

# Contributions to the Life-History of Notothyas.

BY

DAVID M. MOTTIER,

*Associate Professor of Botany, Indiana University, Bloomington, U.S.A.*

—♦—  
With Plates XX and XXI.  
—♦—

THE genus *Notothyas* is represented in Indiana by two species, *N. orbicularis*, Sulliv. and *N. melanospora*, Sulliv. The former, according to Gray's Manual<sup>1</sup>, is pretty widely distributed over the Eastern United States.

*Notothyas orbicularis*, the species with which we are particularly concerned here, grows abundantly upon damp, shady ground where the moisture is tolerably constant during the warmer parts of the year. Not infrequently were specimens found side by side with *Anthoceros*, the lobes of the thallus sometimes overlapping, so that it was often difficult to distinguish one from the other. However, *Notothyas* is of a paler green, the lobes of the thallus smaller and much more irregular than those of *Anthoceros*.

This year, fruiting specimens were collected during the latter part of July and up to August 10, but these are more abundant from October until the plants are frozen in November, when almost every specimen appears to bear

<sup>1</sup> Revised Edition, p. 727.

sporogonia. By gathering specimens earlier in the season one is sure to find both sex-organs and sporogonia in different stages of development on the same semicircular or circular expansion of thallus.

According to the views of Leitgeb<sup>1</sup> and Goebel<sup>2</sup>, *Notothylas* is regarded as representing a transition from *Anthoceros* to the *Jungermannieae*. This view is based mainly upon the structure of the sporogonium, particularly the limited growth of its capsule and the nature and origin of the columella.

Hofmeister has also made careful and extended observations upon the *Hepaticae*, but unfortunately his works are not accessible.

Goebel<sup>2</sup> states that the capsule of *Notothylas* either has not a columella, or has one which is only a secondary differentiation inside the spore-chamber. In another place<sup>3</sup> the same author says that there are species of *Notothylas* which possess a columella similar to that of *Anthoceros*, but of its origin nothing is known; that, in fact, it is uncertain whether this arises, as in *Anthoceros*, with the archesporium though independently of it, or whether it is a product of a gradual differentiation in the spore-chamber.

For a detailed knowledge of these Liver-worts we are indebted to Leitgeb, who, in his admirable work *Untersuchungen über die Lebermoose*, has published the results of careful and extensive investigations.

Four species of *Notothylas* were examined by this author, *N. fertilis*, *N. valvata*, *N. Breutellii*, and *N. melanospora*.

Leitgeb<sup>4</sup> states that in some species of *Notothylas*, there occur capsules, which, in regard to the size (*Mächtigkeit*) of the columella and the difference of its cells from the other sterile cells of the spore-chamber, do not differ from the capsules of *Anthoceros*; and it may be possible, though not probable, that these capsules, in respect to the origin of their

<sup>1</sup> *Untersuchungen über die Lebermoose*, Heft V, 1879.

<sup>2</sup> *Outlines of Special Morphology*, p. 158, English translation (1887).

<sup>3</sup> Schenk in *Handbuch der Botanik*, Band II, p. 357 (1882).

<sup>4</sup> *Loc. cit.*, Heft V, p. 7.

columella and spore-forming layer, agree with *Anthoceros*. That, moreover, in all species one finds capsules in which a columella is present, but where the cells of the columella are formed throughout similarly to the other sterile cells of the capsule, and may be very easily separated from one another. The columella of such capsules is not developed, as in *Anthoceros*, independently of the spore-forming layer, but arises as a secondary differentiation within the spore-chamber, and in this respect agrees with the columella of the Moss-capsule. Again, in his conclusions he says: 'In many (perhaps all?) species of the genus *Notothylas* there are capsules that possess no columella, and the examination of half-ripe sporogonia shows that this is not due to the eventual separation of the cells, but that no columella is ever differentiated.' These conclusions were drawn from a study of the four species mentioned above. In *N. fertilis*, as many capsules were found without the columella as with it, and in no case did the columella extend farther up than the middle of the capsule. The other species possess a columella.

