

THE DEVELOPMENT OF THE ANTHEROZOIDS OF ZAMIA.

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IN the June number of this journal the writer discussed some of the phases of development of the pollen tube apparatus of *Zamia integrifolia*.¹ The generative cell was traced through its migration and division up to the time of the breaking down of the centrosome-like body. The discovery of motile antherozoids was also announced, but the method of their development was not explained.

Since then further observations have shown that each of the daughter cells, formed by the division of the generative cell, develops into a motile antherozoid, two thus being formed in each pollen tube; and that they are encircled by a spirally arranged band of cilia developed in a very novel way from the fragments of the centrosome-like body. In the present preliminary paper these features will be considered, together with a short account of observations on the movements of the antherozoids.

The membrane formed by the wall of the centrosome-like body in its disintegration evidently does not separate into two fragments, as I was at first inclined to think,² but forms a single somewhat contorted membrane or band which at this time lies free in the cytoplasm of the cell. In its further development this membrane becomes greatly extended in length, growing in such a manner as to form a spiral band or ribbon which meanwhile moves outward and becomes closely appressed against the *Hautschicht* of the cell. The first turn of the band is located near the equator of the cell nearly at right angles to the direc-

¹ WEBBER, HERBERT J.—Peculiar structures occurring in the pollen tube of *Zamia*. BOT. GAZ. 23:453. 1897.

² *Op. cit.*, Pl. XL, fig. 10.

tion of the spindle formed in the division of the generative cell, and median sections in this stage show the deeply stained sections of the band lying on opposite sides of the cell (*fig. 1 b*). The band gradually elongates and finally forms five or six turns around the cell, which are arranged in a helicoid spiral on the surface, usually about opposite the side of the cell attached to the other antherozoid (*fig. 2*). Viewed from the apex of the spiral the turns, beginning at the apex, run in the direction opposite to that of the hands of a clock. No exceptions to this rule have been observed. In an early stage of its development very numerous protuberances can be discovered on the outer surface of the band. These gradually increase in length and become plainly visible by the time the band has formed one turn around the cell (*fig. 1*). They continue to grow in length and ultimately form the motile cilia of the mature antherozoids (*figs. 2 and 3*). The band varies considerably in width at various stages,

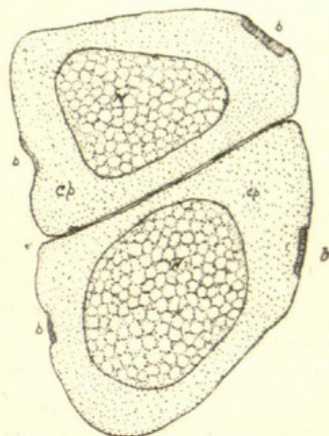


FIG. 1. Young antherozoid formed by the division of the generative cell, showing, in cross section, the spiral band which has developed one turn: *n*, nucleus; *cp*, cytoplasm; *b*, spiral band. $\times 200$.

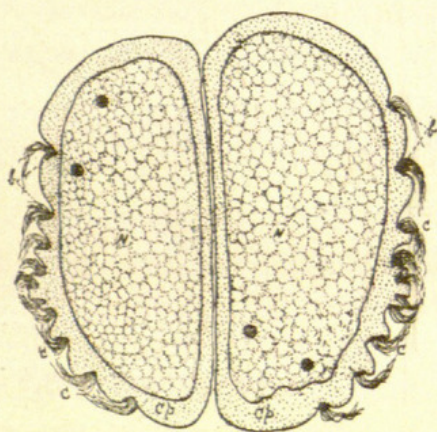


FIG. 2. Cross section of nearly mature antherozoid: *n*, nucleus; *cp*, cytoplasm; *b*, spiral band; *c*, cilia. $\times 200$.

becoming continually narrower as its development proceeds. In a mature stage, just before the antherozoids escape from the pollen tube, it is usually from 5 to 8μ wide. During the development of the spiral cilia-bearing band the cell becomes changed considerably in shape and appearance, as shown by comparing *fig. 2* with *fig. 1*. The band which, as explained above, finally comes to lie in close contact with the *Hautschicht* of the cell, is apparently invariably developed on the side of the cell opposite to the partition wall formed in

the division of the generative cell (*figs. 2 and 5 A*). The *Hautschicht* of the cell is bent in where the band is located, forming a deep spiral groove. By this the band is brought to the surface of the cell, but apparently remains covered by the *Hautschicht* through which the cilia appear to penetrate (*figs. 2 and 3*).



