Contributions to the Morphology of *Sciadopitys verticillata*

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Sciadopitys verticillata SIEB. et ZUCC. is a conifer endemic to Japan. In 1910 LAWSON published an account concerning the gametophyte and embryo of this plant. His observation was not, however, complete, owing perhaps to the paucity of his material. The present study was undertaken primarily to fill in the gap in his investigations. The material used in the present research was obtained from a large tree growing in the grounds of the Kameoka-Hachiman, a small shrine in the suburbs of Sendai. The fixation was made by a strong chrom-acetic solution (1% chromic acid 100 cc, glacial acetic acid 3 cc) after dipping the material into CARNOY's solution for a few minutes. Section 7–25 μ in thickness were stained with NEWTON's gentian violet iodine.

I. The Male Gamete

It is well known, that the sperms found in the Cupressaceae and Taxodiaceae are distinctly organized cells and that the two sperms produced in a pollen tube are exactly alike in size and structure and that both have the function of fertilizing the egg. But in the Abietaceae, the cell division does not follow the nuclear division in the body cell and as the pollen tube is about to discharge the sperms, the boundary of the body cell becomes indistinct and the two sperm-nuclei are left free in the general cytoplasm of the pollen tube. Furthermore, the archegonia being separated from one another by sterile tissue, only one of the two sperm-nuclei can function and the disparity in size of the two sperm-nuclei is distinct.

Sciadopitys verticillata is generally considered as a member of the family Taxodiaceae. But in several important points, this plant differs from the other members of the family. The archegonia, for example, are separated by sterile tissue as in the Abietaceae; the usual members of the Taxodiaceae form an archegonium complex. Concerning the sperms, LAWSON describes them in his paper on *Sciadopitys verticillata*, as follows:

“The division of the body-cell evidently takes place in the tip of the tube immediately over the neck-cells of the archegonium and it results, not in the formation of two cells as in *Sequoia* and

Cupressineae, but in two free nuclei. From the large number of cases examined showing these stages, it became quite evident that there was no cell membrane separating these two male nuclei from one another. The two structures were found lying quite freely in the cytoplasm of the body cell, as is shown in Fig. 12 and one of them is slightly but distinctly larger than the other."

The results of my observations are totally different. The pollen tube growing through the upper part of the nucellus enters into the archegonial chamber a few days before the fertilization. Near the end of the tube, the pollen tube nucleus and the stalk cell nucleus and a short distance behind the elongated body cell are seen. The nucleus of the body cell in this stage of development is always situated near the extremity of the cell. The division of the nucleus in the body cell takes place almost simultaneously with the nuclear division in the central cell of the archegonium to form the egg- and the ventral canal-nucleus. The resulting two nuclei are at first quite alike and between them a cell plate is formed (Fig. 1). Thus a cell division occurs in contradiction to LAWSON's description. Owing to the position of the nucleus of the body cell, the cell is divided into two unequal portions (Fig. 2). Afterwards the difference in size and struc-

Figs. 1-3. 1. The body-cell. Mitosis in telophase. $\times 450$. 2. The two unequal sperm-cells, the pollen-tube nucleus and the stalk-nucleus. $\times 450$. 3. The same in a later stage. $\times 450$.

ture of these two cells becomes more evident and finally in most cases the smaller one becomes entirely disintegrated (Fig. 3).

According to LAWSON's description, the sperm of *Sciadopitys verticillata* closely resembles that of the Abietaceae. But my investigation shows that it is just like the one found in some members of the Taxaceae and Podocarpaceae, where the sperm is a distinctly organized cell and the size-difference of the two sperms produced by the division of a body cell is quite evident.

II. The Ventral Canal Nucleus

As already stated, a few days before the fertilization the nuclear division to form the ventral canal nucleus and the egg nucleus occurs in the central cell of the archegonium. The material collected on the 11th of June of this year has shown a number of these divisions. Fig. 4, a is the metaphase of this division. The spindle is formed just under the neck of the archegonium. A cross section of the ovule has fortunately shown the polar view of the nuclear plate of this division. The chromosome number is clearly determined to be 10 (Fig. 4, b). According to LAWSON the haploid chromosome number of this plant is 8. But recently SAX and BEAL (1934) reported in their paper on the chromosomes of the Cycadales that this plant has 10 pairs of chromosomes in diploid tissues. It is a remarkable fact that all the other members of the Taxodiaceae and Cupressaceae have 11 chromosomes in haploid generation.