According to Gottsche<sup>1</sup>, *N. fertilis* is identical with *N. valvata*, and there is reason to believe that *N. valvata* is the same as *N. orbicularis*<sup>2</sup>, the species here under consideration.

The question to be answered then is what is the origin of the columella? Does it arise as in *Anthoceros*?

This work, the results of which are set forth in the following pages, was taken up at the suggestion of Dr. Douglas H. Campbell of the Leland Stanford Jr. University.

It may be well first to give a brief outline of the method used, as the results will thereby be more fully understood and appreciated. All specimens were fixed in chromic acid (1 per cent. aqueous solution), remaining in the fluid about two hours; then, after washing repeatedly in water for about two days, they were brought gradually into 70 per cent. alcohol, where they remained until wanted. In this way all

<sup>1</sup> Leitgeb, loc. cit., Heft V, p. 40, footnote.

<sup>2</sup> Gray's Manual, revised edition, p. 727.

traces of the acid are removed, a condition so necessary when it is desirable to stain with alum-cochineal. They were then stained *in toto* with alum-cochineal, dehydrated, brought gradually into a solution of turpentine and paraffin, imbedded in paraffin, and sectioned on a Minot-microtome. The sections were counter-stained on the slide with Bismarck-brown dissolved in 70 per cent. alcohol, and mounted in Canada-balsam. The nucleus and parts of the protoplasm are stained by the cochineal, and the cell-walls by the Bismarck-brown. In this way all details are clearly and beautifully brought out.

With the view of contributing something toward the solution of the problem stated above, a study of the development of the sporogonium, especially the earlier stages, was made, together with that of the antheridia, to determine, if possible, whether the latter arise from an epidermal or a sub-epidermal cell.

For the purpose of comparison, a similar study of *Anthoceros* was carried on with that of *Notothylas*.

The first divisions of the embryo correspond to those which regularly follow in all known Liver-worts. The fertilized egg is divided into an upper and a lower cell by the basal wall, which is at right angles to the long axis of the archegonium, the former becoming the capsule and the latter the foot of the sporogonium. Now follow two walls in rapid succession at right angles to the primary wall and to each other, thus dividing the embryo into eight cells disposed as the octants of a sphere. The exact order in which the two latter walls were formed was not determined. Fig. 1 represents three successive vertical sections of such an embryo, which include the whole of it. The eight nuclei were so situated that four came in the first section (*a*) and four in the third (*c*), while in the middle one (*b*) there were no nuclei. Serial sections of a similar embryo, at right angles to the archegonial axis, revealed similar structures.

The embryo, however, is usually more oval in shape (Fig. 16).

The four lower octants develop into the foot, as described by Leitgeb<sup>1</sup>.

The four upper octants are now divided by transverse walls into two tiers of cells disposed as quadrants. It is barely possible that occasionally three tiers are formed (Fig. 2). Each of these cells next divides by a periclinal wall into an inner and a peripheral cell. The inner cells become the columella, while the peripheral cells give rise to the archesporium and the wall of the capsule, precisely as in *Anthoceros*. This will be readily seen by a comparison of Fig. 6 *b* with Fig. 15, which are transverse sections of sporogonia of the same age respectively. The differentiation of the archesporium from the peripheral cells appears to begin at the apex, proceeding toward the base (Fig. 3).

In *Notothylas*, however, a number of difficulties are met with in the demonstration of these facts. Cell-division does not follow with as great regularity as in *Anthoceros*, and the difference between the archesporial cells and those of the columella is not so pronounced. Here the cells of the archesporium, in sporogonia about the size of those in Figs. 4 and 5, are only a little richer in protoplasm than those of the columella, requiring careful staining and exactly straight sections in order to make out the distinction. Considerable difficulty was experienced in orienting specimens to get perfectly straight longitudinal sections through the sporogonia, from the fact that the sporogonia do not stand perpendicular to the surface of the thallus, but slightly inclined forward; and that the small lobes of the thallus do not invariably lie flat, but they are more or less tipped by the wrinkling or wave-like folding of the thallus in its growth. Besides, all sporogonia are disposed radially on the circular or semicircular thallus, no two lying exactly parallel. Several preparations were obtained, however, in which the sections passed exactly straight through the sporogonium with the above results (Figs. 3 and 4).

