PECULIAR STRUCTURES OCCURRING IN THE POLLEN TUBE OF ZAMIA.

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(WITH PLATE XL)

THE recent announcement by Hirase[1] of the occurrence of motile spermatozoids in *Ginkgo biloba*, and that by Ikeno[2] of the occurrence of similar organs in *Cycas revoluta*, render any observations on the phenomena occurring in the pollen tube of Zamia, belonging to the related sub-family Zamieæ, of special interest. In cones of *Zamia integrifolia* shortly before fecundation the writer has observed several remarkable structures, which, so far as can be learned, have never been described.

For a considerable period preceding fecundation in Zamia, as in many other gymnosperms, the pollen tube apparatus remains in almost the same condition, no important changes taking place. In this stage the pollen grains, which lie in the pollen chamber at the apex of the nucellus, are found to have germinated and the germ tubes to have reached a length of 1 to 2^{mm}, penetrating into the tissue of the nucellus. The pollen tube is much greater in diameter than the pollen grain which may be clearly distinguished. The vegetative nucleus of the pollen grain, in every case observed, has wandered into the pollen tube and may usually be found near its lower end (*fig. 1*). In the upper end of the pollen tube, near the pollen grain, two cells are uniformly found, one in close connection with the old pollen grain from which it protrudes or is only slightly separated, and the other immediately in front of this in the more swollen portion of the pollen tube (*fig. 1*). The former cell is spherical or slightly

[1] HIRASE, S. — Untersuchung über das Verhalten des Pollens von Ginkgo biloba. Bot. Centralbl. 69: 33. 14 Ja. 1897.

[2] IKENO, S. — Vorläufige Mittheilung über die Spermatozoiden bei Cycas revoluta. Bot. Centralbl. 69: 1. 30 D. 1896.

elongated and presents a most singular structure. The nucleus of the original cell evidently divides into two, and one of the daughter nuclei forms within the unbroken *Hautschicht* of the mother cell a new and wholly distinct *Hautschicht* which delimits a cell lying entirely free within the mother cell and surrounded on all sides by a layer of protoplasm of nearly uniform thickness (*figs. 1a* and *2*.) The other daughter nucleus remains free within the *Hautschicht* of the mother cell, but is pressed to one side by the interior cell. The free nucleus usually occupies the side of the mother cell opposite the pollen grain. Both the interior cell and the mother cell are crowded full of small spherical starch grains (*fig 2*). The second cell (*fig. 1 gc*) which is probably to be compared with the generative cell in conifers, is much larger then the first or proximal cell above described, and is provided with two small spherical organs, situated at the opposite ends of the nucleus outside the nuclear wall, that somewhat resemble centrosomes (*fig. 3*). They are exactly spherical and stain deep red with safranin, in the Flemming triple stain process, much as the nucleoli. There is no appreciable difference in size between the spheres in the same cell, but those of different cells frequently vary several micromillimeters in diameter, ranging from 7 to 10 μ. Many of the spheres at this stage appear entirely homogeneous, but in some cases a number of small vacuoles have been observed in the interior (*fig. 4*). Numerous long slender threads of kinoplasm radiate in all directions from the spheres, and in many instances may be seen plainly to extend to the *Hautschicht* with which they frequently appear to unite. The threads of kinoplasm are rather coarse and are plainly visible without staining. With the Flemming triple stain they are colored intensely blue. They appear in many instances to be firmly fastened to the *Hautschicht*, which is drawn in more or less where the thread is attached (*fig. 5*). This seeming indentation may, however, be caused by shrinkage during fixing and staining. The spheres are usually 7 to 10 μ from the nuclear wall, which is in most instances strongly indented on the ends next to the spheres. Threads of kinoplasm run from the spheres to the nuclear wall, which at this stage is apparently continuous. In most cases observed the spheres occupy positions on exactly opposite sides of the nucleus, but in a few instances they have been found much nearer together, being only about 135° apart. The nuclei which these bodies accompany are at this stage in a resting condition, the contents presenting a fine granular appearance and usually showing foam-structure more or less plainly. The nuclei are usually elliptical and about 80 μ long by 56 μ wide. The large nucleoli (20 to 24 μ in diameter) contain numerous vacuoles. Frequently several other small nucleoli may be observed in a nucleus.

