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AFLAGELLATE SPERM IN THREE SPECIES OF LEPTOPHLEBIIDAE (EPHEMEROPTERA)*

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Abstract—Immotile spermatozoa of 3 species of Ephemeroptera, *Habroleptoides umbratilis, Habrophlebia eldae* and *Choroterpes picteti* (Leptophlebiidae) are described. In all 3 species, sperm present a coccoidal shape and lack flagella or microtubule systems. *Habrophlebia eldae* shows very atypical sperm with a roundish nucleus and scarce cytoplasm, including an apical acrosome. By contrast, a fibrillar perforatorium and a mitochondrion are present in the sperm of *H. umbratilis* and *C. picteti*. This latter species also shows electron-dense bodies located in the space between the nucleus and the cell membrane. Our findings suggest that sperm/egg interaction should depend in leptophlebiids on the contraction of the genital duct muscles.

Index descriptors (in addition to those in title): Immotile sperm, *Habroleptoides umbratilis, Habrophlebia eldae, Choroterpes picteti,* mayflies, transmission and scanning electron microscopy.

INTRODUCTION

SPERMATOZOA with unusual ultrastructural features, different from the classical sperm model, are occasionally seen in insects (Baccetti *et al.*, 1973a, b; 1974; 1981). Besides their cytological differences, atypical sperm have been used to evaluate both the importance of motility during fertilization (Baccetti, 1972; Phillips, 1974) and the relationships between different insect orders (Dallai, 1979; Dallai and Mazzini, 1989).

Among Ephemeroptera, flagellate sperm represent the most common model. Indeed, there is ultrastructural evidence for flagella in some mayfly families (Baccetti *et al.*, 1969; Phillips, 1969, 1974; Grimm, 1985; Fink and Yasui, 1988; Gaino and Mazzini, 1990a). In light microscopy, aflagellate sperm have been reported in Leptophlebiidae (Soldán,

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1979a, b). This feature has been subsequently confirmed in a single ultrastructural micrograph in *Habrophlebia lauta* (Grimm, 1985). Except for this study, the sperm ultrastructure of leptophlebiids is unknown.

This paper describes the sperm ultrastructure of 3 species of leptophlebiids, each belonging to a different genus. A specific gamete structure is described, which can be useful to evaluate the role of cell shape in fertilization biology.

MATERIALS AND METHODS

Testes and sperm ducts of *H. umbratilis*, *H. eldae* and *C. picteti* were dissected from mature nymphs, subimagoes and adults. Live sperm were observed with a phase contrast light microscope. For electron microscopy, selected material was fixed in Karnovsky's medium (1965) in cacodylate buffer (pH 7.2, 0.1 M), rinsed in the same buffer, postfixed in 1% osmium tetroxide–cacodylate buffer, dehydrated in ethanol series, and embedded in Epon-Araldite mixture. Sections obtained with a Reichert ultratome and mounted on formvar-coated copper grids were stained with uranyl acetate and lead citrate and observed in either a Philips 300 or a Zeiss EM9 electron microscope. Scanning electron micrographs were obtained after sperm squeezing into Karnovsky's primary fixative medium-cacodylate buffer on 1% polylysine-treated glass chips. Specimens were dehydrated in ethanol, critical-point dried with carbon dioxide, gold-palladium coated and observed with a Philips 505 electron microscope.

RESULTS

The spermatozoa of leptophlebiids do not show active movement in biological fluids. In light microscopy, they have a coccoidal shape and include 2 regions differing in size and appearance, a rounded part and a little knob located at the anterior cell pole (Fig. 1).

On scanning electron microscopy (SEM), the sperm of *H. umbratilis* shows a constriction at about ⅓ its length, causing a "figure-eight" appearance (Fig. 2). Sperm are about 2 μm long, and in transmission electron microscopy (TEM), they reveal a complex architecture (Figs 3, 4). The anterior region, corresponding to the apical knob seen in light microscopy, contains the acrosome. The posterior region is wider and almost completely occupied by the nucleus, showing tightly packed chromatin and some translucent areas. The acrosome is rounded and includes a well-defined perforatorium with a fibrillar content (Fig. 4). The perforatorium has a conical shape, with its base facing the apical part of the sperm. Since the perforatorium is included in the central concavity of the acrosome, the latter has thicker lateral regions and a thin apex (Fig. 4), where the acrosomal membrane is juxtaposed to the plasma membrane. A single small mitochondrion, without any crystallized appearance or crystalline-like inclusions, is located beneath the nucleus (see inset in Fig. 3).