In all cases observed it was evident that the archesporial

<sup>1</sup> Loc. cit. p. 49.

cells over the apex of the columella multiplied by tangential divisions, so that this region consisted of a mass of archesporial cells (Figs. 4 and 5), instead of a single layer, as in *Anthoceros*.

Transverse sections reveal the same structures as those seen in longitudinal sections. Fig. 6, *a*, *b*, and *c*, represent cross-sections of a sporogonium of about the same stage in development as Fig. 5, taken at *a-a*, *b-b*, and *c-c* respectively: Figs. 7 to 14, inclusive, represent successive cross-sections, including all of a similar sporogonium above the foot. In Fig. 7 the centre is occupied by four cells, the columella, surrounded by a peripheral row. In Figs. 8, 9, 10 the archesporium is seen, in addition to the other two regions. There can be no question as to its origin. These sections are not unlike Fig. 15, save in the latter the cell-divisions take place with diagrammatic regularity. In Fig. 11 the section passes through the extreme tip of the columella, of which three cells are shown. Figs. 12, 13, 14 embrace the remainder of the sporogonium above the columella.

The sporogonium of *Notothylas* undergoes intercalary growth, just as in *Anthoceros*, and the archesporium and columella extend quite to the foot (Fig. 19). In one case observed (Fig. 19), the cells of the foot were quite regular; they had not grown into the irregular tubes which is almost invariably the case. The intercalary growth is, of course, of comparatively short duration, but as long as it lasts there cannot properly be said to be a stalk in the sense of that term as used in the *Jungermannieae*. It is only when the capsule is mature that the cells immediately connecting foot and capsule elongate and finally break away from the foot, thereby severing all organic connexion with the latter.

As is stated by Leitgeb<sup>1</sup>, the size of the sporogonium is no proof of its age. Not infrequently does one find on the same thallus small sporogonia with others very much larger. Many of these smaller sporogonia, which appear under the dissecting-microscope to be younger stages, have in their apex spore-

<sup>1</sup> Loc. cit.

mother-cells, and sometimes spore-tetrads and ripe spores (Fig. 18). The columella is always present, but much smaller than those of the larger sporogonia (Fig. 18), and its cells are distinguished from those of the archesporium with some difficulty, especially if the section is not straight and more deeply stained. In the case figured in Fig. 18, the cells of the columella were little unlike those of the archesporium in regard to the contents of the cells. In all capsules examined, a columella was found extending up through the centre of the capsule nearly to the apex, varying in size with that of the capsule. From the columella layers of sterile cells extend radially to the capsule-wall, dividing the spore-chamber into more or less irregular cavities in which lie, proceeding downward from the apex, spores, spore-tetrads and spore-mother-cells. By making rather thick longitudinal sections through a nearly mature capsule, and freeing the spores by tapping gently with the end of a camel's-hair brush, one readily finds the columella with few sterile cells and spore-tetrads adhering (Fig. 21). The sterile cells fall out with the spores, and may be found scattered among them in the water. They are derived from the archesporium (Fig. 17). The capsule from which Fig. 17 was taken contained spores in its upper two-thirds: the columella is quite large, and the archesporium extends quite to the boundary between foot and capsule, where, in this case, the cells were just beginning to elongate preparatory to the separation of the capsule. In all robust and well-fed sporogonia (and many were examined) the cells of the archesporium were rich in protoplasm, staining deeply with alum-cochineal, and forming a sharp contrast to those of columella and capsule-wall. The structure of the mature capsule in the Anthocerotae has been carefully described by Leitgeb<sup>1</sup>, so that further details need not be given here. The process of division in the spore-mother-cells, as far as observation went, agrees with that given by Strasburger for *Anthoceros*<sup>2</sup>.

<sup>1</sup> Loc. cit.

