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THE CENTROSOMES OF MARCHANTIA POLYMORPHA.*

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Since Ikeno reported the presence of centrosomes in the antheridial cells of Marchantia polymorpha, in 1903 and 1904, his results have been disputed by several investigators. Ikeno found centrosomes at the poles of the spindle in all the generations of antheridial cells and also observed that these bodies are at the poles of the last division spindle, where they begin to be transformed into the bodies, the so-called blepharoplasts, from which the flagella of the spermatozoid are developed. He came to the same conclusion, therefore, as Belajeff had several years before, through his studies on Gymnogramme and Marsilea, that the blepharoplast is a centrosome.

Miyake, in 1905, failed to see centrosome-like bodies at the poles of the division spindle of the antheridial cells of Marchantia except in the last division, i. e., in the spermatozoid mother cells. From this negative evidence he concludes that Marchantia has no centrosomes. He says: "But my present study seems to show that there is no true centrosome at least in the Hepaticeae, agreeing with the conclusion of the recent study of Gregoire and Bergh. The centrosome hitherto reported in the cells of the Hepaticeae are nothing but a center of cytoplasmic radiation." It is difficult to imagine how one is to distinguish between "true centrosomes" and "centres of cytoplasmic radiation," especially when the bodies in question are situated at the poles of the spindle.

Ikeno, in reply to Miyake, firmly maintains his former position as follows: "Notwithstanding the contrary statement of Miyake, I have no doubt about the real existence of the

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centrosomes in question and so it seemed to me to be quite unnecessary to look for them once more. Nevertheless, in order to make my assertion much firmer, I made, soon after his (Miyake's) paper came into my hands, a special re-examination of my slides and could refind easily almost all stages of centrosomes figured in "Taf." III, of my last paper."

Ikeno showed his slides to Dr. K. Toyama, a zoologist, who was able to see the centrosomes without difficulty. He thus comes to the conclusion that the bodies which he calls centrosomes are evidently distinct and constant structures in the antheridial cells of Marchantia.

Escoyez studying the same subject in 1907, makes the following statement: "Ces corpuscules du Marchantia polymorpha ne sont pas de vrais centrosomes, les porteurs de cils."

Schottlander reported the occurrence of centrosomes in Marchantia in 1892. He appears to have been the first to find these bodies in the Hepaticae and his methods seem to have given more reliable results than some of the later attempts in the same field.

In 1900, Van Hook reported centrospheres with conspicuous radiations in vegetative cells of Marchantia. These centrosomes were observed in the young stalks of the archegoniophores. He says that "They seem undoubtedly to exert a great attractive force from the manner in which certain of the cell contents are drawn to them."

Miyake used the same methods as Ikeno. But different manipulation seems to have given different results. It is curious that with different methods I was able to obtain results similar to Ikeno's. Why were Miyake and Escoyez not able to manipulate the killing and staining processes so as to get the same appearances as Ikeno and myself? It is evident that in microtechnique the personal equation is large and similar methods do not give the same results to all who use them. Therefore, it is useless to attempt to destroy positive evidence by negative results when one cannot produce the positive which others are able to obtain.

After the appearance of Ikeno's first paper, I prepared a large number of slides of the antheridiophores of Marchantia grown in the greenhouse. The material was killed in the weak chrom-acetic acid solution and stained on the slide in various ways.

After much experimenting, I found that I could get the best results by staining with safranin and gentian violet and then restaining in Heidenhain's haematoxylin. About one hundred of what appeared to be the best slides were selected for study. It was found, however, that in only about ten of these had the staining and clearing been done well enough to bring out clearly

the minute structures desired. Accordingly, the observations were made on these ten best slides.

The nuclei of the antheridial cells are only 2–3 microns in diameter and all the cell structures are, therefore difficult to see unless one has good natural light, good slides, a good microscope, and good eyes.

In my preparations, I found centrosomes in the antheridial cells of all stages. In the very early or incipient stages of the antheridium, the cells are somewhat larger but not so clear as in the last stages. The staining must, therefore, be very favorable before many details can be seen. It is not always easy to determine the generation of any given set of cells. Nevertheless, one can come to a fairly good approximation and the exact stage is not of especial importance. The final division and the one preceding can of course be determined without difficulty.

When nuclear division begins, cytoplasmic radiations appear at opposite sides of the nucleus. These asters have very dark-staining centers. These centers are the poles of the future spindle. Their appearance is shown in figures 1, 5, and 22–24. Figure 1 is from a very young antheridium, figure 5 is a great grandmother cell or an earlier stage, while figures 22–24 are spermatozoid mother cells in the final process of division. In the later stages the asters are not developed to any extent while in the earlier generations they are very conspicuous. The same is true for the mother star stage as will appear from an examination of the figures. The most beautiful asters and centrosomes were observed in mother star stages of great grandmother cells (Figs. 10–13). The centrosome is often surrounded by a hyaline zone, the attraction sphere, and the aster is a prominent dark-staining structure forming a cloud-like halo (Figs. 11, 12).

In the daughter star stage the centrosome appears elongated or somewhat double, being probably in the first stage of division (Figs. 4, 14, 15, 20).

In the later stages of the division of the spermatazoid mother cells to form the two spermatids, no doubling of the centrosome was observed (Figs. 31–40) although it becomes elongated and a double nature is probably shown by the development later of the two flagella.

The centrosome appears to begin to enlarge somewhat even in the grandmother cells and in the last division, which is diagonal as Ikeno discovered, the cells become comparatively clear and the centrosome enlarging still more is thus especially conspicuous. As reported by others, the chromosomes were found to be eight in number in the gametophyte (Fig. 25).

