Recent Advances in Insect Embryology in Japan
Edited by H. Ando and K. Miya.
ISEBU Co. Ltd., Tsukuba 1985

Sperm Structure in the Praying Mantis, *Paratenodera aridifolia* (Mantodea, Mantidae)

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Synopsis

The sperm structure of the praying mantis, *Paratenodera aridifolia* (Mantodea, Mantidae) was studied with light- and electron microscopy. The matured spermatozoon is a long filiform in shape, 2.2 mm in length, and consists of the acrosome, nucleus, centriole adjunct, and flagellum composed of the axoneme having the 9+9+2 pattern of microtubule arrangement, mitochondrial derivative, and accessory body. In the seminal vesicle as well as in the seminal receptacle, 500 to 1200 sperms are bound to form a bundle with dense material. The sperm bundle from the female sperm receptacle moved actively, whereas those from the seminal vesicle moved only sluggishly.

Introduction

Many ultrastructural studies have been done on the spermatogenesis and the sperm maturation in various animals (Yasuzumi, 1974). Detailed review on insect sperm was given by Baccetti (1972) and Phillips (1970). The phylogeny and evolution of insects on the basis of sperm structures such as acrosome or flagellum have been discussed (Baccetti, 1979; Dallai, 1979; Dallai *et al.*, 1984; Friedlander, 1983; Grygier, 1982; Virkki, 1969).

Although more than five species of Mantidae are known from Japan, we have no information on the gametes of these mantis. Thus, the ultrastructure of the mature sperm of *Paratenodera aridifolia* was investigated to prove a basis for comparative studies on the mantis sperm.

Materials and Methods

Materials The matured males and females of the mantis, *Paratenodera aridifolia* Stoll (Mantodea, Mantidae) were collected in the vicinity of Nagoya Univeristy at September. Spermatozoa isolated from a seminal vesicle and seminal receptacle were used as materials.
Light microscopy 1. Phase-contrast microscopy. Spermatozoa were isolated in some drops of Hoyle's physiological saline and observed. 2. Fluorescence microscopy. Sperm cells were stained with 0.01% acridinorange solution to clarify a nucleus and an acrosome. After staining with the method described by Fujita and Fukuda (1975), cells were observed and photographed under Olympus BH-RFL microscope. The length of organelles was determined directly on a film.
Electron microscopy 1. Transmission electron microscope (TEM). Whole seminal vesicle and seminal receptacle dissected in balanced saline were fixed in 2% glutaraldehyde buffered to pH 7.2 with 0.2 M sodium cacodylate solution for 1 hr at room temperature. Then they were reimmersed in a fresh fixative for 2 hr. After washing for 20 hr in several changes of the buffer, specimens were post-fixed in 1% OsO_4 in the same buffer for 1 hr at 4°C. Dehydration was carried out in ethanol series and embedded in EPON. Thin sections were stained with uranyl acetate and lead citrate, and examined with a JEM T-8 electron microscope. 2. Scanning electron microscope (SEM). Specimens were fixed in 2% glutaraldehyde buffered in 0.2 M cacodylate. After rinsing, they were dehydrated through ethanol series, critical point dried in CO_2, and subsequently coated with gold and observed by JEM-T20 scanning electron microscope.

Results

The matured spermatozoon of *P. aridifolia* is filiform in shape, which consists of a needle-like head and a long movable flagellum. It measures approximately 2.2 mm in length (Fig. 1). The spermatozoa are deposited in the seminal vesicle and the seminal receptacle as the sperm bundles (Fig. 2). SEM suggests that the sperms are bounded with each other at the heads to be spatulate but the flagella are not bound (Fig. 3). As shown in Fig. 2, the sperm bundle taken out from the female spermatheca began a highly active helical movement immediately after they were left in a saline solution. They continued to move for more than 24 hours in the saline solution. However, the sperm bundles from the seminal vesicles were clustered firmly and they hardly moved even if they were separated mechanically into a single bundle. It was difficult to distinguish the acrosome from the nucleus under a phase-contrast microscope. After staining with acridinorange the acrosome was seen in red and the nucleus is green under a fluorescence microscope, and their length were determined to be 25 μm and 63 μm respectively. Sperm tails were dyed only very weakly (Fig. 4).