<sup>2</sup> Zellbildung und Zelltheilung. Third edition, p. 158, &c. (1880).

As was stated in the preceding pages, there is marked variation in the size of the sporogonia of *Notothylas*. Fig. 20 represents a longitudinal section of a young sporogonium, differing considerably from all others observed—of the same size—especially in regard to the foot, which seems to have developed very little. From the fact that it was in a very slender lobe of the thallus, and that its cells with small nuclei were relatively poor in protoplasm, it is possible that we have here a starved specimen. No other similar case was observed.

From the history of development here observed it is evident that the columella of *Notothylas* (wherever it occurs) is of primary origin, and this, together with other parts of the sporogonium, agrees closely with *Anthoceros*. There is, therefore, a closer relationship existing between this and the other genera of the Anthocerotae than has been previously supposed.

#### THE ARCHEGONIUM.

The development of the archegonium was found to agree with the account of Janczewski and Leitgeb<sup>1</sup>. My preparations, however, showed the various stages very beautifully, and a few of these will be figured for the purpose of comparison (Figs. 22–24). In the mature organ, however, slight differences were noted. In both genera, the neck-cells of the archegonium are quite inseparable from those of the thallus; but in younger stages, in *Notothylas*, some of the neck-cells could be easily distinguished from the adjacent cells of the thallus by their denser contents. Occasionally the neck projected slightly above the surface of the thallus. In *Notothylas*, the neck-canal-cells were always fewer in number than in *Anthoceros*, amounting to three, not including the cap (Deckelzelle of Leitgeb), in the former (Fig. 22); and five or six in the latter, but not exceeding six in any case observed. In this respect, *Notothylas* resembles more closely certain

<sup>1</sup> Loc. cit. pp. 20, 21.



eusporangiate Ferns as *Angiopteris*<sup>1</sup>. Leitgeb<sup>2</sup> states that the lateral walls of the neck-canal-cells during the process of growth become very strongly thickened, and this thickening extends to parts of the cap (Deckelchen) and even to the central cell; that they are finally transformed into a gelatinous layer, while the cross-walls remaining very thin are completely dissolved. It seems to me that the lateral walls are not thickened at all, but the phenomenon is due to the presence of a swelling mucilaginous substance derived from the ectoplasm of the cells (Figs. 22, 23). This mucilaginous formation begins, it is true, at the ventral canal-cell, and proceeds upward. Very frequently, however, the egg is surrounded by a mucilaginous formation staining deeply with Bismarck-brown, while that in the neck stains only very slightly. The ventral canal-cell is usually as large as the egg in the mature organ (Figs. 22, 23).

In one case observed the large cap-cells projected considerably above the surface of the thallus (Fig. 24).

#### THE ANTHERIDIUM.

In regard to the antheridium, I am forced to the conclusion of Waldner and Leitgeb<sup>3</sup>, that this organ arises from a hypodermal cell, and that, if the mother-cell be, in any case, epidermal and become grown over later by the surrounding tissue, this process takes place at a time when the mother-cell cannot be distinguished as such.

Special care was taken in working out this particular detail, and in all the youngest stages recognizable, the mother-cell was found beneath the epidermis. Fig. 25 shows two mother-cells formed by the longitudinal division of an original mother-cell. It will be seen that the formation of the cavity in which the antheridia lie has just set in. Figs. 26 and 27 show two older stages. A further detailed account of the growth of the antheridium would be superfluous here. The

<sup>1</sup> Farmer, *Annals of Botany*, Vol. vi, pp. 265-270, Oct. 1892.

<sup>2</sup> Loc. cit. p. 21.

<sup>3</sup> Loc. cit. p. 17.

antheridial cavity is roofed over by a layer of two, occasionally three, cells in thickness. As the antheridia approach maturity they gradually burst through this roof, the cells of which separate near the centre and turn back, forming the edge of the cup-shaped cavity in which antheridia of different ages stand side by side. The cells of the roof, at the time of opening, appear more rounded, with thinner walls, so that it seems that a dissolving agent acts with the mechanical pressure of the antheridia. Very frequently there are much younger antheridia in the same cavity with the mature ones, a fact which seems to give further evidence of an endogenous origin.

