The Filamentous Appendage in the Spermatozoids of Hepaticae as Revealed by the Electron Microscope*

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佐藤正一**：苔類精子の纖維状附屬物の電子顕微鏡的研究*

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It has been previously reported by the author (Sy. Satô 1954)*** that the spermatozoid of *Conocephalum conicum*, a species of Hepaticae, possesses a filamentous appendage which can be seen with the help of the electron microscope. This filamentous appendage is a long tape along the spermatozoid-body, measuring 0.2μ in width. It can not be observed in the specimens fixed with vapor of 2 per cent OsO_4 solution nor with 4 per cent formalin, because it adheres firmly to the spermatozoid-body. In the specimens treated with hot water, ammonium vapor, or ultra-sonics under a living state, the filamentous appendage is exposed from the body of the spermatozoid, and becomes discernible under the electron microscope. In the unfixed specimen, sometimes, the filamentous appendage parts from the body of the spermatozoid thus comes into sight. Thus the filamentous appendage is a fine and delicate substantial which can not be detected by means of the light microscope, but can be seen under the electron microscope.

In the present paper, it will be reported that a similar filamentous appendage which is found in the spermatozoid of *Conocephalum conicum* exists generally in the spermatozoids of many other species of Hepaticae.

Materials and Methods

The following eight species belonging to three orders of Hepaticae were examined:

Metzgeriales:	<i>Pellia fabbroniana</i> Raddi <i>Makinoa crispata</i> (Steph.) Miyake
Marchantiales:	<i>Marchantia polymorpha</i> L. <i>Conocephalum supradecompositum</i> (Lindb.) Steph. <i>Wiesnerella denudata</i> (Mitt.) Steph. <i>Dumortiera hirsuta</i> (Swartz) Reinw. Bl. et N. ab E. subsp. <i>tatunoi</i> Horikawa
Anthocerotales:	<i>Phaeoceros laevis</i> (L.) Proskauer <i>Notothylas japonica</i> Horikawa

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The spermatozooids of these liverworts were generally fixed with vapor of 2 per cent OsO_4 solution for the observation of each normal form. In order to examine the presence of the filamentous appendage, the spermatozooids were allowed to dry without fixation, or were treated with hot water. Specimens for the electron microscope were prepared in the usual way by drying a drop of the suspension of the spermatozooids on a collodion film. The shadow casting was done with chromium. The electron microscope employed was a Hitachi HU-4 type installed in the Laboratory of Electron Microscopy of the University of Tokyo.

Results and Discussion

Conocephalum supradecompositum—This species is closely related to *Conocephalum conicum*. It differs from *C. conicum* in having a smaller gametophyte, and it matures in autumn in contrast to the spring for *C. conicum*. The body length of the spermatozoid in these two species differs significantly: that is the body length of *C. supradecompositum* is about half long that of *C. conicum*. A filamentous appendage similar to that observed in the spermatozoid of *C. conicum* was also detected in the spermatozoid of *C. supradecompositum* under the electron microscope in the present study. This filamentous appendage does not appear in such specimens fixed with vapor of OsO_4 solution or formalin, but it can be seen in specimens treated with hot water, because the filamentous appendage attached originally along the spermatozoid-body, remains after the destruction of both nucleic and cytoplasmic portions of the spermatozoid-body with this treatment. The filamentous appendage is not destroyed by hot water, but its original form is preserved.

The filamentous appendage of *C. conicum* was a long tape, about 0.2μ in width, which originates from the blepharoplast and is attached along the spermatozoid-body (nucleic portion and cytoplasmic portion) lengthwise (Fig. 1). The filamentous appendage of *C. supradecompositum* has a similar shape, but a slightly less width, 0.16μ (Fig. 2).

Marchantia polymorpha—The spermatozooids of this species are obtained commonly from spring to autumn in Tokyo. In specimens of the spermatozooids fixed with vapor of 2 per cent OsO_4 solution or with 4 per cent formalin, the filamentous appendage could not be detected. However, when specimens were treated with hot water, the filamentous appendage exposed itself clearly and remained after the nucleic and cytoplasmic portions were dispersed by hot water. This filamentous appendage is also a long tape, about 0.2μ in width like *C. conicum* (Fig. 3).

Wiesnerella denudata—The spermatozooids of this species mature in spring. In specimens fixed with vapor of 2 per cent OsO_4 solution or with 4 per cent formalin the filamentous appendage does not appear as in *Marchantia polymorpha*, but, it becomes visible after the nucleic portion has swelled and is destroyed naturally in the unfixed specimens. This filamentous appendage is also a long tape, about 0.25μ in width (Fig. 4).

Dumortiera hirsuta subsp. *tatunoi*—The present sample was an intraspecific polyploid of *Dumortiera hirsuta*, triploid ($n=27$), collected by the present author at Zinmuzi in Miura Peninsula and determined by Dr. S. Hattori. In the spermatozooids of this triploid plant are there two types; one has two flagella, and the other has four flagella. Irrespective of the types, each spermatozoid had a long tape like filamentous appendage, about $0.25-0.35\mu$ in width (Figs. 8 and 9). The spermatozooids of liverworts have generally two flagella, so that it is interesting that *D. hirsuta* subsp. *tatunoi* produces two types of spermatozooids as above mentioned. The filamentous appendage is one in both spermatozooids. Comparative morphological studies of the spermatozooids in triploid plant as well as that of haploid and diploid plants will be reported elsewhere.