TEM observation reveals that the mantis spermatozoon is thread-like in form, which tapered toward both ends; it consists of the acrosome, elongated nucleus, centriole adjunct, and flagellum which is composed of the axoneme, mitochondrial derivatives and accessory bodies. The acrosome is cone-shaped, packed with electron-dense material, situated at the anterior part of the nucleus and elongated inferiorly along one side of the organelle. The striated bands with a periodicity of 8 nm are seen in the longitudinal section of the acrosome (Fig. 5). At the anterior part of the acrosome, the electron dense substances line to cause the

thickening of the inner cell membrane (Fig. 6). Dense granules are seen to surround the acrosomal region like a cap. Therefore the acrosomes are embedded in these materials when the sperm bundle was formed. The acrosome shows the moderate density and the internal space may be found in an area slightly close to the distal end of the head (Fig. 7).

The nucleus occupies major part of the head and contains the most intensive substance. The middle part of the nucleus is cylindrical and 360 nm in diameter. It extends taperingly toward anterior and posterior ends. At the anterior end, the nucleus is lying along the acrosome, while at the posterior end, the nucleus is surrounded by the centriole adjunct terminates between two mitochondrial derivatives (Fig. 8). At a distal level of the nucleus, rod body, measured 430 nm in length, may be observed, but its function is unknown.

The flagellum is comprised three components, namely, the axoneme possessing the microtubules in 9 + 9 + 2 arrangements, mitochondrial derivatives and accessory bodies (Fig. 9). Along the longitudinal axis of the spermatozoon these components may be observed in the following order: 1. nine doublets and peripheral singlets of axoneme which occur in an amorphous substance at the distal level of the nucleus, and then, two central tubules arise from the distal level of these structures (Fig. 8). 2. two mitochondrial derivatives containing crystals which arise from the level of distal end of the amorphous substance beside the axoneme, and 3. three accessory bodies shown as an aggregates of the dense granules found at the most distal ends of the centriole adjunct and the nucleus. Two mitochondrial derivatives and three accessory bodies disappear at the distal part of the tail and the axoneme alone remains, and then it loses the regular arrangement of microtubules at the extreme end of the tail (Fig. 9D).

In this species, as shown in Figs. 6 and 7, about 500 – 1200 spermatozoa are bound with one another to form a sperm bundle, which is often comet-shaped due to the long tails. The direction of running of the axoneme is the same in almost all flagella and the sperms located in the central part of the packet shift their position backward.

Discussion

The spermatozoon of *P. aridifolia* is a long filamentous cell and its full length is 2.2 mm. In general, it has been said that the length of sperm is 300 μm or more in many insects (Chapman, 1969). Although long sperms were found in *Thermobia domestica* (Bawa, 1964), and in *Pseudococcus obscurus* (Ross and Robison, 1969), the sperm of the mantis is extremely long as observed in *Drosophila* (Cooper, 1950). In this mantis the spermatozoa are deposited as a shape of a bundle in the seminal vesicle as well as in the seminal receptacle. The bundles isolated from the spermatheca moved very actively in a saline solution, but those from male did not move so actively. This suggests that the bundles may be activated with substances secreted from the female genital duct. This activated motion of the sperm seems to be very similar to the sperm activation, "capacitation", that has been demonstrated first in a mammalian sperm by Chang (1951) and Austin (1960). Shephered *et al.* (1982) also reported in tick (Acari, Ixodoidea) that the sperm maturation accompanied with the structural changes corresponded to the capacitation.