When the activity, which precedes fecundation, begins, the generative cell does not wander down to the distal end of the pollen tube as might be expected from analogy, but instead the proximal end of the tube (the pollen grain end) with the two cells, shown in *fig. 1*, turns directly downward and grows through the apical tissue of the nucellus (*fig. 6*) and may be seen with a hand lens hanging down into the cavity formed above the archegonia. The pollen grain, constituting the extreme end of the now pendent proximal end of the tube, may be plainly seen, the two cells remaining in the same relation to the pollen grain as shown in *fig. 7*. All of the tubes examined show this same structure, indicating that it must be the normal procedure. The first cell which protrudes from the old pollen grain remains in the same condition, or in the most advanced stages yet studied shows indications of disintegration. The generative cell or second cell in the tube, which now lies above the first cell, has usually divided during this extension of the tube, or may be found in some stage of division. The two centrosome-like bodies which accompany each generative cell and during the resting condition, as described above, occupied the poles of the nucleus corresponding to the major axis of the cell and the longitudinal axis of the pollen tube, usually come to lie, during the migration of the cell into the now extended proximal end of the pollen tube, at two opposite points on the equator, the nucleus corresponding to the minor axis of the cell or transversely in the pollen tube. Whether this change in their position is brought about by the migration of the centrosome-like bodies or by the changed position of the nucleus the writer has not yet been able to surely determine. They have meanwhile greatly increased in size, now measuring usually from 18 to 20 μ in diameter. They now have a clearly distinguishable outer wall of considerable thickness and the contents are evenly and beautifully vacuolate.

When the nuclear spindle is formed these bodies always take up a position directly opposite the polar ends. In the monaster stage of the division (*fig. 7*) the spindle, which occupies a transverse position both to the pollen tube and to the elliptical nucleus, is composed of fine kinoplasmic filaments and seems to be entirely within the still preserved nuclear wall. It is blunt poled, if indeed it is not multipolar, and does not seem to have any kinoplasmic connection with the centrosome-like bodies. In the resting condition of the nucleus the centrosome-like bodies were surrounded by radiating threads of kinoplasm, but no indications of these can now be seen. There is, however, in most cases a slight radial arrangement of the protoplasm immediately bordering the wall. The structure of the bodies has meanwhile undergone considerable change (*figs. 7, 8*). The wall swells up and apparently separates into fragments which in cross-section at this stage show as a broken line. The vacuolated contents meanwhile contract away from the wall, leaving a clear space intervening, which is, however, traversed by a few slender filaments. The body at this stage presents the appearance of disintegration.

When the cell-plate is formed (*fig. 9*) the centrosome-like body is found entirely broken up, the fragments appearing in the cytoplasm as a number of granules mixed with plates or membranes which appear to be fragments of the wall. After the new cell wall is completed and the daughter nuclei have returned to the resting stage, showing a nucleolus, the fragments of the broken-down centrosome-like body present the appearance shown in *fig. 10*. A little cluster of granules appear in the exact location of the body, and the plates, which stain deeply and often appear to be two in number, have separated and moved toward the poles of the daughter nuclei.

The writer has been unable to determine the origin of the centrosome-like bodies described above, and thus cannot be sure as to their nature. In the resting condition of the generative cell (*fig. 3*) they are somewhat similar to the bodies described by Hirase as "attraction spheres"[3] in the pollen cell of *Ginkgo biloba*, but his description and figure differ from what I have seen, in that spherical bodies much larger than the attraction spheres are located between them and the nucleus. No indications of the latter body occur in Zamia. The nucleus, furthermore, is of very different shape from those occurring in my slides of Zamia, and the so-called attraction spheres are apparently much smaller than the similar bodies which I find.