Sperm architecture has been reconstructed with ultrastructural serial sections (Fig. 5).

In *H. eldae*, the sperm (about 1 μm long) shows an elementary architecture (Fig. 6),

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FIG. 1. Live sperm of *H. umbratilis* under phase-contrast microscope. Note little knob (arrows) at anterior spermatozoon pole. × 2500.

FIG. 2. *H. umbratilis*: SEM shows a sperm, consisting of 2 regions that differ in size and shape. × 11,500.

FIG. 3. TEM of variously oriented sections of sperm of *H. umbratilis*, showing their architecture. A = acrosome; FP = fibrillar perforatorium; M = mitochondrion; N = nucleus. × 16,000. Inset shows nucleus (N) and mitochondrion with some cristae. × 50,000.

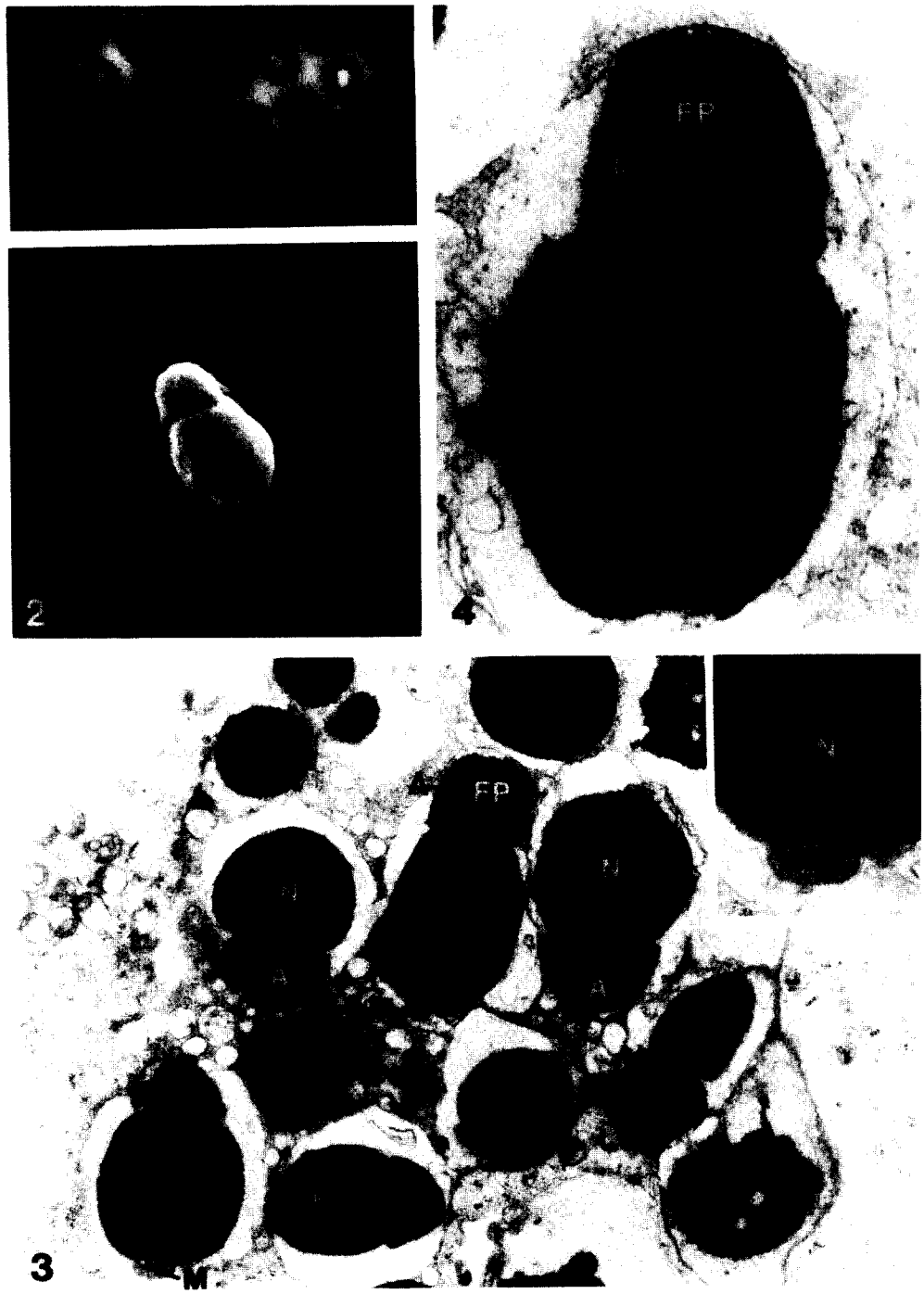

FIG. 4. Architecture of a sperm of *H. umbratilis*. Note acrosome (A) in the anterior region including fibrillar perforatorium (FP). Posterior region is almost completely occupied by nucleus (N). × 47,000.

