Electron Microscopical Studies on Reproductive Cells of Plants

III. Structure of Spermatozoid of *Sargassum Horneri* Ag.*

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佐藤正一：電子顕微鏡による植物生殖細胞の研究 III. アカモクの精子の構造

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Sargassum Horneri Ag. is an alga belonging to the family Fucaceae in the phylum Phaeophyceae. On the spermatozoids of Fucaceae some previous observations have been reported. Kylin (1920) has reported upon the structure of the spermatozoid of *Fucus Areschougii* studied by means of the light microscope; it has two flagella and a body consisting of a nucleus, chromatophore and plastomere. Shortly after, Kunieda (1924) published a report upon the spermatozoid of *S. Horneri* under the light microscope, but, he did not observe the structure in detail. The fine structure of these spermatozoids cannot be observed under the light microscope. By means of the electron microscope, however, a more detailed structure can be seen. With respect to electron microscopical studies of the spermatozoid of Fucaceae, Manton and Clarke (1950) were the first to report about the spermatozoid of *Fucus serratus*. In this paper, the results obtained with reference to the fine structure of the spermatozoid of *S. Horneri*, by means of the electron microscope, will be described.

Materials and Methods

The spermatozoid samples of *S. Horneri* were obtained on the sea shore near the Misaki Marine Biological Station in April 1951 and 1952. The specimens were prepared as follows: sufficiently matured male receptacles were taken into a watch-glass half filled with sea water, and the Diatoms, Protozoa or debris attached to the surface of the male receptacle were carefully brushed off. The receptacle was then transferred to a new watch-glass, and a few drops of sea water were added. Numerous spermatozoids rush out energetically from the conceptacles of a ripened receptacle and swim actively in the medium. This feature is easily observed under the light microscope. Then, from this medium a microdrop is drawn up by a pipette, and placed on a slide glass and fixed with either 4 per cent formalin diluted in sea water, or the vapour of a 2 per cent solution of osmic acid, or Altmann's solution. After fixing, a microdrop of the spermatozoid suspension on the slide glass is then

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placed on the collodion membrane supported by a sheet mesh holder used for insertion into the electron microscope. These specimens are dried in the air, and washed by the distilled water, and dried in the air again.

Specimens treated with either the vapour of chloroform or ammonium vapour for a short time were also used. At the same time, a specimen after being fixed with 4 per cent formalin, centrifuged at 2500 r/min for 3 minutes, was also used.

A comparative study by means of the light microscope was also carried out. The fixing method for preparations was nearly the same as used for the electron microscope. These were stained in five ways; the aceto-carmin, Heidenhain's iron-haematoxylin, gentian-violet, carbol-fuchsin and Feulgen's reaction.

The HU-4 type electron microscope of the Hitachi Company, installed in the University of Tôkyô, was used. The electron micrographs in this paper were taken by Mr. S. Sakata.

Results

The spermatozoid of *S. Horneri* consists of an egg-apple like body, two flagella attached at the sides and a blepharoplast (Figs. 1 and 2). The anterior portion of the body is somewhat narrow and the posterior roundish. The length of the body is 2.7 to 4.8 μ , and the width is 1.6 to 2.4 μ at its middle region. The spermatozoid has two flagella. The front flagellum is ca. 11 μ in length, and the hind one is ca. 15 μ , a little longer than the front.

The body of the spermatozoid is too thick for the electron beam penetrance in the light field of the electron microscope. The image however obtained in the dark field of the electron microscope becomes somewhat transparent and more details are obtained. The degree of transparency for the electron beam differs in the different regions of the body. The anterior portion is most transparent, the middle portion is semitransparent, and the posterior portion is not transparent. These facts are due either to the thickness or the density of each region. By means of the light microscope the spermatozoid, when stained with either aceto-carmin, Heidenhain's iron-haematoxylin, gentian-violet or Feulgen's staining method, indicated that the staining reaction of these three portions of the body was graduated in a way similar to the image obtained in the dark field method of the electron microscope (Figs. 3 and 9 a, b, c, d, e).

The author suggests that this round dense mass in the body (Fig. 3) seems most likely to be a nuclear portion and the other portion to be the cytoplasm. The cytoplasm is semitransparent in the dark field image of the electron microscope.