The development of the antheridium of *Notothylas* is precisely like that of *Anthoceros*.

#### SUMMARY.

The results of the foregoing statements may be summed up as follows :

1. The capsules of *Notothylas orbicularis*, Sulliv. possess a columella varying in size with that of the capsule.
2. The columella originates, as in *Anthoceros*, primarily in the young sporogonium with the archesporium, and independently of it, and consequently it is not a secondary differentiation within the spore-chamber.
3. The archegonium of *Notothylas* resembles more closely that of the eusporangiate Ferns than does the archegonium of *Anthoceros*.
4. The antheridium arises from a hypodermal cell, a process occurring nowhere else in the whole group of Bryophytes.

I desire to acknowledge my indebtedness to Dr. L. M. Underwood for the use of important and necessary literature.

## EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Mr. Mottier's paper on *Notothylas*.

Fig. 1. The three successive longitudinal sections of an eight-celled embryo of *Notothylas orbicularis*; the arrow pointing toward the fore edge of the thallus.  $\times 520$ .

Fig. 2. Longitudinal section of an older embryo.  $\times 520$ .

Fig. 3. Longitudinal section of a young sporogonium of *Notothylas*, showing columella and four archesporial cells arching over it; *I-I*, basal wall.  $\times 372$ .

Fig. 4. Longitudinal section of an older sporogonium of *Notothylas*; the cells of the archesporium with contents indicated. Those just over the apex of columella have divided by tangential walls.  $\times 372$ .

Fig. 5. Similar to Fig. 4, but a little older.  $\times 372$ .

Fig. 6. Transverse sections of sporogonium of about the same age as 5; *a* taken at *a-a* (5); *b* at *b-b*, archesporium indicated; *c* at *c-c*.  $\times 372$ .

Figs. 7-14. All the successive cross-sections of a sporogonium of *Notothylas* a little younger than Fig. 5, from the basal wall upwards.  $\times 372$ .

Fig. 15. Transverse section of a young sporogonium of *Anthoceros*, showing the four central cells, the columella surrounded by the archesporium, and then the peripheral row.  $\times 372$ .

Fig. 16. Longitudinal section of an eight-celled embryo of *Notothylas* with the surrounding cells of the thallus.  $\times 372$ .

Fig. 17. Longitudinal section, showing half of the lower part of a nearly mature capsule of *Notothylas*, in which the origin of the sterile cells from the archesporium is evident. At the boundary between foot and capsule the cells are becoming slightly elongated preparatory to the separation of the capsule.  $\times 250$ .

Fig. 18. Longitudinal section of a small sporogonium of *Notothylas* with a portion of the foot omitted. Several spore-mother-cells lying loosely in the spore-chamber are rounded off and almost ready to divide. The columella is small, but unquestionably present; the archesporium extends to the region of the basal wall.  $\times 250$ .

Fig. 19. Longitudinal section of sporogonium of *Notothylas*, showing foot and lower portion of capsule. The cells of the foot are quite regular; the columella and archesporium extend entirely to the foot.  $\times 350$ .

Fig. 20. Longitudinal section of an apparently starved embryo of *Notothylas*, from a very slender lobe of the thallus.  $\times 372$ .

Fig. 21. Portion of a columella to which adhere several sterile cells and three spore-tetrads. Fresh preparation in dilute glycerine.  $\times 94$ .