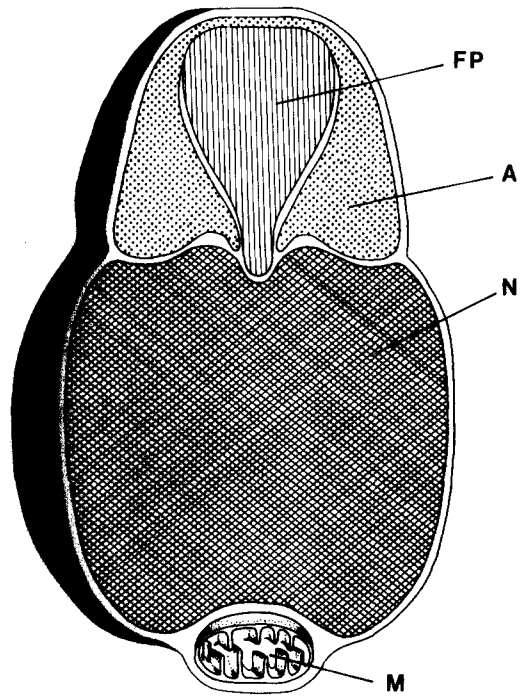

FIG. 5. Schematic drawing of sperm of *H. umbratilis*. A = acrosome; FP = fibrillar perforatorium; M = mitochondrion; N = nucleus.

with only the nucleus and the overlying acrosome. The chromatin is condensed, except for the central nuclear part. No mitochondrion, mitochondrial derivatives or perforatorium are seen.

In *C. picteti*, the sperm (about 1.5 μm long) shows a small knob-like acrosome (Fig. 7) surrounding a fibrillar perforatorium. The nucleus is separated from the cell membrane by a sheath of cytoplasm, containing some electron-dense bodies (Fig. 7), which in some cross-sections constitute a discontinuous peripheral ring. Even though in insects with aflagellate sperm, mitochondria are usually located at the posterior end, in this species, a small mitochondrion is present in the anterior portion, at the base of the acrosome (Fig. 7).

DISCUSSION

Most mayflies show motile sperm. The lack of flagella is a peculiar trait of leptophlebiid spermatozoa, which can be regarded as a final stage of evolution (Baccetti, 1972). These findings suggest that Leptophlebiidae may be considered a specialized group of Ephemeroptera. The well-differentiated fibrillar perforatorium, hitherto unknown in other mayfly sperm models, and the uncrystallized mitochondrion represent, however, primitive structures. In most insects, an evolutionary trend toward the loss of perforatorium and the final loss of acrosomal complex has been described (Baccetti, 1979). The mitochondrial derivative with no crystalline inclusions, present also in

