ULTRASTRUCTURE OF THE FLAGELLUM IN SPERM OF *COCCINELLA SEPTEMPUNCTATA*

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Abstract Using cell whole mount preparation and ultrathin section technique, the ultrastructure of the flagellum in the sperm of *Coccinella septempunctata* L. was examined with transmission electron microscope. The flagellum is made up of a classic 9+9+2 axoneme containing two similar crystallized mitochondrial derivatives, two accessory bodies, which are divided in to two portions, an osmiophilic dense crescent and a spongy one, and a non-crystalline body. At the end of the flagellum, only the axoneme is present, it loses the two central microtubules but retains the nine doublets with dynein arms and the nine accessory microtubules.

Key words sperm, flagellum, axoneme, *Coccinella septempunctata*

1 INTRODUCTION

Coccinellidae includes about 5 000 species. Some Coccinellids are of great economic importance, and useful for biological control (Hodek 1973). So far very few of these forms have been subjected to ultrastructural observation on spermatogenesis and spermatozoon morphology. In *Coccinella septempunctata* L., which is an important predator of aphids, there is little information on spermatogenesis and sperm ultrastructure although Nath *et al.* (1951) had observed the sperm formation by light microscopy. The present study focuses on the ultrastructure of the sperm flagellum of *C. septempunctata* using cell whole mount preparation with positive and negative staining and thin-section.

2 MATERIALS AND METHODS

Lady beetles, *Coccinella septempunctata* L., were collected from wheat field in the suburb of Beijing and reared with aphids in the laboratory. The mature sperms were taken from the spermatheca of female adults. They were placed on the blind end of a thin glass rod and slightly ground with another glass rod and touched onto the surface of redistilled water in a trough (14 cm × 4. 5 cm × 1. 0 cm) (Wang 1994). Sperms being spreaded out were picked up with grids coated with formvar and carbon fily. They were stained in 2% uranyl acetate solution, rinsed in distilled water,

passed through a series of graded ethanol and finally infused twice in amyl acetate for 10 min. Then samples were allowed to dry in air at room temperature. Also germ cells spreaded by cell whole mount technique were stained negatively with 2% PTA in 0.03 sucrose phosphate buffer, pH 7.4.

For thin-section, mature sperms from the seminal vesicle of the male and from the spermatheca of the female were fixed in 4% glutaraldehyde solution in phosphate buffer (pH 7.4) at 4℃ for 2 h, and then rinsed in the same buffer and post-fixed in 1% OsO_4 solution for 1 h. Finally they were dehydrated in ethanol and embeded in Epon 812. Ultra-thin sections obtained by LKB ultramicrotome were stained with 2% aqueous uranyl acetate and lead citrate. Samples were examined by Hitachi H-300 electron microscope.

3 RESULTS AND DISCUSSION

As in other Coleoptera (Baccetti and Daccordi 1988, Burrini *et al.* 1988, Bawa and Kanwar 1975, Bao 1991, Dallai and Afzelius 1987), the flagellum of sperm in *C. septempunctata* is asymmetrical. It is made up of a conventional axoneme, 2 crystallized mitochondrial derivatives, 2 accessory bodies and a non-crystalline body (Figs. 1 and 2 transverse section). The two accessory bodies are lateral to the axoneme, and each has two portions, an osmiophilic dense crescent and a spongy one. The two mitochondrial derivatives flanking the axoneme and next to the spongy portion of the accessory body are almost in same size (cross-section about 120 nm in diameter) and have cristae arranged parallel in double helix (Fig. 1)

The axoneme belongs to the classic 9+9+2 pattern. It includes an outer layer of 9 accessory microtubules full of dense osmiophilic material, 9 doublets with evident dynein arms and radial spokes on their A-microtubules and 2 central microtubules within the central sheath (Fig. 2 transverse section). An accessory microtubule, which is thicker than the central singlet and the A microtubule (16 nm), has an diameter of 22 nm, and its wall made up of protofilaments is about 7 nm in thickness (Fig. 2).

Figure 3 shows a row of outer arms which connect the A-microtubule to the B-microtubule of the adjacent doublet. A lateral view of part of the outer arms arrangement with 16-32-40 nm periodicity is depicted in Fig. 4, in the left a doublet is linked to the B-microtubule of the adjacent one by the large arm heads, and in the right the heads (possible rich in ATP) are detached from the B-microtubule. Each outer arm is composed of a large globular head (~12 nm in diameter), an associated stem (~16 nm in length) and a small foot.