The sperm bundle within a testis has been reported in various insects such as *Chortophage viridifasciata* (Payne, 1933), *Locusta migratoria* (Szöllösi, 1975), *Leptocoris trivittatus* (Itaya *et al.*, 1980), *Pseudococcus obscurus* (Nur, 1962; Ross and Robison, 1969), *Parlatoria*

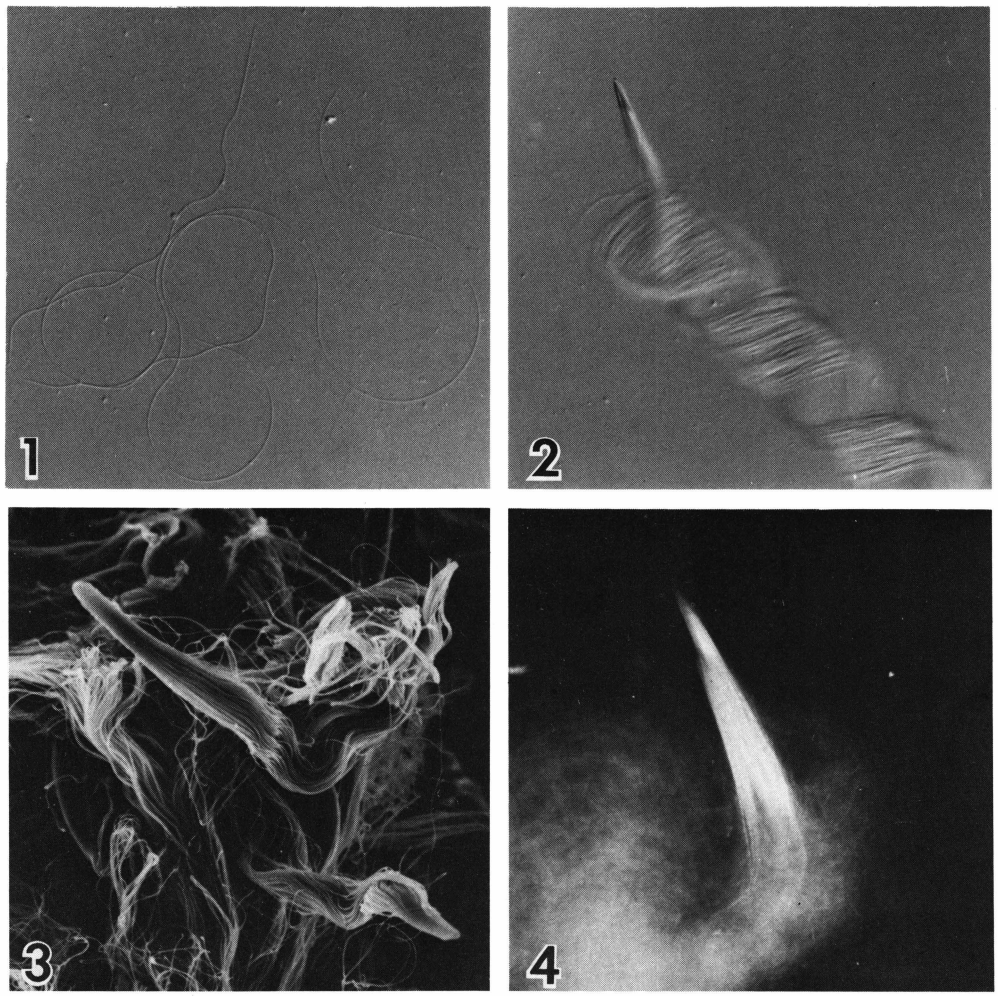

Fig. 1 Phase-contrast micrograph of living spermatozoon in seminal receptacle of *Paratenodera aridifolia.* Needle-like head seen upper middle. x 190.

Fig. 2 Phase-contrast micrograph of living sperm bundle dissociated from seminal receptacle. Helical movement of flagella may be seen clearly. x 190.

Fig. 3 SEM micrograph of sperm bundle in seminal vesicle. x 350.

Fig. 4 Fluorescence micrograph of sperm bundle stained with acridinorange. Most bright part corresponds to acrosome followed by nucleus and fuzzy tails. x 360.

