Decomposed surplus cytoplasm is usually emitted



Figs 3-4. *Lestes sponsa*: (3) Electron micrograph from the testis, showing immature intracyst sperm cells with accumulation of a cap of periacrosomal substance (asterisk) on individual cells (a: acrosome; – cd: segregated surplus cytoplasm from spermatids; – uranyl acetate and lead citrate). – (4) Electron micrograph from the vas deferens, demonstrating clusters of sperm cells cut transversely and longitudinally. Caps of periacrosomal material on individual sperm cells have coalesced to form a common cap (asterisk) of a cluster (a: acrosome; – n: nuclear head; – uranyl acetate and lead citrate). – [Scale bars = 5 μm].



Figs 5-6. *Calopteryx virgo*: (5) Electron micrograph from the distal region of the vas deferens: sperm cells are seen embedded in a homogeneous substance forming clusters of various sizes, most of which are here seen in cross-section. At the bottom left, the top of the epithelial coat (E) bordering the lumen has a brush-border of microvilli. (Uranyl acetate and lead citrate). – [Scale bar = 10 μm]. – (6) Electron micrograph from the vas deferens, showing a group of sperm cells, longitudinally sectioned: complete sperm cells are seen embedded in an intercellular matrix (asterisk). Note the spiral arrangement of sperm flagella (t) and the reverse packing of some sperm cells (arrowheads). (n: nuclear head; – uranyl acetate and lead citrate). – [Scale bar = 5μm].

from the cysts together with the sperm cells. Secretory products are added from the epithelial cells of the spermiducts. So far, the bundling and clustering of sperm cells in anisopterans and zygopterans, respectively, appear to develop along different lines.

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