Sperm ultrastructure of the honey bee (Apis mellifera) (L) (Hymenoptera, Apidae) with emphasis on the nucleus-flagellum transition region

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Abstract
The flagellum of Apis mellifera (Hymenoptera, Apidae) consists of two mitochondrial derivatives, an axoneme and two accessory bodies. The mitochondrial derivatives are of unequal size and lie parallel to the axoneme. In the larger derivative four regions can be distinguished while in the smaller, only three. The region occurring only in the larger derivative consists of paracystalline material. The smaller mitochondrial derivative terminates anterior to the larger one. An extremely long centriolar adjunct is observed between the nucleus and the smaller mitochondrial derivative. This adjunct is compact, very electron dense and gradually tapers from base toward apex, finishing at the anterior extremity of the axonemal microtubules. In this flagellar region, there is only one accessory body present between the larger mitochondrial derivative and the axoneme. Anteriorly, the tips of the axonemal microtubules are inserted in a well developed mass of granular appearance. This material surrounds the nuclear base, separating it from the anterior end of the larger mitochondrial derivative. We believe that the structure identified here as a centriolar adjunct is homologous to that observed in Formicidae, Ichneumonoidea and Symphyta. Therefore, very probably, it is common to most Hymenoptera.

Keywords: sperm, ultrastructure, centriolar adjunct, honey bee

Introduction

The ultrastructure of the spermatozoa has been extensively used in taxonomic and phylogenetic studies of various animal groups, including the insects (Baccetti, 1972; Dallai, 1979; Dallai & Afzelius, 1990; 1995; Carcupino, et al., 1995; Jamieson et al., 1999). In Hymenoptera, the structural diversity of the spermatozoa seems to be sufficient to furnish character sets, which could be used in phylogenetic studies (Quicke et al., 1992). In the hymenopteran sperm, the nucleus-flagellum transition is a complex region and there are still many uncertainties in relation to its structural organization. Recently the possibility has been raised that this region might provide new phylogenetic indicators (Newman & Quicke, 1999a). However, to apply these indicators to phylogenetic studies, the structures must be positively identified so that the homology can be correctly established.

In the hymenopterans, the honey been is, surely, the species in which the spermatozoa have best been studied.

They were briefly described by Rothschild (1955), Hoage and Kessel (1968) studied their spermiogenesis and CruzHöfling et al. (1970) and Lensky et al. (1979) examined in detail

the mature sperm ultrastructure. Woyke (1984) compared the ultrastructural differences between haploid and diploid sperm. Peng et al. (1992; 1993) studied the ultrastructure of the sperm, specially the acrosomal complex, submitted to high-pressure freezing fixation, and the integrity of these cells after rapid freezing and thawing. Also, an excellent revision of this subject can be found in Jamieson et al. (1999). The honey bee sperm, as in most insects (Phillips, 1970), are quite long and filamentous and about 250–270 µm long. The acrosomal complex is formed by a conical acrosomal vesicle and internally, the perforatorium which extends from a deep fossa in the anterior nuclear tip. The total length of the acrosomal complex is 5 µm (Lensky et al., 1979). The nucleus is homogeneous and strongly electron dense, measuring 5 µm in length. Its posteriorly tapering nuclear cone is eccentric, where the anterior extremities of the axoneme and the two mitochondrial derivatives are attached. The tail is formed by an axoneme, two mitochondrial derivatives and two accessory bodies. The axoneme, as is the rule for insects, has the typical 9 + 9 + 2 arrangement of microtubules. The mitochondrial derivatives are of unequal diameter and length and lie parallel to the axoneme. Their matrix is composed of amorphous and paracrystalline materials (Cruz-Höfling et al., 1970; Lensky et al., 1979; Peng et al., 1992; 1993). Two accessory bodies (deltoid structures in Cruz-Höfling et al., [1970] or triangular rods in Lensky et al., [1979]) are situated between the axoneme and each mitochondrial derivative. The present study provides some additional information about the ultrastructure of mature spermatozoa of Apis mellifera, specially of the nuclear-flagellar transitional region.