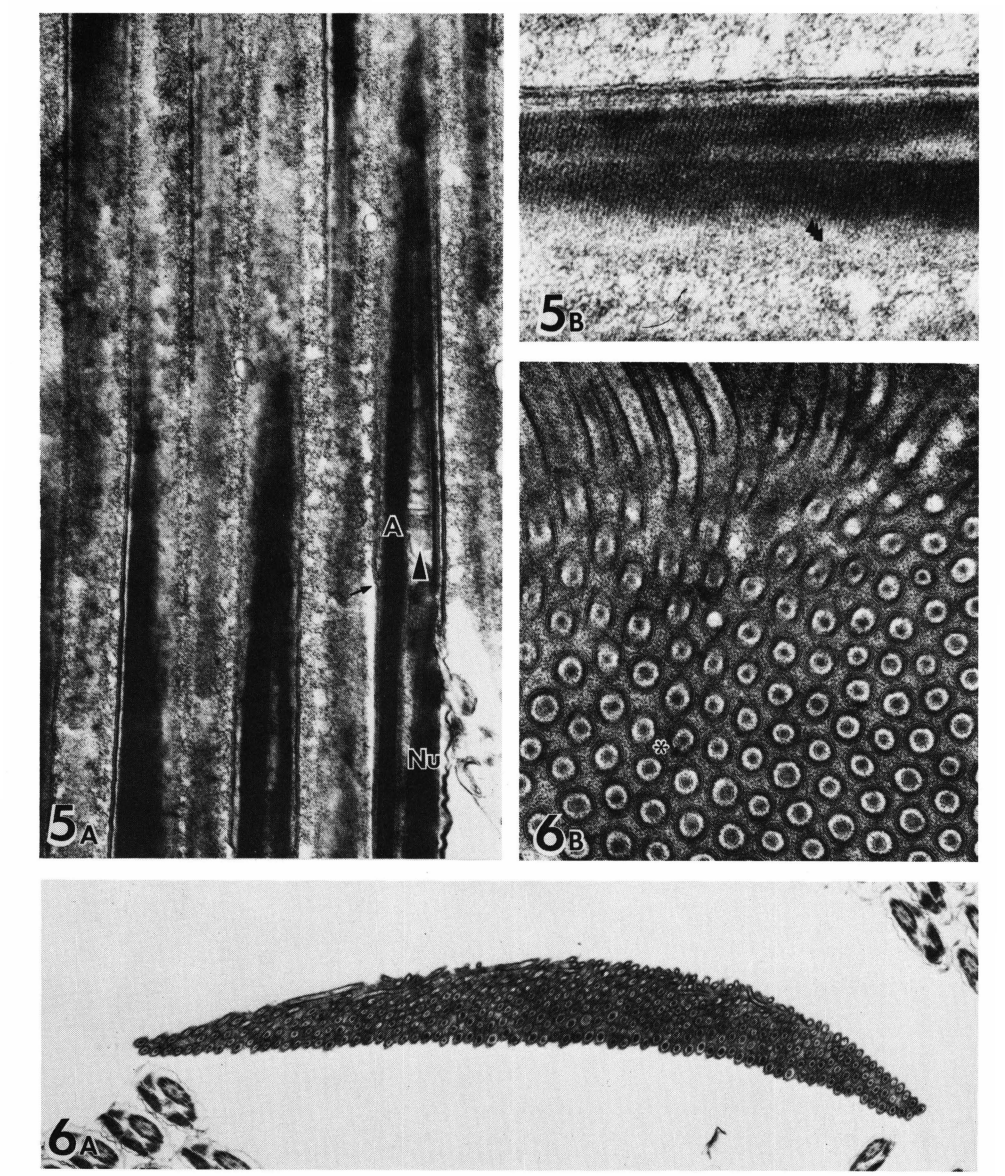

Fig. 5 A. Longitudinal section showing acrosome and nucleus. Cell membrane narrows slightly at the part of end of nucleus (small arrow) and bears no dense substances. × 35,000. B. Enlargement of a part showing striations (arrow heads). × 100,000. A acrosome (arrow head, acrosomal space), Nu nucleus.

Fig. 6 A. Cross section of sperm bundle showing acrosomal region. 500 cells are bounded with dense material. × 9,000. B. Cross section of sperm bundle at its anterior part showing the tip of acrosome and dense material (*) around cells. × 35,000.