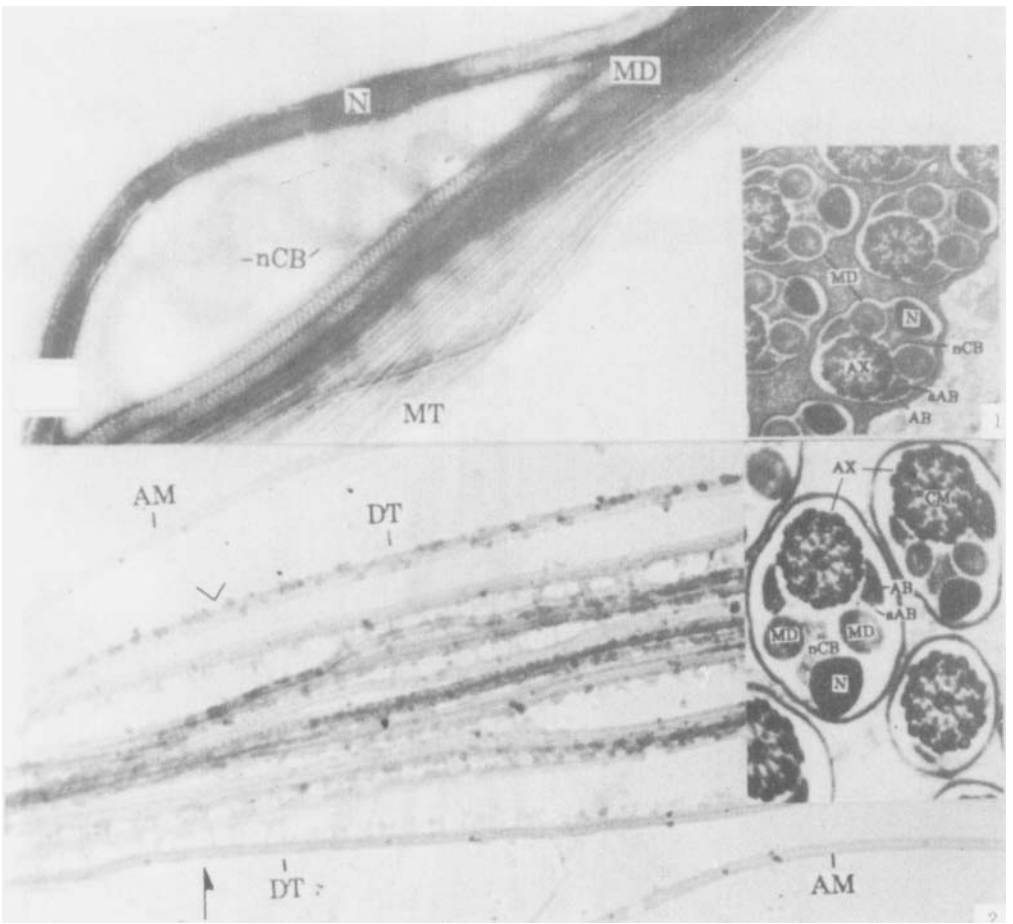

Fig. 1 The sperm flagellum; the micrograph with negative staining shows two mitochondrial derivatives (MD) with same size which have cristae arranged parallel in double helix, numerous microtubules (MT) of the axoneme, a non-crystalline body (nCB) and nucleus (N). ×21 000.

Transverse section in inset and Fig. 2 in inset show that the flagellum consists of a classical 9+9+2 axoneme (AX), two accessory bodies (AB), two spongy portions of the accessory bodies (sAB), two mitochondrial derivatives (MD), a non-crystalline body (nCB). N: nucleus. ×40 000.

Fig. 2 The sperm axoneme: The portion of an axoneme prepared by spreading technique shows accessory microtubules (AM) and different views of doublets (DT). Arrow points the lateral view of a doublet facing the central microtubules. ×78 000.

Transverse section in inset shows that an axoneme comprises nine accessory microtubules (AM), nine doublets (DT) and two central microtubules (CM). ×50 000.