The researches of Farmer,[4] Osterhout,[5] Mottier,[6] and Strasburger[7] have thrown much doubt on the occurrence of centrosomes in the pteridophytes and phanerogams, where the nuclear spindle in its earlier stages is multipolar, at least in the spore or pollen mother cells. In view of these facts their occurrence in Ginkgo and Zamia may well be doubted. The spheres in the generative cell of Zamia resembles centrosomes in that they have the kinoplasmic filament centered upon them during a large part of their existence, and have an important relation to the formation of the spindle, being uniformly located near the poles and always having a definite orientiation with reference to the axis of the spindle. They differ, however, so materially from the centrosomes described by Farmer,[8] Swingle,[9] Strasburger,[10] and others, and

[3] HIRASE, S.— Notes on the attraction sphere in the pollen cells of *Ginkgo biloba* The Botanical Magazine **8**: 359. 1894.

[4] FARMER, J. BRETLAND.— Ueber Kerntheilung in Lilium-Antheren besonders in Bezug auf die Centrosomen-Frage. Flora **80**: 56. ——.

[5] OSTERHOUT, W. J. V.— Ueber Enstehung der karyokinetischen Spindel bei Equisetum. Jahr. f. wissenschaft. Bot. **30**: 159. 1897.

[6] MOTTIER, DAVID M.— Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahr. f. wissenschaft Bot. **30**: 169. 1897.

[7] STRASBURGER, EDUARD.— Ueber Cytoplasmastructuren, Kern- und Zelltheilung. Jahr. f. wissenschaft. Bot. **30**: 375. 1897.

[8] FARMER, J. BRETLAND.— On spore formation and nuclear division in the Hepaticæ. Annals of Botany **9**: 469. 1895.

from the centrospheres found by Harper[11] in the Ascomycetes, that in the present state of our knowledge they must be considered to be distinct organs. What they are cannot be determined till their origin and functions are better known.

The further highly remarkable history of these organs the writer hopes soon to discuss, together with other details of the fecundation, in a future number of this journal.

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NOTE.— As this paper is passing through the press Mr. Webber sends word that he has discovered motile antherozoids in Zamia. They were found in a sugar solution and kept moving for two hours and forty-four minutes.— EDS.

EXPLANATION OF PLATE XL.

Zamia integrifolia Willd.

FIG. 1. Pollen tube growing in the nucellus of the ovary; *pg*, pollen grain; *vn*, vegetative nucleus; *a*, cell protruding from old pollen grain and containing a free interior cell and nucleus; *gc*, generative cell. × 120.

FIG. 2. Proximal cell shown at *a* in preceding figure. × 550.

FIG. 3. Generative cell showing centrosome-like bodies with radiating filaments and kinoplasm. × 450.

FIG. 4. Centrosome-like body during resting stage of nucleus, showing vacuoles. × 1800.

FIG. 5. Centrosome-like body seen from above looking toward the nucleus showing kinoplasmic filaments connected with *Hautschicht*. × 600.