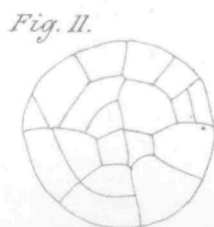
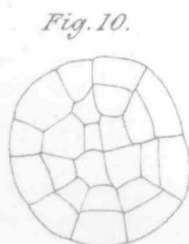
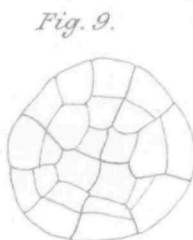
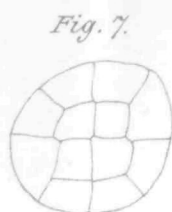
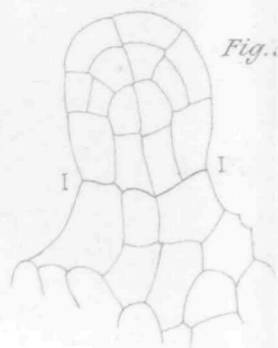
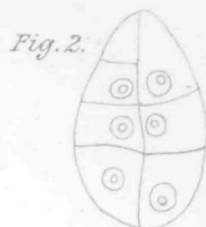
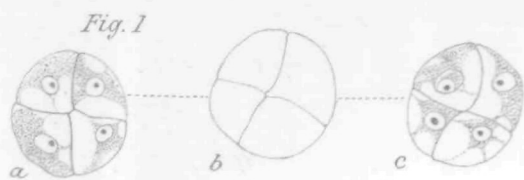
Fig. 22. Longitudinal section of an archegonium of *Notothylas* shortly before opening. The contents of the cells immediately surrounding the archegonium are indicated.  $\times 750$ .

Fig. 23. Longitudinal section of an archegonium of *Anthoceros*; the wall between egg-cell and ventral canal-cell has disappeared; the rounded contents of these two cells are surrounded by gelatinous substance, a condition almost invariably present when the organ is ready to open.  $\times 520$ .

Fig. 24. Similar to Fig. 23, but with high projecting cap-cells.  $\times 520$ .

Figs. 25-27. Longitudinal vertical sections representing three early stages in the development of the antheridium of *Anthoceros*. In Fig. 25 may be seen two antheridium-mother-cells formed by longitudinal division of a primary cell.  $\times 520$ . Figs. 26 and 27,  $\times 372$ .





*Fig. 19.*

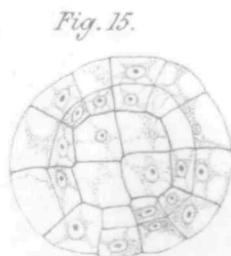
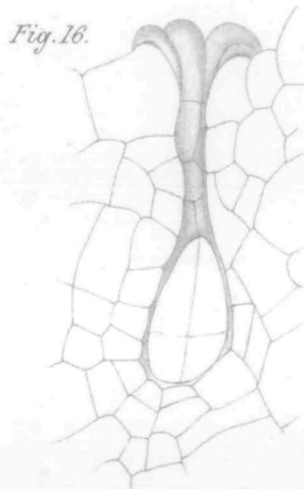
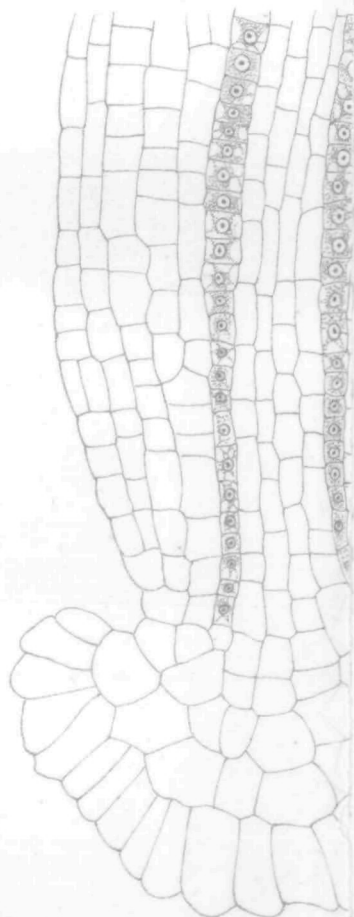
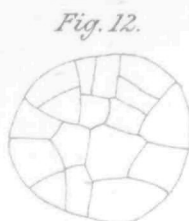


Fig. 4.