FIG. 3. Cross section of spiral band, showing attachment of cilia $\times 900$.

While the generative cell and the antherozoids are developing as above described, the proximal ends of the pollen tubes, which, as noted in my previous paper,³ grow downward through the apical tissue of the nucellus into a cavity formed in the prothallium above the archegonia, have increased in length until the ends almost or quite touch the neck cells of the archegonia, which protrude into the same cavity. It is interesting to note that the pollen tubes when they enter the prothallium cavity, which is filled with air, do not grow at random, but bend slightly outward and grow directly toward the archegonia. Frequently several turn toward the same archegonium. The end of the pollen tube is occupied by the proximal cell, described in my previous paper, which remains intact till the pollen tube bursts in the act of fecundation. The antherozoid cells at this stage invariably occupy a position in the pollen tube immediately above the proximal cell (*fig. 5 A*). The end of the tube is wider than the upper portion and is evidently under considerable tension. The protruding tip formed by the old pollen grain (*fig. 5 pg*) is plainly visible with a hand lens, and is evidently the point which first comes in contact with the neck cells of the archegonia. The neck cells are also distended and turgid, and are evidently easily broken. If in this stage the end of a pollen tube be touched very lightly with the flat side of a scalpel it bursts, and the antherozoids together with a drop of

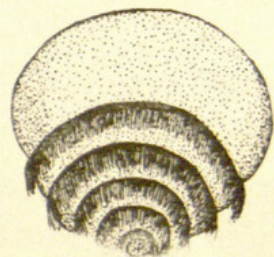


FIG. 4. Mature antherozoid. $\times 90$.

³ *Op. cit.*, 455, *Pl. XL, fig. 6*.

the watery contents of the pollen tube are quickly forced out, and the pollen tube immediately shrivels up into a shapeless mass. This is probably what happens naturally in the course of fecundation. The pollen tube evidently grows down until the end is forced against the neck cells, when the tube bursts, discharging the mature antherozoids and the watery contents of the tube which supplies a drop of fluid in which the antherozoids can swim. I have several times observed the antherozoids after they were discharged over the archegonia, but studying them in this position is difficult and unsatisfactory. For purposes of microscopic study the pollen tubes were carefully cut off some distance above the antherozoid cells and placed in water, but this proved unsatisfactory, as the antherozoids soon died and burst, evidently from the difference in density of water and the contents of the pollen tube. Solutions of cane sugar of several strengths were then tried, and a 10 per cent. solution proved thoroughly satisfactory. By the use of this solution I was able to keep the antherozoids living and moving for a considerable time, usually from thirty to sixty minutes, and in one case two hours and forty-four minutes. When mature pollen tubes are placed in sugar solution the proximal cell, protoplasm, etc., can at first be seen to have their normal shape, the antherozoid cells usually still adhering to each other (*fig. 5 A*). In a few minutes, however, when the sugar has had time to diffuse into the pollen tube, the proximal cell and protoplasm break down into a shapeless mass, and the antherozoids, under the stimulation of the sugar solution, gradually begin to awaken into life, as it were. The cilia begin to move, and the cells round up and slowly pull apart (*fig. 5 B*). When swimming free without pressure the antherozoids are slightly ovate, nearly round, or compressed spherical (*fig. 4*).

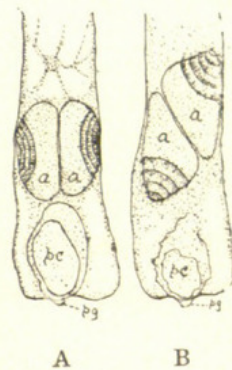


FIG. 5. Separation of antherozoids under influence of 10 per cent. sugar solution: *A*, mature pollen tube immediately before fecundation placed in sugar solution; *B*, the same tube about five minutes later; *pg*, remains of pollen grain at lower end of tube; *pc*, proximal cell; *a, a*, antherozoids. $\times 50$.

