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ULTRASTRUCTURE OF THE SPERM OF *DOLANIA AMERICANA* EDMUNDS AND TRAVER (EPHEMEROPTERA : BEHNINGIIDAE)

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Abstract—The ultrastructure of the mature sperm of the mayfly, *Dolania americana* Edmunds and Traver (Ephemeroptera : Behningiidae), is described from scanning and transmission electron microscopy. The head is 0.7–1 μm wide and 4.6–6.9 μm long, rodlike, and topped by a short, rounded acrosome 0.4 μm long and 0.6 μm wide. The flagellum is 5–6 times the head length and is flattened, except for a thin, tubelike terminal portion. The axoneme pattern is 9–9–1 (9 outer singlet microtubules, 9 doublet microtubules, and a central dark element) and is new for Ephemeroptera. The inner dynein arms are conspicuous and outer arms are lacking, and radial spokes and a central sheath are prominent. A densely-staining and bi-lobed accessory body lies adjacent to the axoneme. A mitochondrial derivative with regularly arranged transverse-to-oblique cristae lies adjacent to the accessory body.

Index descriptors (in addition to those in title): Unique ephemeropteran 9–9–1 axoneme pattern, central dark axonemal element.

INTRODUCTION

MAYFLIES are the most primitive extant pterygote insects. However, our knowledge of the ultrastructure of mayfly sperm was until 1985 based on studies of only 4 species from 4 families in 3 of the extant superfamilies†: *Cloeon dipterum* (Linnaeus) (Baetidae, Baetoidea) (Baccetti *et al.*, 1969); and *Pentagenia vittigera* (Walsh) (Palingeniidae, Ephemeroidea), *Hexagonia* sp. (Ephemeridae, Ephemeroidea), *Tricorythodes* sp. (Tricorythidae, Ephemerelloidea) (Phillips, 1969, 1970). These sperm were similar and possessed an axononeme with a 9–9–0 microtubule pattern (9 outer singlets, 9 doublets, and no central singlets). This axoneme pattern is unusual in insects, and was generalized

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†In this paper we follow the phylogeny proposed by Edmunds (1984) and McCafferty and Edmunds (1979).

to the entire order of mayflies (e.g. Baccetti, 1972, 1979, 1985; Baccetti and Afzelius, 1976; Dallai, 1979; Jamieson, 1987) even though representatives of the remaining 15 extant families (total of 19) and 3 superfamilies (total of 6) remained to be studied.

A second or aflagellate type of mayfly sperm was suggested by light microscopical observations of 8 Leptophlebiidae (Leptophlebioidea) species (Soldán, 1979a, b). Grimm (1985) confirmed in an ultrastructural study that the sperm of the leptophlebiid, *Habrophlebia lauta* Eaton was aflagellate, and that the axonemal pattern of *Siphlonurus croaticus* Ulmer (Siphlonuridae, Baetoidea) resembled that of other non-leptophlebiid mayflies.

Our study describes ultrastructurally the sperm of a 7th species of mayfly, *Dolania americana* Edmunds and Traver, in a 7th family (Behningiidae, Ephemeroidea). We demonstrate that the mature sperm of *Dolania* possesses a new or 3rd type of axonemal pattern in mayflies.

MATERIALS AND METHODS

Transmission electron microscopy

The posterior abdomens of 5 mature last instar larvae (black wing padded larvae soon to emerge to the subimago) were cut into a fixative of 2% paraformaldehyde and 2.5% glutaraldehyde in Millonigs phosphate buffer at pH 7.4, postfixed in 1% osmium tetroxide in Millonigs phosphate buffer at pH 7.4, dehydrated in acetone, and embedded in Spurr medium. Ultrathin sections were stained with uranyl acetate and lead citrate.

The penes (containing extruded sperm) from an imago were placed into a 1.5% glutaraldehyde–cacodylate buffer fixative (pH 7.2, 0.1M) and sent by overnight mail from Florida to Salt Lake City. Immediately upon arrival, the specimen was postfixed in 1% osmium tetroxide–cacodylate buffer, and further prepared as described above for the larvae.