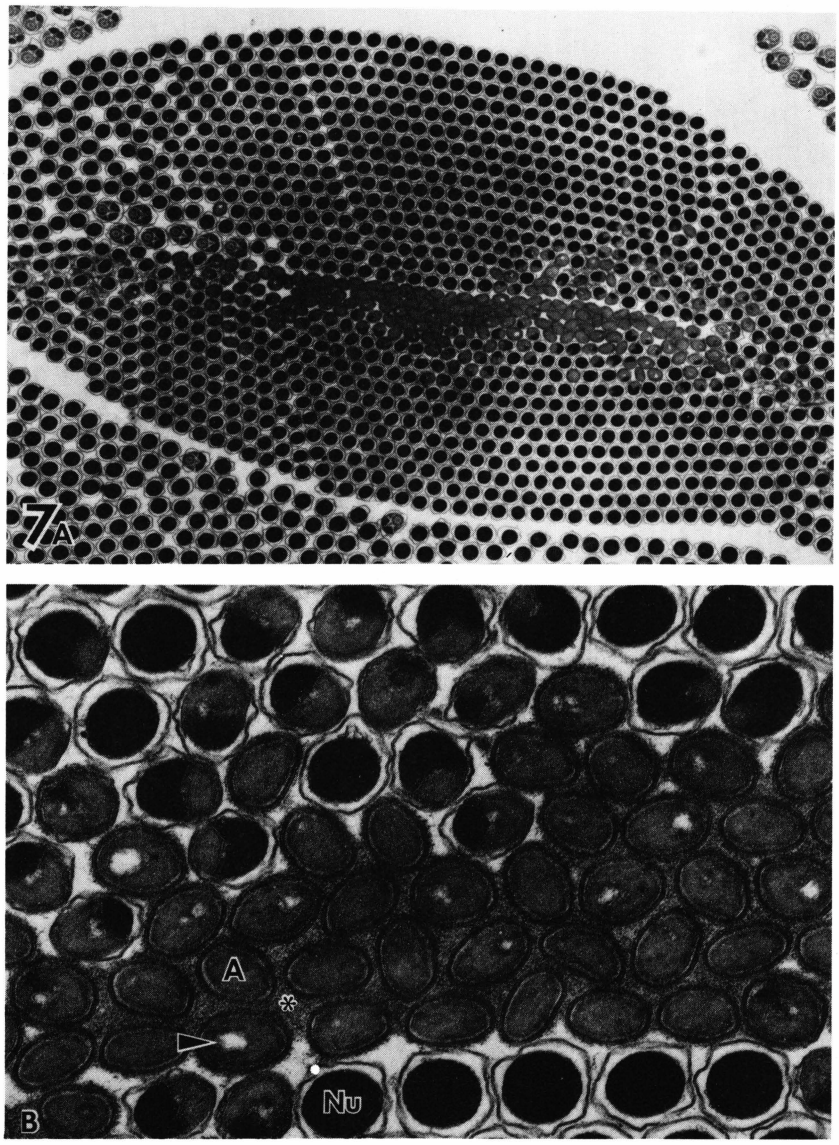

Fig. 7 A. Cross section of sperm bundle at its posterior part shown in Fig. 6. At the center of bundle various levels of sperms from acrosome to nucleus may be seen. Numerous nuclei are seen in periphery. In this bundle, sperm number in 1200. × 6,000. B. Cross sections of sperms distributing at the center of bundle. A acrosome (arrow head, acrosomal space), Nu nucleus, (*) dense material. × 35,000.