Materials and Methods

The adult honey bee drones used in this study were obtained from colonies maintained in the Apiary of the Federal University of Viçosa, MG, Brazil.

Seminal vesicles were dissected and treated as follows: a. fixed in a mixture of 2.5% glutaraldehyde, 1% tannic acid, 1.8% sucrose in 0.1 M phosphate buffer followed by block staining in 1% uranyl acetate in distilled water (Afzelius, 1988). The specimens were dehydrated in acetone. b. For the detection of basic proteins, seminal vesicles were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, dehydrated in alcohol and treated ‘en bloc’ with a solution of 2% phosphotungstic acid in absolute ethanol (E-PTA). The dehydrated samples were embedded in Epon 812 resin and the ultrathin sections, stained with uranyl acetate and lead citrate a. or unstained b., observed by transmission electron microscopy (Zeiss, Leo 906), operating at 40 or 80 kV.

To help compare transverse sections of flagella, the micrographs were always reproduced with the dynein arms oriented counterclockwise.

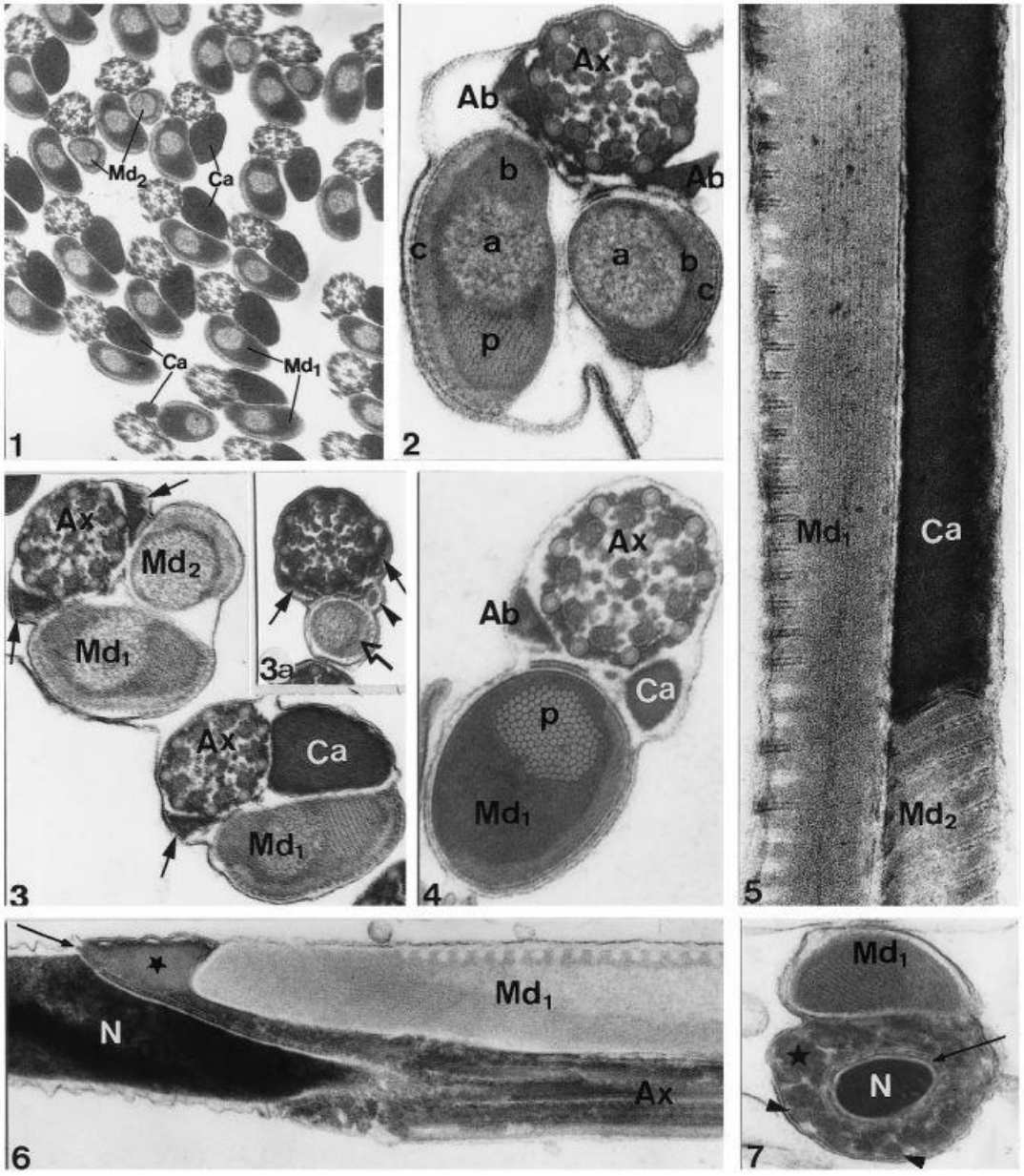
Results

The flagellum of honey bee spermatozoa consists of two mitochondrial derivatives, an axoneme, and two triangularshaped accessory bodies (Figs. 1–4, 10 D). In cross sections, the larger derivative has an oval form and the smaller is more or less circular (Figs. 2–3, 10 D). In the former, four different regions can be distinguished while only three are found in the smaller one (a, b, c and p in Figs. 2, 8). The region designated a is circular, central, unstructured and less electron dense. It occupies almost all the minor mitochondrial derivative. The p region is only present in the big mitochondrial derivative and is formed by paracrystalline material. The