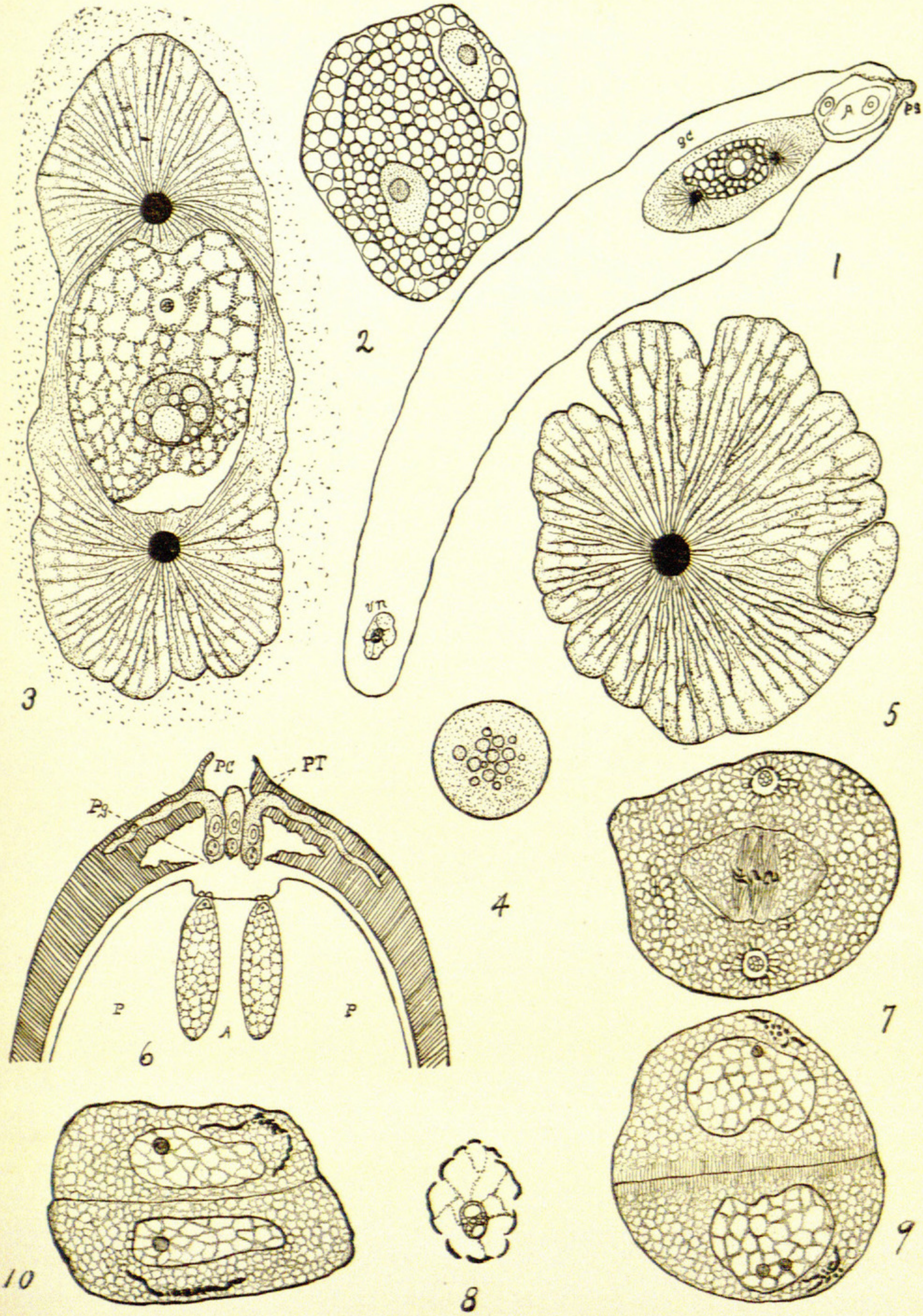
FIG. 6. Diagrammatic outline of the upper end of nucellus, showing the proximal end of the pollen tubes growing down into the cavity just above the archegonia; *p*, prothallus; *a*, archegonia; *pc*, pollen chamber; *pt*, pollen tubes; *pg*, pollen grain.

FIG. 7. Generative cell in monaster stage of division, showing the breaking down of the centrosome-like body and the absence of the radiating kino-

[9] SWINGLE, WALTER T.— Zur Kenntniss der Kern- und Zelltheilung bei den Sphacelariaceen. Jahr. wissenschaft. Bot. 30: 297. 1897.

[10] STRASBURGER, EDUARD.— Kerntheilung und Befruchtung bei Fucus. Jahr. f. wissenschaft. Bot. 30: 351. 1897.

[11] HARPER, R. A.— Kerntheilung und freie Zellbildung in Ascus. Jahr. wissenschaft. Bot. 30: 249. 1897.



WEBBER on ZAMIA.