Makinoa crispata—The spermatozoid of this species is the largest one in liverworts, having 70μ in body-length. This filamentous appendage is a long tape, about $1-1.2\mu$ in width (Fig. 5). Fig. 5 shows a part of a spermatozoid near the blepharoplast, and shows that the filamentous appendage originates from the blepharoplast and separates from the spermatozoid-body by chance. It was found that the filamentous appendage of this plant is wider than the nucleic portion near the blepharoplast, and the filamentous appendage is very thin.

Pellia fabbroniana—The spermatozoid of this species belongs also to the largest in liverworts such as *Makinoa crispata*. It is about 70μ in body-length. It seems that this filamentous appendage does not separate easily from the body of the spermatozoid. Fig. 6 shows that a filamentous appendage separated partially from the body. In the specimens treated with hot water, the width of the filamentous appendage was about 0.4μ . This is small exceedingly as compared with that of *Makinoa crispata*.

Notothylas japonica and *Phaeoceros laevis*—The form of the spermatozooids of Anthocerotales is very different from those of the other orders, Marchantiales and Metzgeriales. The spermatozoid-bodies of *N. japonica* and *P. laevis* show a long dumb-bell shape, and each nucleic portion has a slender portion which scarcely stains with Feulgen reaction or Heidenhain's iron alum hematoxylin. The nucleic portion attaches a blepharoplast to its front part, and attaches a cytoplasmic portion to its posterior respectively. The filamentous appendage exists in both *N. japonica* and *P. laevis*, and the form is tape like, about 0.12μ in *N. japonica* (Fig. 7) and about 0.08μ in *P. laevis*.

The spermatozooids of nine species of three orders in Hepaticae have been observed by means of the electron microscope. And it becomes clear that the filamentous appendage exists in the spermatozooids of liverworts without exception so far, and that it originates in the blepharoplast stretching along the nucleic and cytoplasmic portions forming a long tape. Previously it was found by the present author that the filamentous appendage of the spermatozoid of *C. conicum* is composed of ten fine fibrils (unpublished). In the present study it was also found that

the filamentous appendage of other species among liverworts most probably consists of many fibrils, the number of which could not be confirmed yet.

It is obscure as yet what function the filamentous appendage has. This structure may serve to support the structure of the spermatozoid-body.

The chemical differences between the filamentous appendage and flagellum of the spermatozoid of *C. conicum* have not yet been made clear, but morphologically from the electron microscopical observations, the flagellum is composed of eleven fibrils forming a cylindrical array, and the filamentous appendage is composed of ten fibrils which keep side by side in a plane. The width of the fibrils of the filamentous appendage differs significantly from that of the flagellum. This will be explained in the future. It is a question whether the presence of the filamentous appendage is specific to the spermatozooids of liverworts or universal in plants, mosses and ferns. This is now under investigation.

Summary

The filamentous appendage similar to that found in the spermatozoid of *Conocephalum conicum* has been confirmed in the following eight species belonging to three orders of Hepaticae: Metzgeriales: *Pellia fabbronia*, *Makinoa crispata*, Marchantiales: *Marchantia polymorpha*, *Conocephalum supradecompositum*, *Wiesnerella denudata*, *Dumortiera hirsuta* subsp. *tatunoi*, Anthocerotales: *Phaeoceros laevis*, *Notothylas japonica*.

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Explanation of Plates

Electron micrographs of spermatozooids of Hepaticae. Chromium shadowed. A: Filamentous appendage, B: Flagellum, C: Spermatozoid-body. Scale shows 1 μ . Plate IX. Fig. 1. *Conocephalum conicum*. Treated with hot water. A filamentous appendage(A) remained along the spermatozoid-body(C) which dispersed due to the treatment of hot water. Fig. 2. *Conocephalum supradecompositum*. Unfixed specimen. When the spermatozoid is allowed to dry without fixation, it is destroyed partly, and a filamentous appendage separates from the body. Fig. 3. *Marchantia polymorpha*. Treated with hot water. Fig. 4. *Wiesnerella denudata*. Unfixed specimen. Plate X. Fig. 5. *Makinoa crispata*. Unfixed specimen. Part of a spermatozoid near the blepharoplast, showing a wide tape like filamentous appendage(A). Fig. 6. *Pellia fabbronia*. Unfixed specimen. Part of a spermatozoid does not separate completely from the body, seen as the fin. Fig. 7. *Notothylas japonica*. Unfixed specimen. Showing a body of a dumb-bell like shape and a filamentous appendage(A). Figs. 8 and 9. *Dumortiera hirsuta* subsp. *tatunoi*. Treated with hot water. Fig. 8 shows the spermatozoid having two flagella attached to it, and Fig. 9 shows the spermatozoid having four flagella. Each has a filamentous appendage.

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