b region is unstructured and quite electron dense. This region is well developed in the larger mitochondrial derivative and reduced in the smaller. The c region is semi-circular, located on opposing faces of the mitochondrial derivatives and contains the mitochondrial cristae. When treated with E–PTA, only the b region is E–PTA–positive (Fig. 8). When the flagellum is sectioned next to the nucleus, the larger mitochondrial derivative becomes more or less circular, the b region making up almost the totality with the a region disappearing (Fig. 4. 10 B). Observing Fig. 3A, it is possible to deduce that, of the two mitochondrial derivatives, it is the smaller that terminates anterior to the larger one.

In most flagella, the smaller mitochondrial derivative (Md2) is 'replaced' by a more or less triangular structure, which is uniformly compact and very electron dense (Ca in Figs. 1, 3, 10 B–C). In a favourable longitudinal section, this structure can be observed to be long, and to have a truncated and concave posterior extremity, in which the tip of the smaller mitochondrial derivative fits (Fig. 5). We believe that it corresponds to the centriolar adjunct. In negative staining preparations (not shown) we observed that this centriolar adjunct is very long, about 30 µm. It gradually tapers anteriorly from base toward apex (Figs. 1, 4, 10B), and finishes at the level of the anterior extremity of the axonemal microtubules. When treated with E–PTA it appears electron dense (Figs. 8–9). This centriolar adjunct can yet be differentiated from the minor mitochondrial derivative since it does not have membranes, lies very close to the axoneme and, principally, because of the absence of the accessory body between it and the axoneme (Fig. 3, 10B–C).