As stated above, after the final division the centrosome becomes elongated and appears as an oval, dense, dark-staining body from which the flagella develop. It is evident that this

enlarged centrosome or blepharoplast is of exactly the same nature as the centrosomes at the spindle poles in the earlier divisions. In the antheridial cells of Marchantia, therefore, we have normal centrosomes appearing at each division from the very earliest stages through the great grandmother, grandmother and mother cell stages preceding the formation of the spermatozoids and according to Van Hook they are also present in the vegetative cells.

The lack of prominent asters in the last division is no doubt due to the decrease in size of the cell with corresponding decrease in the amount of cytoplasm. There is not sufficient space or material in which an aster could be developed.

It is needless to give further observations, for they would only be a repetition of the observations so thoroughly reported by Ikeno. In conclusion it is only necessary to repeat that anyone with proper perseverance may obtain preparations which will verify the results here given; and further it is evident that the blepharoplast of Marchantia is only a slightly modified centrosome which can be traced back through many cell generations and which is probably present in all the cell divisions of the entire ontogeny.

This study was completed at the Botanical Garden of the University of Zürich, and I desire here to express my thanks to the director, Professor Dr. Hans Schinz, for many favors shown me while working in his laboratory.

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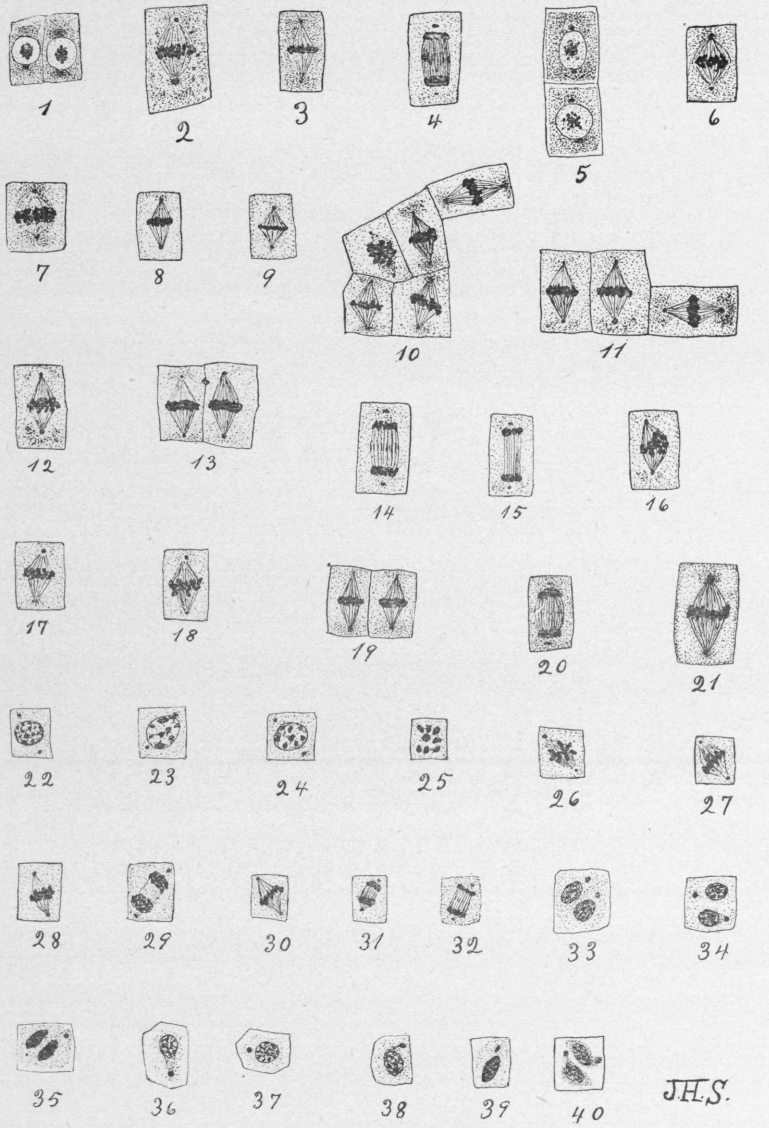

Schaffner on Centrosomes of Marchantia.

EXPLANATION OF PLATE XXI.

The drawings were made with a No. 12 compensating ocular and a 1-12 oil immersion objective, the original magnification being 2250 except figure 21 for which a No. 18 compensating ocular was used. The plate is reduced one-third in reproduction.

Fig. 1. Two cells from a very young antheridium, showing centrosomes on opposite sides of the nucleus.

Figs. 2–3. Mother star stage from a very young antheridium, with centrosomes at the poles.

Fig. 4. Daughter star stage showing elongated centrosomes, from very young antheridium.

Fig. 5. Great grandmother cells, or earlier, showing centrosomes on opposite sides of the dividing nuclei.

Figs. 6–9. Great grandmother cells, or earlier, with centrosomes at the poles of the spindle.

Figs. 10–15. A series of great grandmother cells, showing the appearance of the centrosomes and in some cases dark-staining asters.

Figs. 16–20. Grandmother cells in various stages of division, showing centrosomes at the poles.

Fig. 21. A grandmother cell with prominent centrosomes.

Figs. 22–32. Spermatozoid mother cells in process of division, showing the same kind of centrosomes as are in the earlier divisions. Fig. 25 shows eight distinct chromosomes.

Figs. 33–40. Spermatids or incipient and young spermatozoids, showing the increase in size of the centrosome (blepharoplast) as it is being transformed into an elongated cilia-producing organ.