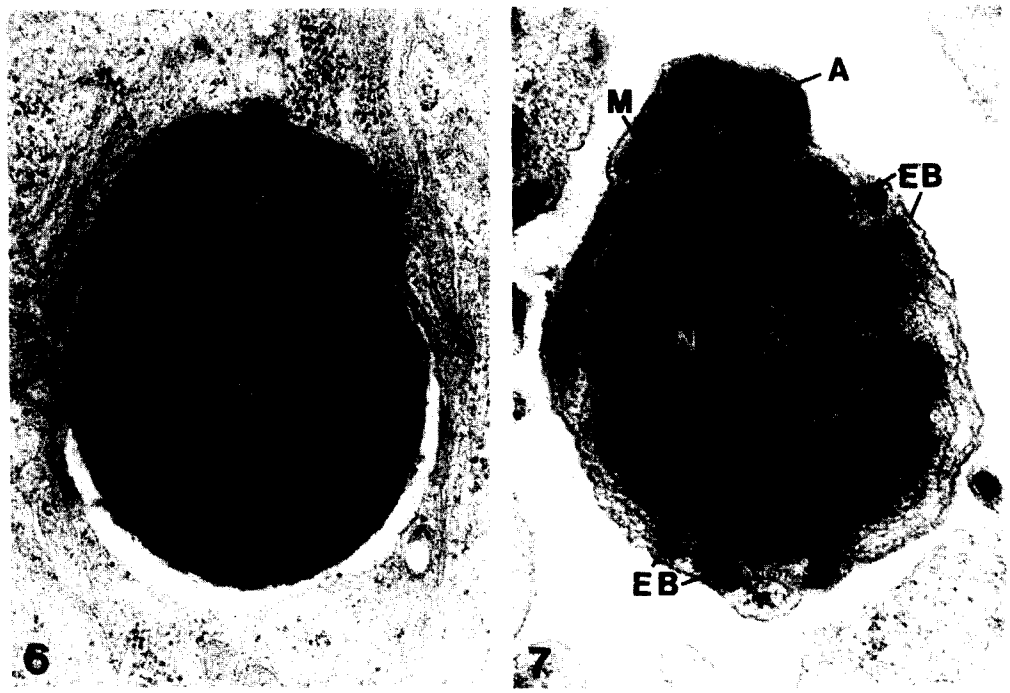

FIG. 6. Sperm of *H. eldae*. A = acrosome; N = nucleus. × 61,000.
FIG. 7. Sperm of *C. picteti*. A = acrosome; EB = electron-dense bodies; M = mitochondrion; N = nucleus. × 49,000.

flagellate sperm of other mayflies (Baccetti *et al.*, 1969; Phillips, 1969, 1974; Fink and Yasui, 1988; Gaino and Mazzini, 1990a), is another character of the most primitive insect groups. Sperm length and structure show ultrastructural characteristics peculiar to each species examined. As a result, sperm may be useful for recognizing different species and for outlining their relationships. The aflagellate sperm of *H. lauta*, described by Grimm (1985), lacked typical cytoplasmic organelle. Such atypical conditions could be due to this author's examining few sections. Nevertheless, the genus *Habrophlebia* does show a simplified sperm model, compared with that of other leptophlebiids.

The loss of the flagellum or of dynein arms from the *A* axoneme subfibers may cause the sperm to be immotile. Immotile sperm have been observed in different species of Protura (Baccetti *et al.*, 1973b; Dallai *et al.*, 1990), in termites (Baccetti *et al.*, 1974), in psychodids (Baccetti *et al.*, 1973a) and in several cecidomyiids, in which immotility is due to the absence of dynein arms in the microtubular doublets (Dallai, 1979; Dallai and Mazzini, 1989). Progressive axoneme degeneration takes place during spermatogenesis in Homoptera, an evolutionary trend leading to an aflagellate and non-motile sperm in Coccoidea (Baccetti and Dallai, 1977). Aflagellate sperm were recently found also in Coleoptera (Baccetti and De Coninck, 1989).

As remarked by Afzelius (1979), sperm organization affects reproduction biology, and it is reasonable that in species characterized by immotile sperm, fertilization should differ considerably from the classical sperm model. The absence of spermathecae in the 3

species examined and the immotility of sperm call for direct gamete interactions. Ultrastructural observations of the female reproductive system architecture of the leptophlebiid *H. eldae* showed the presence of a wide muscle sheath enveloping the ovipositor (Gaino and Mazzini, 1990b), a structure which is believed to facilitate the movement of sperm. Furthermore, in the psychodid family immotile sperm are deposited in the female genital ducts. Contractions of the wall of the ducts allow them to reach the eggs (Burrini and Dallai, 1975). Peristaltic action may contribute to egg/sperm interaction even with motile sperm.

A peculiar sperm pump has been described in leptophlebiids (Grimm, 1985). It consists of a muscle ring surrounding the caudal part of the vasa deferentia. Sperm are transferred through peristaltic muscle contraction which allows fertilization. Investigation of the morphology of the genitalia is important for understanding their function, as recently stressed by McCafferty and Bloodgood (1989) for the coupling system in *Tortopus* mayflies.

In conclusion, sperm morphology reflects not only phylogenetic relationships, but also fulfills functional necessities related to fertilization biology. In leptophlebiids, sperm may be propelled to reach eggs by muscle sheaths around the genital ducts, a feature that compensates for sperm immotility. This finding represents a peculiar mating strategy in mayflies.

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