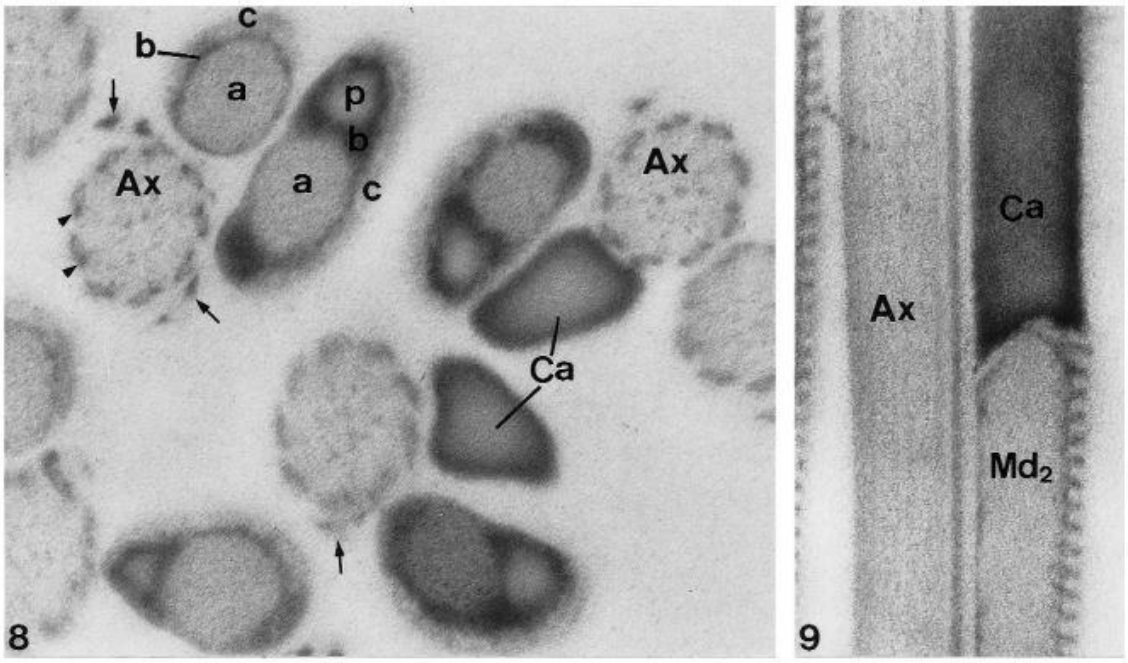
The axoneme follows the typical pattern of 9 + 9 + 2 arrangement of microtubules; including 9 outer single accessory tubules, 9 doublets and 2 single central microtubules (Figs. 2–4, 10B–D). The presence of electron dense material is also observed in the axoneme

between the accessory microtubules (Figs. 2–4). It is E–PTA–positive (arrowhead in Fig. 8). Anteriorly, the tips of the axonernal microtubules are inserted in a material with a granular appearance (Fig. 7, 10A). This material is abundant and surrounds the posterior nuclear projection (Figs. 6–7, 10A). The anterior extremity of the large mitochondrial derivative, which lies beside this nuclear projection, is separated from the latter by the same material (stars in Figs. 6, 7, 10A). This material also forms a triangular expansion, which fills the space between the large mitochondrial derivative tip and the baso-lateral region of the nucleus (star in Fig. 6). Finally, between the basal nuclear projection and this material there exists another lamellar structure (arrows in Figs. 6, 7, 10A).



Figs. 1–7 1. Various flagella cross sectioned anteriorly. Observe the presence of flagella sectioned through the centriolar adjunct (Ca) and more posteriorly, at the level of the smaller mitochondrial derivative (Md2). × 43,000. 2. A flagellum cross sectioned at the level of two mitochondrial derivatives

shows the central region, unstructured and less electron dense a., the paracrystalline region p, a unstructured, electron dense one b and the region of the cristae c. × 135,000. 3. A flagellum sectioned through the two mitochondrial derivatives (left upper corner) and another through of the centriolar adjunct. Arrows indicate the accessory body. × 83,000. 3a. Cross section of the flagellar extremity showing the larger and smaller mitochondrial derivatives (⇒and →, respectively) and the accessory bodies. (→). × 65,000. 4. A flagellum cross sectioned anteriorly. Observe the reduced diameter of the centriolar adjunct. × 142,000. 5. Longitudinal section of a flagellum at the transition of the centriolar adjunct (Ca) and smaller mitochondrial derivative (Md2). × 130,000. 6.–7. Longitudinal and transverse sections, respectively, of the nucleus-flagellum transition showing the posterior nuclear extremity (N) surrounded by a lamellar structure (→) and the material of granular appearance (stars), where the tips of the axonemal microtubules ( ) are inserted. 6: × 83,000; 7: × 110,000. Ab, accessory bodies; Ax, axoneme; Md1 , larger mitochondrial derivative.



Figs. 8–9 Transverse and longitudinal sections, respectively, of flagella treated with E–PTA. The arrows indicate the accessory bodies and the , the material between the accessory microtubules. Observe in the mitochondrial derivatives that the b region is E-PTA-positive, while a, c and p are not. Ca, centriolar adjunct; Ax, axoneme; Md2 , smaller mitochondrial derivative. 8: × 117,000; 9: × 95,000.