Figs. 4-5. 4, a. Mitosis in metaphase to form the ventral canal nucleus and the egg nucleus. $\times 400$. b. The same in polar view. $\times 1600$. 5, a. The same in anaphase. $\times 400$. b. The same in polar view. $\times 1600$.

In anaphase the two halves of the chromosomes move toward opposite poles. The v-shaped chromosomes assembled at the inner pole form a compact group, while those at the outer pole are scattered loosely and lie almost in a plane, just as in the metaphase (Fig. 5, a). So the polar view of the latter enables us again to determine easily the exact number of the chromosomes (Fig. 5, b). In telophase the nucleus formed at this pole is much compressed and soon disorganizes. Cell division does not follow this nuclear division, as is usual with the members of the Taxodiaceae.

III. The Proembryo

My observations regarding fertilization are still incomplete and will be reported in a subsequent paper.

The first mitosis after the fertilization was met with several times in my preparations. Its position within the archegonium varies according to the circumstances. It may lie near the middle of the archegonium or a little higher up in the same. In the metaphase of this division the membrane of the fusion nucleus has already disappeared and the spindle lies in a mass of dense cytoplasm. At the beginning of this stadium we see two chromosome-groups. But these soon come together and form a spindle of the usual appearance.

The two nuclei resulting from the first mitosis are at first small, but soon enlarge and divide simultaneously to form four nuclei. These four nuclei pass to the base of the archegonium, where three more free nuclear divisions take place also simultaneously and 32 free nuclei are produced (Fig. 6-10). These 32 nuclei then become arranged in three tiers, each of which contains about 10 nuclei. The cell-wall formation has its beginning in this stage. But this process is restricted to only the middle and lower parts of the embryo. Meanwhile the 6th nuclear division takes place almost simultaneously. Text-fig. 11 shows the prophase of this division. Although I could not see the metaphase of this division, it is certain that the long axis of the spindle of the division in the middle tier should be placed mainly parallel to the long axis of the archegonium, because we see in a cross section of this part of the embryo about the same number of cells fore the beginning and after the end of this division.

After this nuclear division the formation of the cell-wall becomes completed. But in the uppermost tier, always some of nuclei remain free without forming the cell-wall and often, not always, some are found in the cells, distal ends of which are open to the general cytoplasm of the archegonium. Later the cells of the middle tier become much elongated and send the group of cells of the lowest tier toward the center of the endosperm.

Figs. 6-11. The development of the proembryo. $\times 350$. 6. The 3rd. mitosis after the fertilization. 7. 8-nucleate stage. 8. 16-nucleate stage. 9. 5th mitosis. 10. 32-nucleate stage. 11. 6th mitosis.

In 1931 BUCHHOLZ published a paper concerning the later embryogeny of this plant. The remarkable cleavage polyembryony described and figured by him could be easily confirmed by dissecting the fresh material in sugar solution and staining it with ruthenium red.

LAWSON's description regarding the development of the embryo is rather simple. He believed that only 8 free nuclei could exist before the formation of the cell-wall. In the Conifers the number of free nuclei in early embryogeny varies according to the family. Four free nuclei are common in the Abietaceae, the Taxodiaceae and the Cupressaceae. The highest number is reported in the family Araucariaceae. BURLINGAME found 32 in *Araucaria brasiliensis* and EAMES found 32 or 64 in *Agathis australis*. Therefore *Sciadopitys verticillata* also differs, in this characteristic from other members of the Taxodiaceae. It stands just at the level of the Araucariaceae.

IV. Summary

1. In *Sciadopitys verticillata* the cell division follows the mitosis in the body cell. The nucleus being situated near the extremity of the cell, the two resulting cells show a remarkable dissimilarity in size and structure.

2. The mitosis to form the neck canal nucleus and the egg nucleus occurs just under the neck cells of the archegonium. The polar view of the spindle clearly shows 10 chromosomes. In the anaphase, the v-shaped chromosomes in the inner pole form a compact group, while those at the outer pole are scattered loosely and lie almost in a plane similar to those in the metaphase. The polar view of the latter enables us to determine easily the exact number of the chromosomes.

3. After fertilization 5 successive nuclear divisions occur and produce 32 free nuclei before the formation of the cell-wall.

In conclusion I wish to express my hearty thanks to Mr. K. OIKAWA for the assistance given me during the course of the present investigation.

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