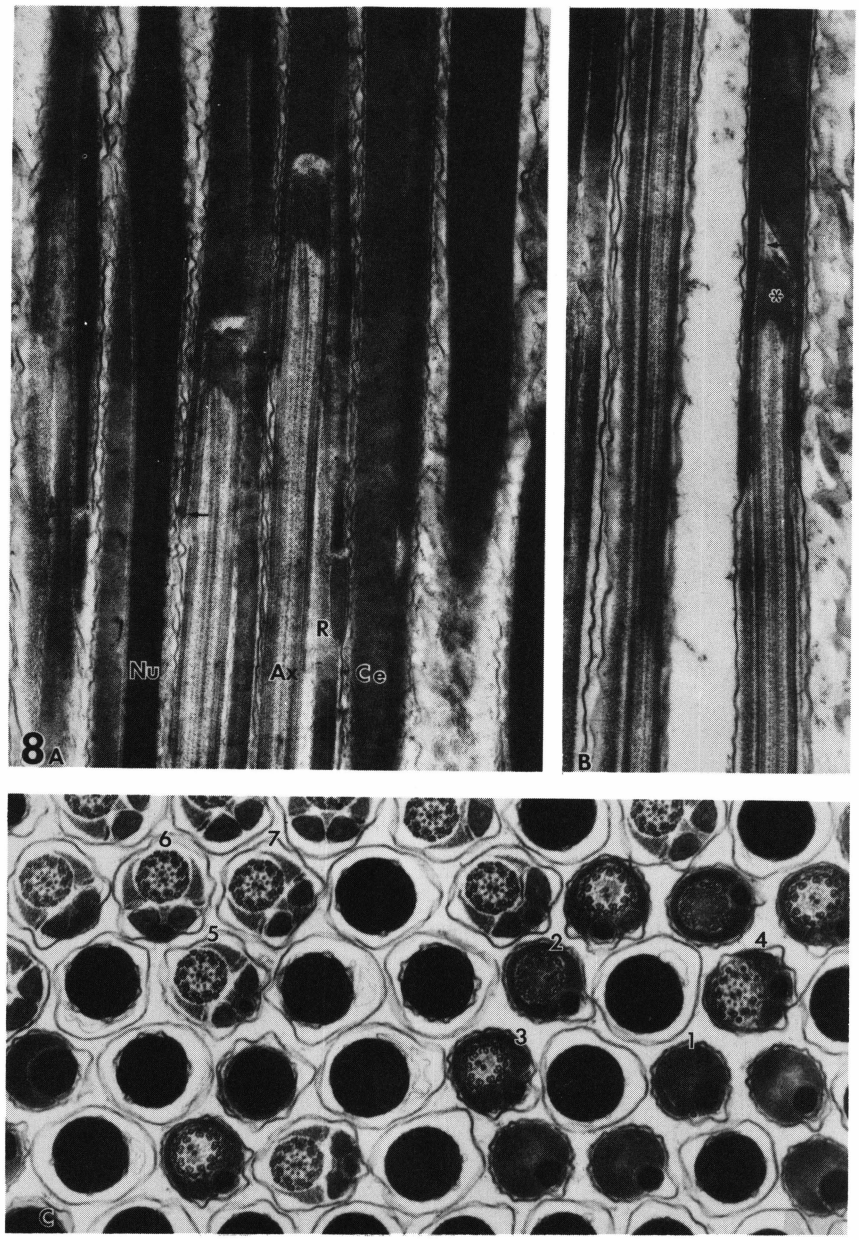

Fig. 8 A. Longitudinal section showing the distal part of head connected to tail with centriole adjunct. Arrow indicates the terminal of centriole adjunct. x 35,000. B. Longitudinal section showing sperm cut at another plane of cells illustrated in A. Peripheral singlet (arrow) expands through amorphous substance (*). x 35,000. C. Cross section showing various levels of head of the sperms. Numbers (1 → 7) indicate levels shifting in position anterior to posterior. x 35,000.
Ax axoneme, Ce centriole adjunct, Nu nucleus, R rod body.

