ON THE NUMBER OF CHLOROPLASTS IN THE CELLS OF THE SPOROPHYTE OF ANTHOCEROS LAEVIS.

ATHA A. PINNICK, Indiana University.

In a study of the morphology of the gametophyte and sporophyte of *Anthoceros laevis* L., the writer was somewhat surprised to find in stained microtome sections of the sporophyte the presence of but one chloroplast in each cell, since in the literature one is given the impression that sporophyte cells contain regularly two chloroplasts (Campbell '18, pp. 121, 142, 597). Inasmuch as the single chloroplast in each cell of the gametophyte, in all North American species, seems to be an organ as permanent as the nucleus, it would be natural to suppose that the cells of the sporophyte might contain two chloroplasts, one being derived from the egg and the other form the sperm in the form of a very small primordium.

Hofmeister, as early as 1862, stated in regard to the number of chloroplasts found in the sporophyte that cells of the upper part of the young fruit (*A. laevis* L. and *A. punctatus* L.) contain without exception two chlorophyll bodies; but that in the inner tissue of the stem (gametophyte) the appearance of two chlorophyll bodies is unusual.

Campbell ('18, p. 142), in describing the sporophyte of *A. fusiformis* and *A. Pearsoni,* says that there is a doubling of chloroplasts in the sporophyte, and specifically that "each epidermal cell contains two large chloroplasts like that of the gametophyte." He refers also to Schimper ('85) as having noted that the chloroplasts double in the sporophyte of *Anthoceros laevis.* Schimper (1 c., p. 21) states that most cells of the sporophyte contain two chloroplasts; those of the epidermis, however, several.

The spore mother-cell shows regularly only one chloroplast. Therefore the presence of two chloroplasts in the vegetative cells of the sporophyte would be strange and not easily explained. Strasburger ('80) and Davis ('99) have traced the history of the spore mother-cell and its two divisions to form the tetrad. Barring nuclear details, it is not at all difficult to observe all phases of the division of the spore mother-cells in the fresh material, since the spores mature gradually and the spore mother-cells readily separate from one another.

In a large spore mother-cell the chloroplast is very conspicuous, and is seen as a thickened mass in one side of the cell. This large chloroplast is yellowish green in color and contains starch and other granules, which become more numerous and prominent with further development. The chloroplast finally elongates and resembles a thick crescent curved about the nucleus. As the chloroplast divides the two equal portions move apart but are connected by cytoplasmic filaments.

The spore mother-cell now elongates and the second division of the chloroplast is exactly like the first. According to Davis ('99) both chloroplasts may be active at the same time, or one may be in a more

"Proc, Ind. Acad. Sci., vol, 34, 1925 (1926),"

advanced state of fission than the other. The cell finally appears broader, in proportion to length, and when the four chloroplasts are formed they are grouped in an orderly arrangement about the centrally placed nucleus. These chloroplasts are all formed before nuclear division occurs in the spore mother-cell. After final nuclear division one nucleus remains in contact with each chloroplast.

A. laevis and *A. punctatus* are the only two species common in the vicinity of Indiana University. All observations mentioned pertain to the former species.

Comparisons were made between chloroplasts of the gametophyte and of the sporophyte. Each cell of the gametophyte shows one very large chloroplast with the characteristic pyrenoid in the center. This chloroplast is granular and somewhat globular, while the contents differentiate prominently with stains. Hofmeister (62) has described the chloroplasts in elongated cells of older shoots as being flattened and sometimes spindle-shaped, while they also appear flattened in the epidermis. Observations seem to verify these conclusions. Hofmeister ('62, p. 7) also found that the chloroplasts in the interior of the sporophyte are smaller than those in the epidermis. Campbell ('18), however, seems to imply that epidermal chloroplasts are as large as in any other cell, for he compares the two large chloroplasts of the epidermis with those of the gametophyte in *A. fusiformis* and *A. Pearsoni.*

McAllister ('14) in his study of the structure of the pyrenoid of Anthoceros finds that the sporophyte chloroplasts do not differ from those of the gametophyte, although they average considerably smaller. The writer verified this conclusion; moreover, the smaller chloroplasts of the gametophyte can in no way be distinguished from the larger ones of the sporophyte. McAllister also agrees that each archesporium cell of *A. laevis* contains a minute chloroplast which is difficult to distinguish from granular cytoplasmic cell contents.