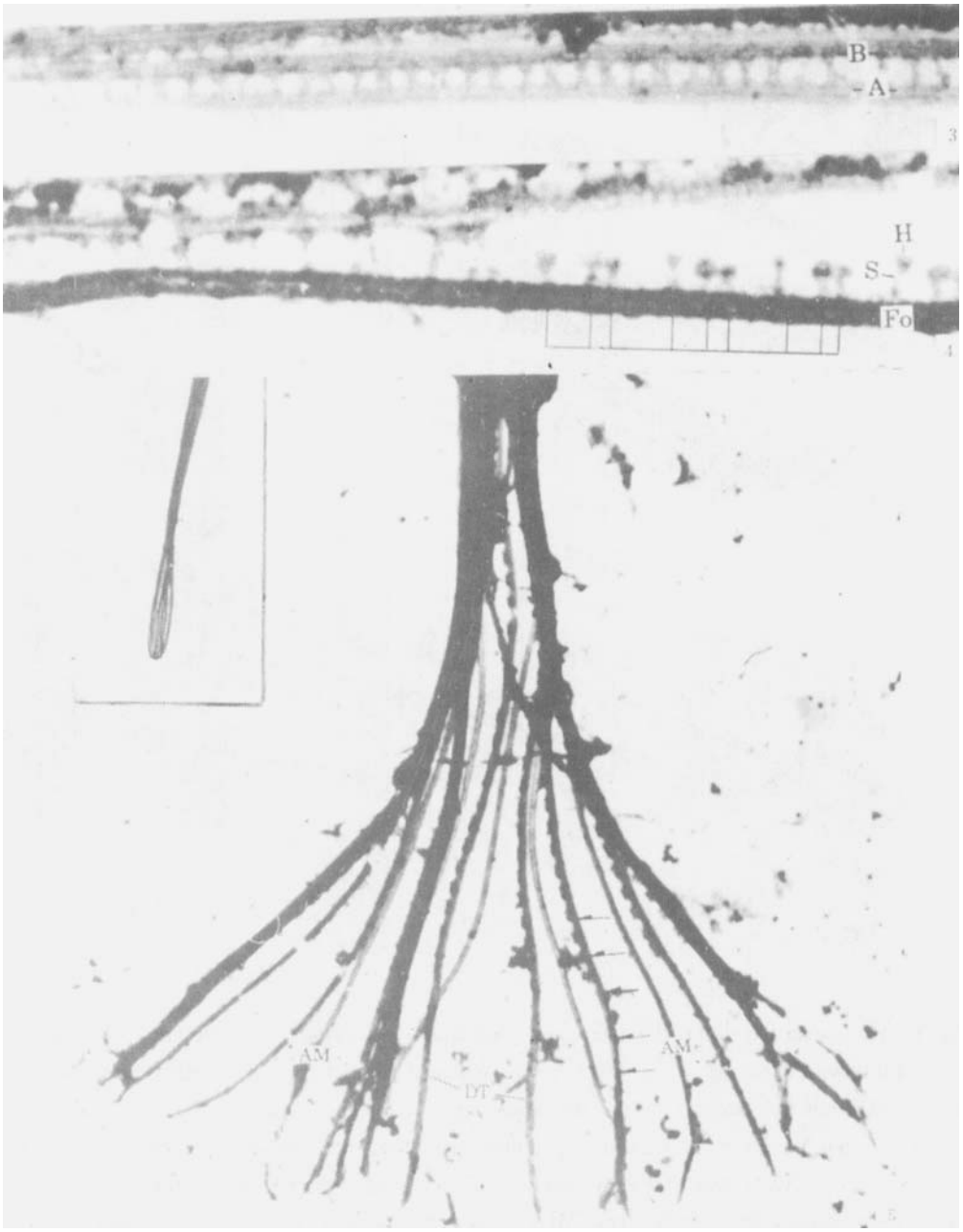

Fig. 3 A row of outer arms connect A-microtubule of a doublet to B-microtubule of the adjacent doublet. ×100 000. **Fig. 4** A lateral view of a portion of the outer arms with 16-32-40 nm periodicity H-head; S-stem; Fo-foot. ×182 000. **Fig. 5** The tail of a sperm flagellum looks like an oar (inset, ×16 000) having nine accessory microtubules (AM) and nine microtubular doublets (DT). Arrows point dynein arms on the doublet. ×42 000.

It is most interesting to finding that outer arms bind to the A-microtubule with a 16-32-40 nm periodicity rather than 24 nm period as in *Chlamydomonas*, *Tetrahymena*, *Strongelocentratus* and *Mnemiopsis* (Goodenough and Heuser 1985). Regarding morphology of outer arms, that of *C. septempunctata* flagellum is different from those of urchin sperm flagellum, *Chlamydomonas* flagellum and *Tetrahymena* cillum (Yano and Miki-Noumura 1981, Sale *et al.* 1985, Witman *et al.* 1983).

At the end of the tail, as an oar in appearance (Fig. 5, inset), the axoneme loses the two central microtubules and the nine doublets with dynein arms and nine accessory microtubules are retained (Fig. 5). The doublets are devoid of links and spokes.

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七星瓢虫精子鞭毛的超微结构

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应用细胞整装制备和超薄切片技术,在透射电子显微镜下检查了七星瓢虫成熟精子鞭毛的超微结构。精子鞭毛是由一个典型的9+9+2轴丝,两个同形结晶的线粒体衍生物,两个附体(每个附体具有两部分,一个嗜锇致密月牙体和一个海绵月牙体)和一个非结晶体组成,在鞭毛终端部,仅存的轴丝失去了两个中央微管保留了9个具有动力蛋白臂的双微管和9个附微管。

Errata : Volum 4, Number 4 Fig. 1 facing page 360. The numbers above B should be reversed, i. e. 1, 2, 3, 4, 5, 6, 7, 8 should be changed into 8, 7, 6, 5, 4, 3, 2, 1.