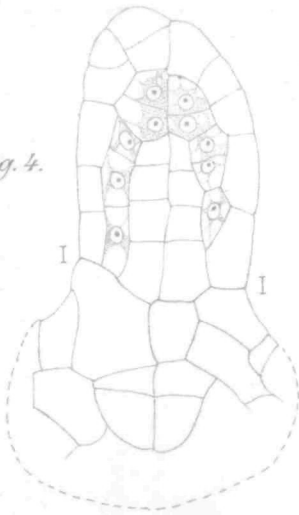


Fig. 5.

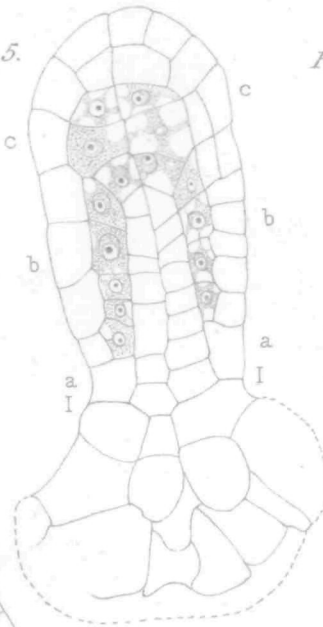


Fig. 6.

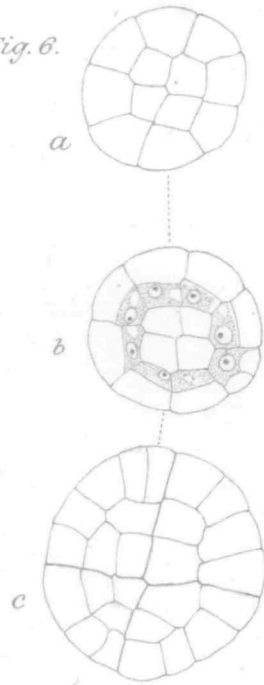


Fig. 18.

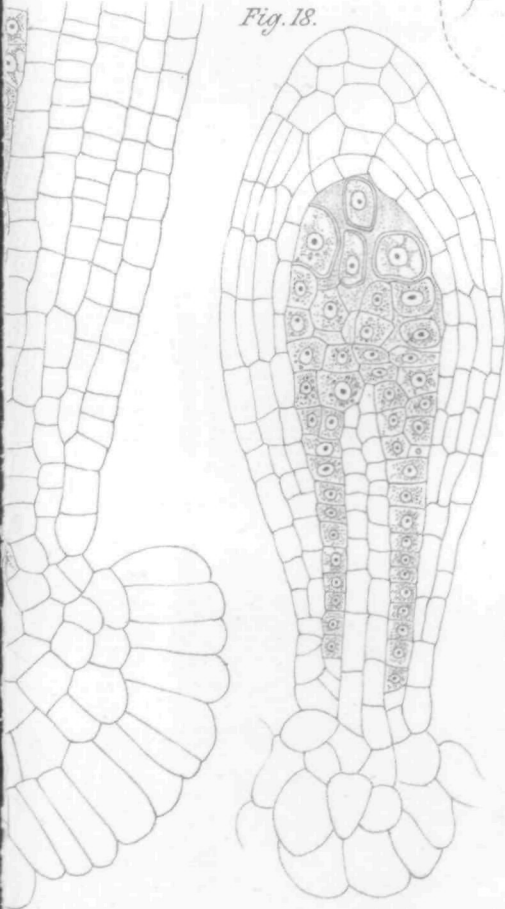
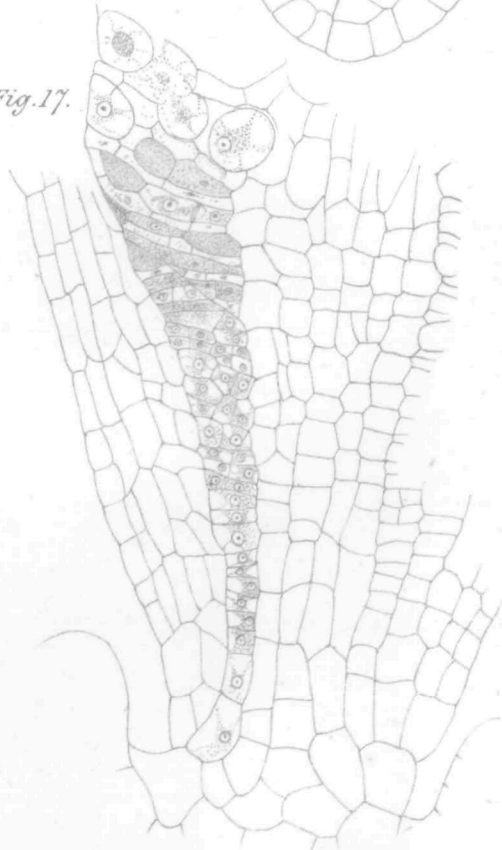