The cells at the base of the sporophyte, adjacent to the cells of the gametophyte, show regularly one large chloroplast identical with that of the gametophyte.

While examining preserved material the writer became interested in the number of chloroplasts found in the sporophyte, since the preparations showed only one chloroplast in each cell. After careful examination material was found which showed a very short section of sporophyte cells where two chloroplasts seemed visible in each cell; but all surrounding cells in the same section had only one chloroplast in each cell. Fresh material was then collected and with free-hand sections observations were continued with the result that occasionally two chloroplasts seemed to be visible in certain rows of cells. However, the majority of the cells in the same section showed only one chloroplast to the cell.

It seemed reasonable to question whether certain parts of the sporophyte might contain two chloroplasts to a cell while other parts would contain only one. Since the stained microtome sections, in a great number of preparations, showed almost without exception only one chloroplast to the cell in the sporophyte, this conclusion was drawn: that probably the free-hand sections of fresh material had the walls

and layers of cells so obscured that the contents of a single cell would be difficult to determine exactly. As some of the oblique walls are very delicate, and do not show clearly in fresh material, one cannot rely upon free-hand sections to give positive results. Fresh material was treated with two per cent osmic acid and mounted in dilute glycerine. The results showed that if two chloroplasts were occasionally found it was the exception rather than the rule. All stages of young and mature sporophytes were examined in fixed and stained sections, and the above conclusion was confirmed.

The writer is convinced after obtaining the above results, that only one chloroplast is present in the cells of the sporophyte of *A. laevis.* Therefore it may be possible that sources of error due to methods of observation are responsible for the report of the presence of the double chloroplast in the sporophyte of Anthoceros as published by Campbell ('18), Hofmeister ('62), and Schimper ('85).

One might explain the presence of an occasional double chloroplast by assuming that after the chloroplast divided the cells failed to divide. It seemed strange, on the one hand, to expect two chloroplasts regularly in the vegetative cells of the sporophyte when only one is found in the spore mother-cell. But, on the other hand, the sporophyte is formed by the union of two cells or gametes; it therefore would seem that the sporophyte should have two chloroplasts, if the sperm furnished one or its primordium, and the egg another.

In the egg cell a chloroplast is present, although somewhat reduced in size as compared with those of many of the vegetative cells. The presence of a chloroplast in the egg was pointed out by Schimper (1. c., p. 7) for *A. laevis* and for the moss, *Atrichum undulatum*, while Scherrer ('14, p. 18) has demonstrated the presence of a well developed chloroplast in the egg of *Anthoceros Husnoti* and in *A. punctatus.*

It seems that all observers agree that in the development of the sperm the chloroplast disappears, and that the chloroplasts of the sporophyte are derived from that in the egg. It may be reasonably concluded, therefore, that the chloroplast in Anthoceros is transmitted in heredity by the female gamete or egg cell, and that in all probability the chloroplast is a permanent organ of the cell.

Whether a primordium of a chloroplast is attached to the sperm in any of the Bryophyta or Pteridophyta remains to be seen. Primordia of plastids are very small structures, and it is not impossible that such structures may be included in the cytoplasmic part of male gametes, although in the plants in question the presence of a plastid primordium in the sperm will be difficult to demonstrate.

LITERATURE CITED.

Campbell, D. H. The Anthocerotes. The structure and development of mosses and ferns (Archegoniatae) 120-159, 620-622. New York. 1918. (3rd Ed.)

Davis, B. M. The spore mother-cell of Anthoceros. Bot. Gaz. 28: 89-109. pl. 9, 10. 1899.

Hofmeister, W. On the germination, development, and fructification of the higher Cryptogamia, and on the fructification of the Coniferae. Transl. F. Currey. London. 1862.

McAllister, F. The pyrenoid of Anthoceros. Am. Jour. Bot. 1:79-95. pl. 8. 1914.

Scherrer, A. Untersuchungen über Bau und Vermehrung der Chromatophoren und das Vorkommen von Chondriosomen bei Anthoceros. Flora 107: 1-56. pl. 1-3. 1914.

Schimper, A. F. W. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. Wiss. Bot. 16:1-247. pl. 1-5. 1885.

Strasburger, E. Zellbildung und Zelltheilung. Jena. 1880. (3rd Ed.)