The flagella are ensheathed by a somewhat thick plasmic membrane. This plasmic sheath readily disintegrates and exposes an axis which appears to be a thin-walled cylinder (Fig. 6). The plasmic sheath disintegrates when the spermatozoid is swimming in sea water. This plasmic sheath of the flagella disintegrates, also, after fixation with 4 per cent formalin and centrifuging. Figs. 1 and 2 show that

the flagella do not have the plasmic sheath. Fig. 5 shows the flagella with the plasmic sheath. In Fig. 7, the plasmic sheath has ruptured partially on account of some mechanical stress and the axis appears as a consequence.

On the front flagellum the hairy appendages, the mastigonemes, are present, but are absent from the hind flagellum (Figs. 4 and 5). The front flagellum bears the mastigonemes, which are attached to the wall of the axis through the plasmic sheath.

The axis of the flagellum, normally, has a cylinder-shape. However, after fixation with 4 per cent formalin, and centrifuging, it shows clearly that this axis consists of a bundle of many fibrils (Fig. 8). The number of these fibrils was not determined.

The two flagella unite into a single thread at the blepharoplast as seen in Fig. 8. Such figures were obtained in the specimens treated with either ammonium or chloroform vapour for a short period. After such treatment the body swells up and breaks to show the connection of the front and hind flagella at the blepharoplast.

Discussion

No electron microscopical studies of the spermatozoid of *S. Horneri* have been reported so far the author is aware. The body of the spermatozoid is seen as a homogeneous dark mass when observed through the electron microscope in the ordinary method. But it becomes differentiated when a special dark field method is applied, a dark sphere becomes to be seen in the posterior portion of the body (Figs. 3 and 9e). Considered from the point of view of cytological nuclear stain reaction, this sphere is a nuclear portion of the spermatozoid (Figs. 9a, b, c and d). The spermatozoid of *Fucus Areschougii* studied by Kylin (1920) consists cytologically of three parts, the nucleus, chromatophore and plastomere as illustrated in Fig. 9f.

Figs. 9a-e. Spermatozooids of *Sargassum Horneri*; a-d, stained with various "nuclear stains". Dotted portion shows nuclear reaction. a, Aceto-carmin staining. b, Altmann's fixation and Heidenhain iron-haematoxylin staining. c, Osmic acid vapour fixation and gentian-violet staining. d, Formalin fixation and Feulgen's staining. e, By means of the electron microscope. A spherical dense mass (dotted portion) in the posterior portion is seen.

9f. A reproduced sketch of the spermatozoid of *Fucus Areschougii*, from Kylin, 1920. Altmann's fixation and rosanilin staining; dotted portion shows a nucleus.

The state of the nucleus differs from that of *S. Horneri* (Fig. 9, a, b, c and d) and has a large nucleus supplemented with a small mass of chromatophore and of plastomere, while the spermatozoid of *S. Horneri* has a rather small nuclear sphere in the cytoplasm. This may depend on the difference of species.

The front and hind flagella are connected with each other at the blepharoplast and they seem forming a single thread as observed in the centrifuged specimens after treatment with either ammonium or chloroform vapour (Fig. 8). This is one of the most interesting in this study.

The axis of the flagellum of the spermatozoid of *S. Horneri* is enveloped by a plasmic sheath. A similar appearance was observed in *Marchantia polymorpha* which had been reported by the author (1951). The flagellum of Protozoa (Brown, 1945) and Flagellata (Pitelka, 1949), and the tail of spermatozoa of many animals have generally a plasmic sheath. In case of *S. Horneri*, the origin of plasmic sheath has not yet been confirmed.

Manton and Clarke (1950) have reported that the mastigonemes are seen on the front flagellum of the spermatozoid of *F. serratus* by means of the electron microscope. Both *Sargassum* and *Fucus* belong to the same family Fucaceae, and the presence of the mastigonemes on the front flagellum seems to be a character of this family. However, the mastigoneme can be also seen in Protozoa generally. Brown (1945) has reported that a single row of mastigonemes exists on the flagellum of both *Euglena gracilis* and *Astasia klebsii*, and the mastigonemes exist either on both sides or on all sides of the flagellum of *Ochromonas variabilis*. Further, Pitelka (1949) has reported in *Euglena gracilis*, *Astasia longa* and *Rhabdomonas incurvum* that the flagellum consists of an axis in the center and plasmic sheath surrounding the axis, and one row of mastigonemes on the sheath.