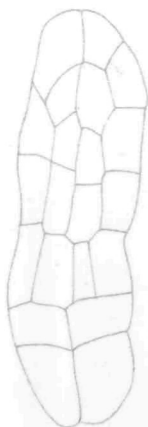
Fig. 17.



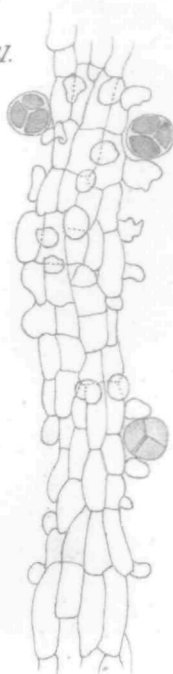




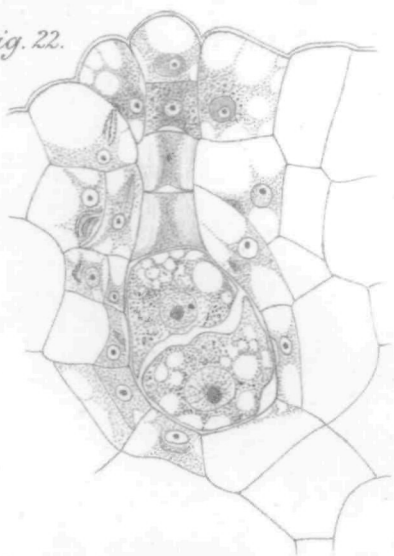
*Fig. 20.*



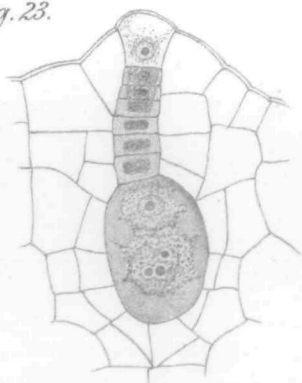
*Fig. 21.*



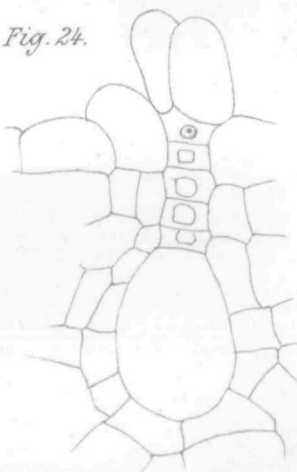
*Fig. 22.*



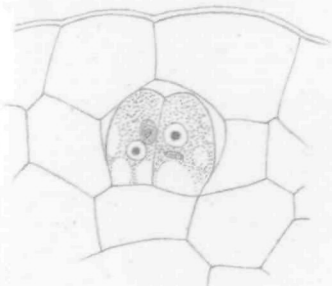
*Fig. 23.*



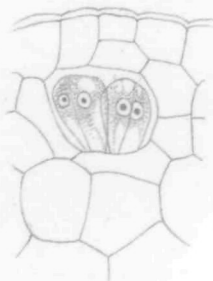
*Fig. 24.*



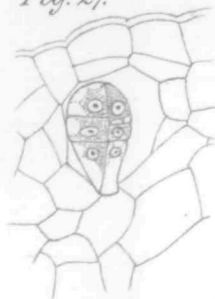
*Fig. 25.*



*Fig. 26.*



*Fig. 27.*



University Press, Oxford.