Scanning electron microscopy

Sperm were squeezed from 10 imagoes (squeezed posterior abdomen towards penes) into the paraformaldehyde–glutaraldehyde primary fixative. After fixation, sperm were allowed to settle onto glass chips previously treated with 1% polylysine to attach them to the chips, dehydrated in ethanol, passed through an amyl acetate–ethanol series, and critical-point-dried with carbon dioxide. Specimens on tabs were coated with gold–palladium. Some sperm were mixed with eggs, dissected from an adult female, in Millonigs phosphate buffer for 2 min, and then prepared as for sperm only.

Light microscopy

Aldehyde-fixed sperm were observed with phase contrast and eggs with brightfield or interference contrast optics.

RESULTS

External morphology

The sperm of *Dolania* (Fig. 1) has a rod shaped head 4.6–6.9 μm long and 0.7–1.0 μm wide, and is topped by a short rounded acrosome 0.3–0.6 μm long and 0.4–0.7 μm wide (Fig. 1, insert). The flagellum is 5–6 times the head length (24–39 μm) and is flattened, except for a thin tubular terminal portion. The greatest and least width dimensions of the flagellum are 0.6–0.9 and 0.1–0.2 μm, respectively. Total length of the sperm is 29–45 μm.

Ultrastructure

The head (Figs 2; 4) contains a very dense nucleus with highly compacted chromatin.

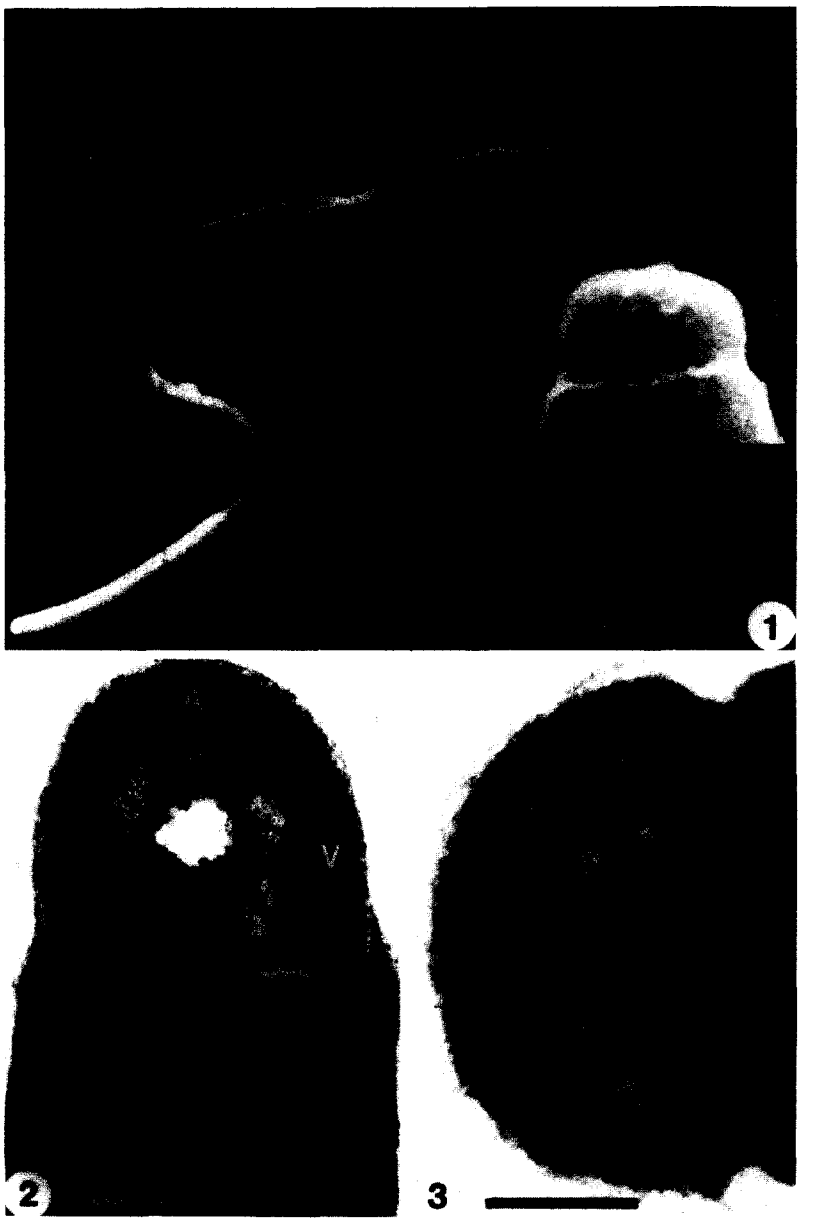


FIG. 1. *Dolania americana* sperm (inset shows acrosome).
FIGS 2; 3. Longitudinal sections of *Dolania* acrosomes showing light vesicular like areas. A = acrosome; F = flagellum; H = head; V = vesicular-like areas in acrosome. Scale bars: Fig. 1 = 5 μm (inset = 0.25 μm); FIGS 2; 3 = 0.2 μm.