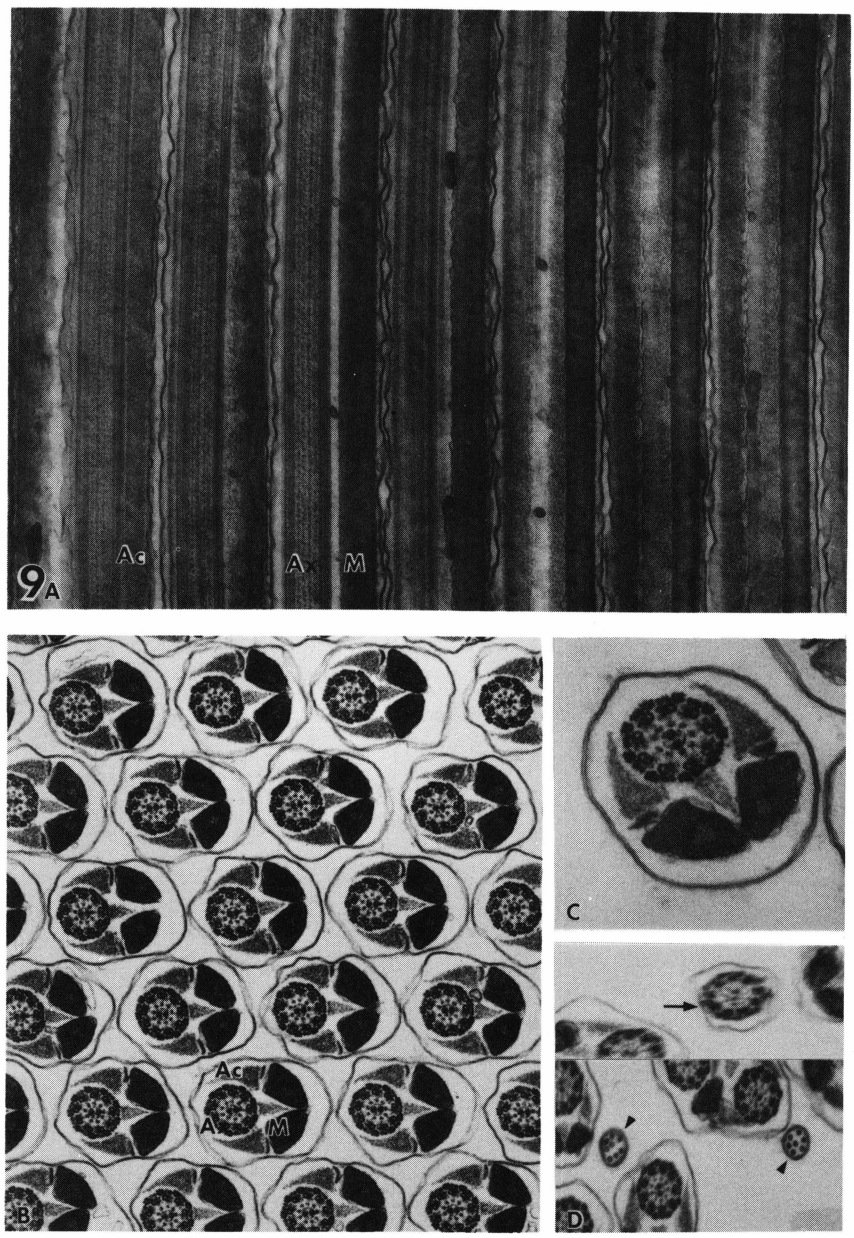

Fig. 9 A. Longitudinal section of middle part of flagella. × 35,000. B. Cross section of middle part of flagella. × 35,000. C. Cross section of flagellum showing 9 + 9 + 2 arrangement of microtubules. × 80,000. D. Cross sections at the level close to the distal end of tail (arrow and arrow heads). × 35,000. Ac accessory body, Ax axoneme, M mitochondrial derivatives.