In cross section, two accessory bodies are observed between axoneme and mitochondrial derivatives (Figs. 2–4, 10D). These structures, are electron dense using routine staining methods (Figs. 2–3) and have E–PTA–positive regions (Fig. 8). As mentioned above, an accessory body does not exist between the axoneme and the centriolar adjunct; in this region, only one accessory body is located between the axoneme and the larger mitochondrial derivative (Figs. 3–4, 8, 10B–C).

Discussion

Although the centriolar adjunct has already been described for many insects (Cantacuzene, 1970), in hymenopterans only recently a structure positioned between the nucleus and one or both mitochondrial derivatives has been identified as a centriolar adjunct

in mature sperm of the ant (Wheeler et al., 1990), braconids (Newman & Quicke, 1998), Symphyta (Newman & Quicke, 1999a) and cynipoids (Newman & Quicke, 1999b). No structure homologous to those of other hymenopterans was identified in bees. In Apis mellifera, Lensky et al. (1979) identified an electron dense 'triangular body' located between the anterior tip of the larger mitochondrial derivative and the nucleus, opposite to the axoneme. This triangular structure was tentatively considered to be a centriolar adjunct (Jamieson, 1987; Jamieson et al., 1999). However, when observed in an appropriate section it is, in fact, an antero-lateral expansion of the material that surrounds the tapered nuclear base (see Fig. 6, asterisk). Therefore, it is not homologous to those structures that have been called centriolar adjuncts in other hymenopterans (Wheeler et al., 1990; Quicke, 1997; Newman & Quicke, 1998; 1999a; 1999b).

In spite of not having been previously identified, the drone spermatozoa also have an electron dense structure anteriorly located in relation to the smaller mitochondrial derivative, which we believe to be homologous to the centriolar adjunct observed in other hymenopterans (q.v.). However, in Apis mellifera, differing from those hymenopterans, this structure is extraordinary long and anteriorly tapered (see Fig. 4). This shape and position probably made the structure difficult to observe or it was misinterpreted (Hoage & Kessel 1968). Also in other hymenopterans, this structure has been initially interpreted as being the anterior tip of one of the mitochondrial derivatives (Quicke et al., 1992) or a region where the axoneme, nucleus and mitochondrial derivatives overlap (Chauvin et al. 1988). In the majority of those species, if not in all, only one accessory body was observed near the nuclear base, associated to one of the mitochondrial derivatives (Quicke et al. 1992); the other structure was interpreted also as a mitochondrial derivative, probably corresponding to the centriolar adjunct. In those chalcidoids, where sperm ultrastructure already has been observed in detail, the centriolar adjunct covers the nuclear base, separating it from the axoneme and mitochondrial derivatives. This adjunct also presents an anterior projection that overlies the basal nuclear region (Lino Neto et al. 1999; 2000). However, the centriolar adjunct in these wasps differs morphologically from most hymenopterans (Wheeler et al., 1990; Newman & Quicke, 1998; 1999a; 1999b), including Apis. Further, this structure in honey bee presents a predominance of basic proteins (E–PTA–positive), even though it differs from chalcidoids in which it is E–PTA–negative (Lino Neto et al. 1999; 2000). Based on the various hymenopteran species, from Symphyta to the bees, where a centriolar adjunct has been described, we believe that it is now possible to admit that this is a structure common to most or all species of this order. Moreover, it occurs with strong variations in shape (ex., chalcidoids) and dimensions (ex., Apis), and may be a good phylogenetic indicator.