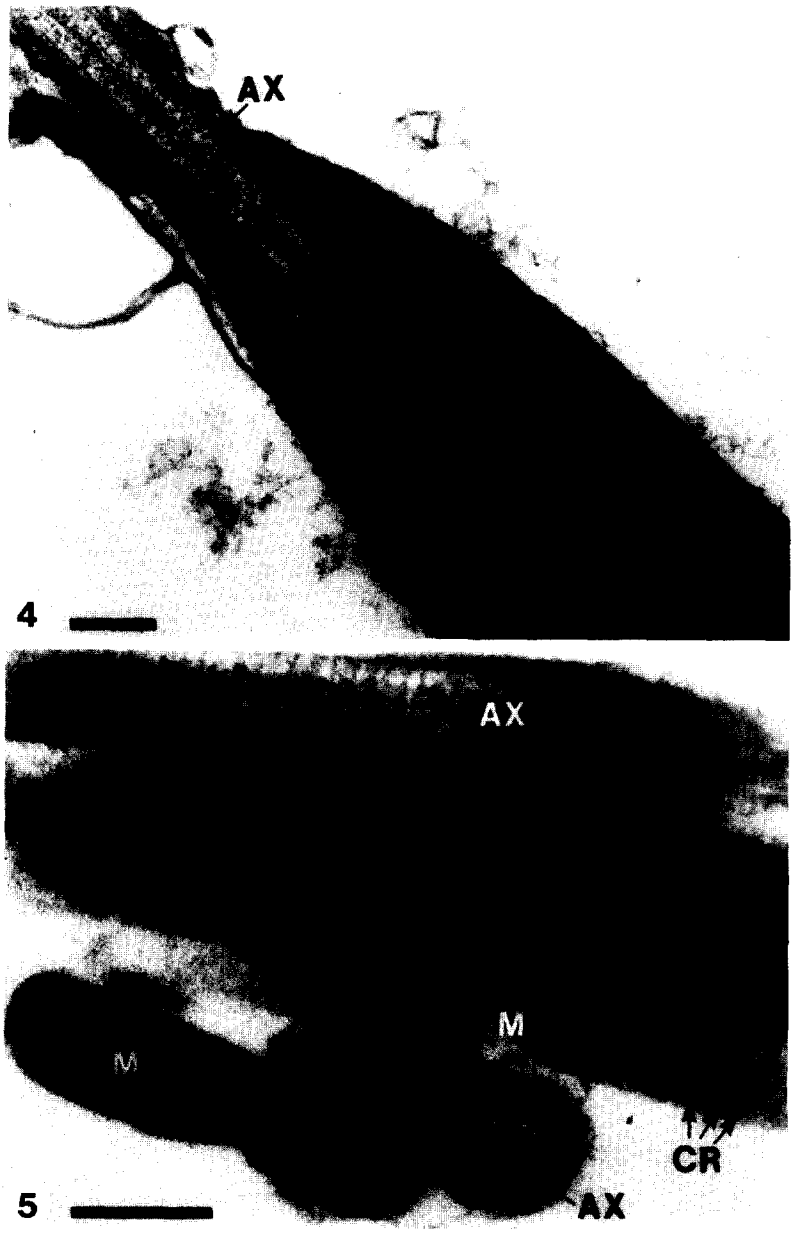
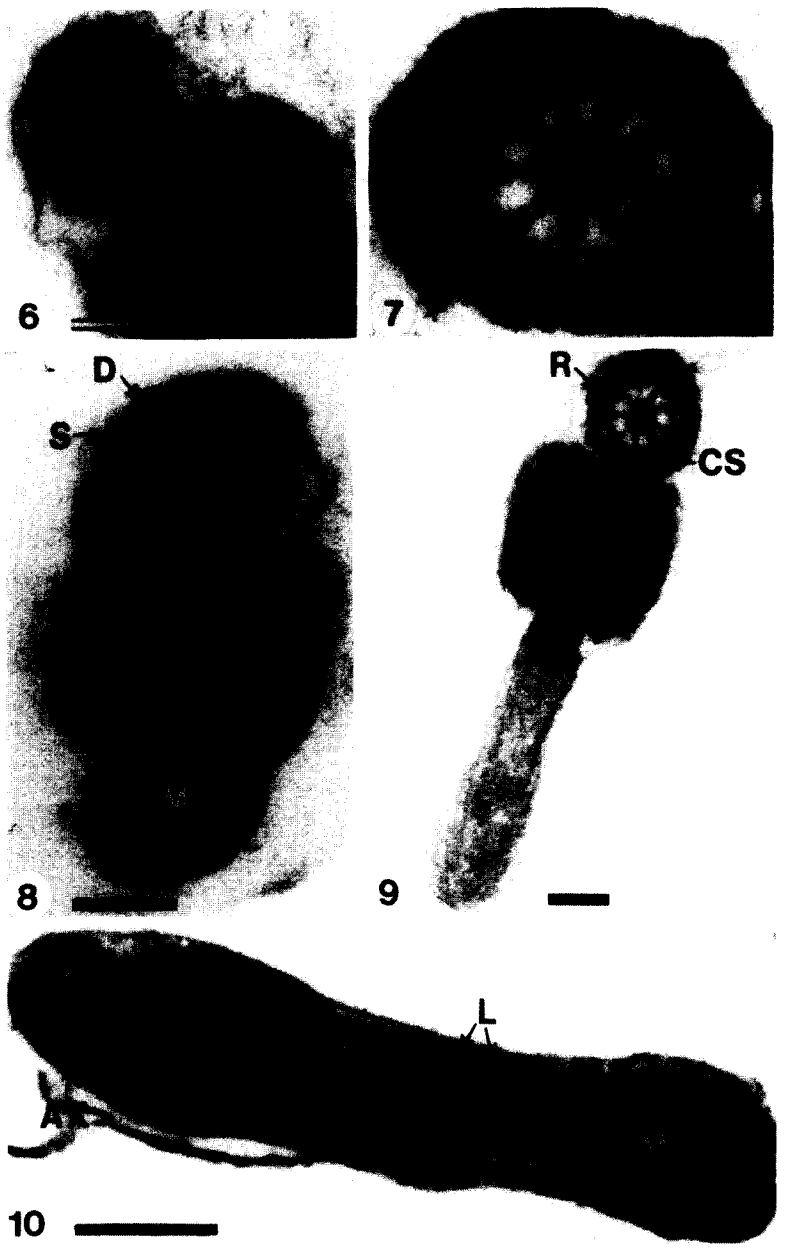