oleae (Robison, 1966), *Bombyx mori* (Katsuno, 1978), *Papilio xuthus* (Numata and Hidaka, 1980), *Drosophila* (Liebrich *et al.*, 1982), *Popillia japonica* (Anderson, 1950), and *Apis cerana indica* (Bawa and Narwaha, 1975). Approximately 500 – 1200 spermatozoa make a bundle in *P. aridifolia*. However whether these abundant cells have been derived from a single spermatogonium or not may not be known definitely till the spermatogenesis is analyzed. In Odonata, Omura (1953) found that a single follicle of the testis contained 500 – 2000 sperms, which were formed after a single spermatogonium repeated cell division 11 times in each cyst. Robison (1966) has shown that the sperm bundle in the scale insect, *Parlatoria oleae*, containing 16 sperms in encysted form, was derived from a single spermatogonium. According to Bawa and Marwaha (1975), the sperm bundle of the honeybee, *Apis cerana indica*, is comprized on an average of 72 sperms. Each sperm bundle has a hyaline cap at its anterior region. Szöllösi (1982) has described on the formation of the sperm bundle in locusts, in which acrosomes of the spermatids become tightly linked by a cap of glycoprotein in the cyst during spermiogenesis. In the sperm bundles of *P. aridifolia*, the acrosome is coated with electron dense material and bounded with each other (Figs. 5, 6). It is very likely that this substance may contain some kind of protein, because the bundle may be released by a treatment with trypsin (Iwaikawa, unpublished). Bawa and Marwaha (1975) pointed out that, in the honeybee, the hyaline cap disappeared and the sperms were no longer seen in bundle as the sperms entered into the seminal vesicle. In this mantis, on the other hand, the sperm bundles were preserved not only in the seminal vesicle but also in the seminal receptacle immediately after oviposition. These results suggest that the cap substance may be different among the insects and male mantes may be able to transfer many sperms safely into the female genital duct in the form of the bundle.

It is well known that the ultrastructures of insect sperms are different from species to species (Baccetti, 1972; Phillips, 1970). TEM observation revealed that the matured sperms of *P. aridifolia* tapered toward both anterior and posterior ends. The head region of sperm of this insect consists of the acrosome, nucleus, and centriole adjunct. The acrosome is cone-shaped, within which an internal space may be found. The rod-like structure previously known from the sperms of the cockroach, *Phycnoscelus indicus* (Shahaney *et al.*, 1972) was not found in *P. aridifolia*. The posterior part extends laterally along the nucleus. This resembles with that of the sperm in the orthopteran, *Conocephalus saltator* (Cruz-Landim and Ferreira, 1977). It is clear that a periodic striation is seen in the acrosome sectioned longitudinally (Fig. 5). We assume that this repeated pattern is characteristic of this mantis acrosome. Electron-dense material is accumulated over the inner cell membrane at more anterior part of the acrosome. This dense layer may correspond to an extraacrosomal layer of triple-layered acrosomal complex exhibited by Baccetti (1972). The nucleus is a solid homogeneous and the most intensive densely packed organelle as observed in other insect sperms. The distal part of the nucleus is extensively surrounded by the centriole adjunct, as occurred in various other species. In *P. aridifolia* the centriole adjunct may connect the nucleus to the flagellum which has the axoneme arranged in 9 + 9 + 2 pattern of microtubules. In various animals including mammals, it has been shown that the centrioles of the sperms which have nine triplets exist near the nucleus (Yasuzumi, 1974). In the insects, however, Phillips (1970) stated that no centriole exists in the sperms of many species. We also failed to verify nine triplets beneath the nucleus in the matured sperms of *P. aridifolia*. In this species only a mass of an amorphous material, 300 nm in length, may be found, instead of the centriole and an origin of nine doublets and peripheral singlets of flagellar

axon. Two mitochondrial derivatives were seen to extend along the axon till the distal end of the tail. This extension resembles closely to that of the cockroach sperm (Shahaney *et al.*, 1972) except that three accessory bodies lie along the axoneme (see also Baccetti, 1972). Microtubules found in the axons of all sperms in the bundles are arranged in such a way that they are pointing the same direction (Fig. 9B). This might be correlated with the synchronous helical movement of the sperm flagella in the bundle.

In a preliminary study using another mantis, *Statilia maculata* Thunberg, a positive reaction for acridinorange staining was detected at the base of the flagellum, as occurred in the acrosome. This suggests that the ultrastructural variations of the sperms might be present in another species of mantes, which will be usefull to discuss the sperm structures in future.

Acknowledgments

We wish to thank Dr. M. Takaoka, Professor Emeritus, Nagoya University, for his critical reading and suggestions in preparing the manuscript.

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