The term of "mastigoneme" employed in this paper is the same as used by Brown (1945) for the first time, and corresponds to "Flimmer" (Fischer, 1894), "hairy appendage" (Manton and Clarke, 1950), and "cilia" (Foster et al., 1947). Mainx (1928) has reported on the mastigonemes of some euglenoids, and Vlk (1938) classified successfully Chrysophyta, Chlorophyta, Euglenophyta and Zoomastigophora after Deflandre's method (1934), which divides the flagella into 5 types, according to the presence or absence of the mastigonemes as well as their position. Thus, the presence or absence of the mastigonemes could be a useful character in classifying Protozoa, Flagellata, and the spermatozooids of Phaeophyceae or other plants.

Pitelka (1949), in the flagella of *Euglena gracilis*, *Astasia longa* and *Rhabdomonas incurvum*, has reported that the sheath included fibrillar elements, and that under some conditions, was apparently always associated with death of the organism, the fibrils of the sheath fraying out along one side only of the flagellum into fine lateral filaments, mastigonemes. However, in case of *Sargassum*, the mastigonemes are different from Euglenida. The author considers the mastigonemes of *S. Horneri* to be a natural hairy structure, and not such a fibrillar one frayed out from the

plasmic sheath as Pitelka asserted.

Manton and Clarke (1950) have reported, in the spermatozoid of *F. serratus*, that the "thumbmark" appendage or the beaklike organ curving away from the base of the flagellum at the front of the body could be seen. In *S. Horneri*, such a pattern could hardly be seen in Figs. 4 and 5, so it is doubtful whether such a pattern really exists in *Sargassum*.

The axis of the flagellum or the cilium, generally, consists of many fibrils, for example, in case of *M. polymorpha*, the axis of the flagellum consists of several fibrils, while the cilium of *Isoetes japonica* consists of about twenty fibrils (Satô, 1951). The flagellum of Protozoa or Flagellata consists of many fibrils (Korschikow, 1923; Brown, 1945; Foster, Baylor, Meinkoth and Clark, 1947; Saxe, 1947; Pitelka, 1949). Moreover, the tail of spermatozoa of various animals consists of many fine fibrils also (Baylor, Nalbandov and Clark, 1943; Schmitt, Hall and Jakus, 1943; Schmitt, 1944; Yasuzumi, 1950; Rötheli, Roth and Medem, 1950; Hanson, Randall and Bayley, 1952). In case of *Sargassum*, the number of fibrils making up the flagellum could not be determined.

Summary

The structure of spermatozoid of *Sargassum Horneri* has been studied by means of the electron microscope.

1. The body of the spermatozoid is too thick for the electron beam penetrance in the light field of the electron microscope. In the image however obtained in the dark field of the electron microscope, a round dense mass could be seen. This dense mass may be a nuclear portion of the spermatozoid, which is considered also from the data obtained by the cytological nuclear stain reactions.
2. The two flagella of spermatozoid have a plasmic sheath, and only the front flagellum has natural distinct hairy structures, mastigonemes.
3. The axis of the flagellum consists of many fine fibrils.
4. The front and hind flagella are connected with each other at the blepharoplast of the body.

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Figs. 1-8. Electron micrographs of spermatozooids of *Sargassum Horneri*. 1. Dark field images; formalin fixation. 2. Light field images, normally used in the electron microscope; formalin fixation. 3. Dark field image; in posterior portion a spherical dense mass is seen; formalin fixation. 4. Osmic vapour fixation, followed by formalin. Mastigonemes are seen on the front flagellum. 5 and 6. Mastigonemes extended through the plasmic sheath which envelops around the axis; formalin fixation. 7. Plasmic sheath broken partially, and the axis appears. 8. Treated with centrifuge. Flagellum consists of many fine fibrils, the front and hind flagella are connected with each other at the blepharoplast of the body.