FIG. 4. Longitudinal section of *Dolania* sperm head (base) and basal portion of flagellum.
FIG. 5. Longitudinal (above) and transverse views of *Dolania* sperm flagella. AX = axoneme; B = accessory body; CR = mitochondrial cristae; M = mitochondrial derivative; N = nucleus. Scale bars = 0.2 μm.



FIGS 6–9. Transverse sections of *Dolania* sperm flagella (sperm collected from an imago). In the center of axoneme is a dark central element which gives *Dolania*, among Ephemeroptera, a unique 9–9–1 axoneme pattern.

FIG. 10. Transverse section of an immature *Dolania* sperm from a very mature last instar larva. Unlabelled arrow = dark central element at center of axoneme; AX = axoneme; B = accessory body; CS = central sheath; D = doublet microtubules of axoneme; L = lateral microtubules; M = mitochondrial derivative; R = inner dynein arm of *a* microtubule of a doublet; S = outer singlet accessory microtubule of axoneme. Scale bars: Figs 6–9 = 0.1 μm; Fig. 10 = 0.2 μm.

The acrosome often had large, light staining vesicle-like structures (Figs 2; 3).

The flagellum is attached at the base of the head as shown in Fig. 4. The axoneme occurs at one side of the flagellum and is bordered by a densely-staining and bi-lobed accessory body. A single, large mitochondrial derivative occurs on the other side of the flagellum (Figs 5; 6; 8–10), and shows in longitudinal section many transverse-to-oblique parallel cristae (Fig. 5). Neither the accessory body nor the mitochondrial derivative appeared crystalline-like, or contained any crystalline-like inclusions.