They vary greatly in size, but are commonly longer than broad, ranging in length from 258 to 332 μ and in width from 258 to 306 μ . The antherozoids of Ginkgo, as described by Hirase,⁴ are egg-shaped and 82 μ long by 49 μ wide. Compared with these, the antherozoids of Zamia are surprisingly large, being plainly visible to the unaided eye. In Cycas, according to Ikeno, they are somewhat larger than in Ginkgo. I judge from this that they are also much larger in Zamia than in Cycas. The numerous cilia which are developed from the spirally arranged band, as described above, give the pointed end of the antherozoid a striking helicoid appearance. There is no free tail in Zamia, as is said by Hirase to occur in Ginkgo. The nucleus is very large (*fig. 2 n*), occupying a large portion of the body, and is surrounded on all sides by a thin layer of cytoplasm (*fig. 2 cp*). The vibrations of the cilia in vigorous antherozoids are exceedingly rapid and difficult to study. Judging from observations made on certain antherozoids just starting motion and others which had nearly exhausted their energy, there would seem to be a rhythmic contraction of the cilia which passes quickly from one end of the band to the other. A tremulous vibration of the cilia, apparently independent of the rhythmic contraction, can be observed in the weaker motion of extreme youth and age. Whether this occurs in the period of vigorous rapid motion could not be determined. The motion of the antherozoid is comparatively slow and sluggish. In pollen tubes placed in sugar solution the two antherozoids frequently move around very vigorously, bumping against each other and the wall of the pollen tube. They seldom escape from the upper cut end of the pollen tube, although they as frequently swim toward this end of the tube as the other end, so far as could be observed. In a number of instances the pollen tubes were cut so that the antherozoids escaped and their unobstructed motion was thus observed. The *Hautschicht* of the antherozoid is very tender, however, and is commonly broken in attempting to remove the latter from the

⁴ HIRASE, DR. S.—Untersuchungen über das Verhalten des Pollens von *Ginkgo biloba*. Bot. Centralb. 69: 34. 14 Ja. 1897.

tube. The motion when swimming free in the sugar solution is in no way different from the action observed in the pollen tube. The general motion is a continuous rotation of the body, always in the same direction around an axis passed through the apex of the helicoid spiral. Viewed from the head end or apex of the spiral the rotation is in the direction of the hands of a clock, and contrary to the turns of the spiral band. They roll round, first here, then there, resembling in this respect the motion of *Pandorina*. After moving about rapidly for from five to ten minutes they usually cease all progressive motion, but continue to rotate for a considerably longer period. The rotary motion also soon ceases, but the cilia continue to vibrate for a considerably longer time.

In fecundation the entire antherozoid unchanged swims into the archegonium, passing between the ruptured neck cells. Several antherozoids commonly enter each archegonium, from two to three having been found in almost every archegonium examined, and in one case four. Only one of these takes part in fecundation, and the others may be found presenting a perfectly normal appearance or in some stage of disintegration.

The study of the centrosome-like body, which formed the main feature of my previous paper, has continued to grow in interest as the investigation has progressed. No case is known, so far as I am informed, where true centrosomes fulfill such functions as are performed by the centrosome-like body in *Zamia*. The very large size of the organ; the loss of the radiating filaments of kinoplasm in an early stage of the division of the generative cell; the swelling and breaking down of the body in the anaphases of the same division; the disintegration of the central part of the body; and the final growth of the membrane, formed by the broken wall of the body, into the spiral band which bears the motile cilia of the antherozoid—are features and functions peculiar to the centrosome-like body of *Zamia*. It is interesting in this connection to note that the substance of the band stains a dense blue black with Heidenhain's iron hæmatoxylin, which is primarily a centrosome stain,

although it also stains nucleoli deeply. The method of antherozoid formation found in *Zamia* is also very different from any known case, so far as I can learn, but is probably similar to what occurs in *Ginkgo* and *Cycas*.

In a future number of this journal the writer hopes to discuss the phenomena of fertilization in *Zamia* together with further notes on the homologies and functions of the centrosome-like body.

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