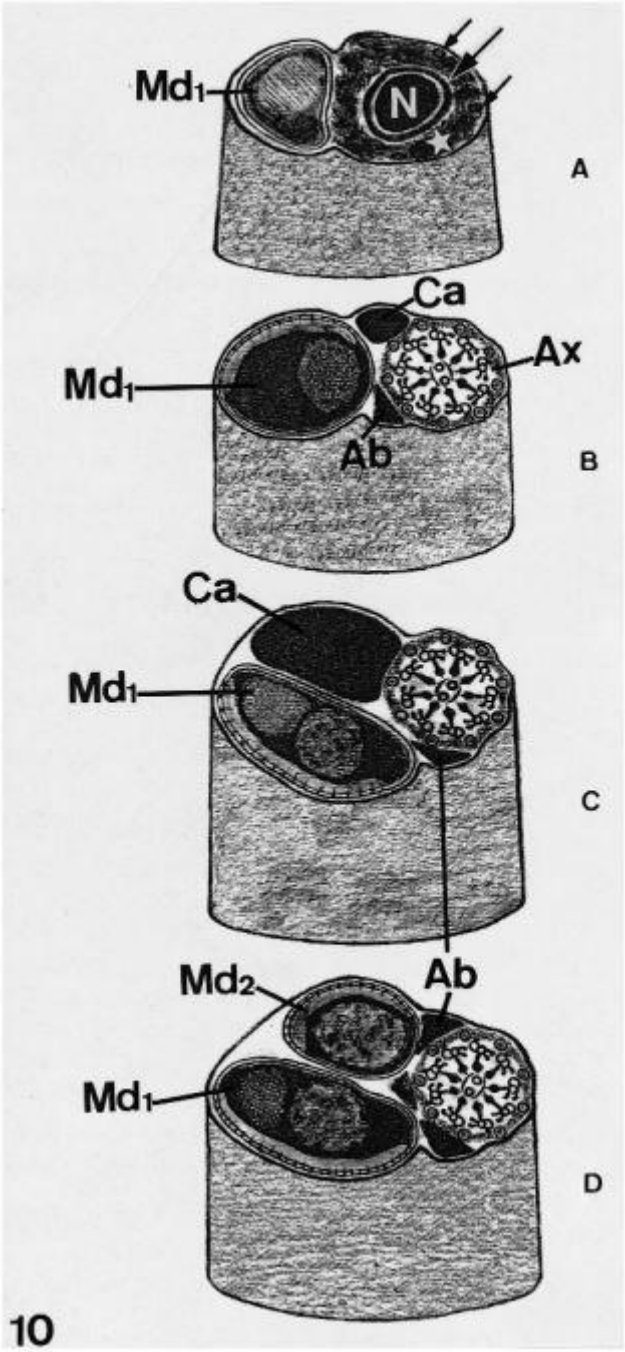


Fig. 10 Schematic diagram of the nucleus-flagellum transition region. The smaller arrows indicate microtubules; and the larger → indicates the lamellar structure. The star, appears showing the material of granular appearance. N, posterior nuclear extremity; Md1 , larger mitochondrial derivative; Ca, centriolar adjunct; Ax, axoneme; Ab, accessory bodies; Md2 , smaller mitochondrial derivative. × 75,000.

In Apis mellifera, the mitochondrial derivatives, which have already been described (Cruz-Höfling et al., 1970; Lensky et al., 1979; Peng et al., 1992; 1993), are unequal in diameter and length and lie parallel to the axoneme. However, only the larger mitochondrial derivative has a paracrystalline region, differing from previous descriptions.

Newman and Quicke (1998; 1999a; 1999b) proposed that the presence of a centriolar adjunct between nucleus and one of the mitochondrial derivatives could explain why, in the

final flagellar region, one mitochondrial derivative terminates before the other. However, observing the position of the mitochondrial derivatives in relation to the orientation of the axoneme's dynein arms in figures 1–4, it becomes evident that the smaller derivative terminates first, which is exactly the one located abutting the centriolar adjunct. In the eulophid, Trichospilus diatraeae, and in other chalcidoids (Lino Neto et al., 1999, 2000), the two derivatives always begin together a small distance from the nucleus and in contact with the basal region of the centriolar adjunct. Although, in these species a difference in length is always observed for the mitochondrial derivatives, this difference is unusually large in the case of T. diatraeae (Lino Neto et al., in preparation). Therefore, contrary to the proposition of Newman and Quicke (1998), we believe that the different lengths of the mitochondrial derivatives observed in the final flagellar region, are not directly related to the centriolar adjunct's position and, therefore, this should be another hymenopteran sperm characteristic to be considered in phylogenetic analyses.

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