The axoneme (Figs 6–9) possesses 9 outer singlet accessory microtubules, and 9 doublet microtubules. In fully mature sperm, a dark "central element" occurs at the center of the axoneme so that the axoneme pattern is 9–9–1. We can not confirm, based on our present ultrastructural results, whether the central element is or is not a singlet microtubule. It appeared more variable in shape and size than the outer singlet microtubules. Surrounding the central element is a central sheath. A radial spoke (link) connects the central sheath to each *a* tubule of a doublet. Each *a* tubule possesses an inner dynein arm, but lacks an outer arm.

Sperm observed from last-instar larvae were not fully mature (Fig. 10) as noted by cytoplasm exterior to the axoneme, accessory bodies and mitochondrial derivative. These sperm, unlike the mature sperm, also possessed microtubules surrounding the mitochondrial derivative. The fixation of the axoneme appeared inferior to that of the mature sperm, and it was impossible to determine whether a central element occurred or was forming at the center of the axoneme. Generally, however, a central element did not appear to be present in these immature sperm (Fig. 10).

DISCUSSION

Mayfly sperm are much more variable than originally expected in the early ultrastructural studies. The axoneme pattern was first characterized to be 9–9–0 (Baccetti *et al.*, 1969; Phillips, 1969, 1970). A 2nd or "no" pattern was found in the aflagellate sperm of the Leptophlebiidae (Soldán, 1979a, b; Grimm, 1985). Our study demonstrates in mature *Dolania americana* sperm a 3rd axoneme pattern, the 9–9–1, in which there are 9 outer singlet microtubules, 9 doublet microtubules, and central element. *Dolania* is a member of the isolated lineage Behningiidae in the superfamily Ephemeroidea. Thus, within the Ephemeroidea alone there are 2 axoneme patterns.

The number, shape, location and crystalline appearance of accessory bodies also varies in mayflies. *Dolania* sperm appear to have a single bi-lobed accessory body, which does not appear crystalline-like. Other non-Leptophlebiidae mayflies have 2 accessory bodies (Phillips, 1969, 1970; Baccetti *et al.*, 1969; Grimm, 1985), and in *C. dipterum* they were crystalline-like (Baccetti *et al.*, 1969). *S. croaticus* sperm, when viewed in transverse section, displayed accessory bodies, which were triangular in shape, widely separated and situated laterally around the mitochondrial derivative (Grimm, 1985). In contrast, in non-leptophlebiid mayflies, the accessory bodies lie close together and directly between the mitochondrial derivative and the axoneme. The axoneme lies closer to the mitochondrial derivative in *S. croaticus* sperm than in other flagellate mayfly sperm due to the different location of the accessory bodies.

The aflagellate sperm of Leptophlebiidae are the most unusual. *H. lauta* lacked an acrosome and mitochondria, or mitochondrial derivative. These sperm were approximately 1.5 μm in diameter and consisted of a nucleus with condensed chromatin

and a central light staining area (Grimm, 1985).

Mayfly sperm even differ at the light microscopy level. Soldán (1979a, b) studied the mature sperm of 51 species in 25 genera, 11 families, and in 5 of the 6 extant mayfly superfamilies and designated 4 types of sperm based on relative head and flagellum sizes. Some species showed polymorphic sperm, which differed in size, shape and degree of stainability (with Pappenheim's stain). Mayfly sperm varied in size from 1 to 25 μm, which is small compared to many other arthropods.

The apparent lack of a central element in the axoneme of immature sperm in very late instar *Dolania* larvae may have been due to inadequate preservation, or this structure may form very late. Young and even some late spermatids of *Psocus* sp. (Insecta, Psocoptera) did not yet possess the central dark rod observed in mature sperm (Phillips, 1969). Sperm maturation in *Dolania* probably occurs very late in the last larval instar, because male subimaginal and imaginal lifespan prior to mating is only about 45 min (Peters and Peters, 1977). Our larval specimens were very close to emergence to the subimago as judged by swollen wingpads, containing fully formed subimaginal wings. We hypothesize that the threshold temperature responsible for the synchronized mass emergence in this species (Peters *et al.*, 1987) also stimulates final sperm maturation.

The variability now known in mayfly sperm warrants ultrastructural studies of additional families, especially in the superfamilies Caenoidea and Prosopistomatoidea, which have not yet been investigated.

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