Campbell, D.H.

Studies on the Flower and Embryo of Sparganium. 1899.

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OF THE

CALIFORNIA ACADEMY OF SCIENCES.

THIRD SERIES.

BOTANY. VOL. I, NO. 9.

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Studies on the Flower and Embryo of Sparganium.

BY

DOUGLAS HOUGHTON CAMPBELL,

Professor of Botany, Leland Stanford Junior University.

WITH THREE PLATES.

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Issued July 21, 1899.

SAN FRANCISCO:

PUBLISHED BY THE ACADEMY.

1899.

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STUDIES ON THE FLOWER AND EMBRYO OF SPARGANIUM.

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Professor of Botany, Leland Stanford Junior University.

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THE genus *Sparganium* Tourn. includes about a dozen species distributed over the northern hemisphere, but also represented in New Zealand and Australia. The genus is a peculiar one, and there has been much diversity of opinion as to its affinities with other Monocotyledons. It has usually been associated with *Typha* in the family Typhaceæ, but the present view (Engler and Prantl, 1889) is that it should be considered the type of a separate family, Sparganiaceæ, with the Pandanaceæ or Screw-pines as its nearest allies.

The genus has not been extensively studied, but our knowledge of the development of the flower and embryo of the European *S. ramosum* is fairly complete (Hegelmaier, 1874; Dietz, 1887). The other species have, so far as I know, been treated from the standpoints of the descriptive botanist only, and no account of the embryo-sac has

yet been published. Some years since I collected a small amount of material of the flowers of the common eastern species, *E. eurycarpum* Englm., and an examination of this material revealed several puzzling peculiarities of the embryo-sac, which were not clearly understood at the time. Figures of a few of these were published two years ago (Campbell, 1897), but lack of proper material, as well as other work, prevented any further study at the time. During the summer of 1896 a trip to Japan enabled me to procure a few specimens of the Japanese *S. longifolium* Turcz., and during the past season a good supply of *S. simplex* Huds. was collected in August at Tallac on Lake Tahoe. This species proved very satisfactory for study, and was made the principal subject of my investigations. In September, through the kindness of Miss Eastwood, a quantity of *S. Greenii* Morong. collected at Lake Merced, near San Francisco, was sent me, and served to supplement the work done on *S. simplex*. My material of the latter species was much more complete than that of any of the others, and moreover it proved easier to handle. The results obtained from a study of this species have been compared, so far as was possible, with those from the other species mentioned.

A preliminary statement in regard to *S. simplex* has already been published in a recent number of the "Botanical Gazette" (Campbell, 1899); but this refers simply to certain of the most striking points concerning the embryo-sac, and no account of the embryo is included.

Unfortunately all of the material collected was too old to show the earlier stages of development of the flower, so that the early history of the pollen and embryo-sac could not be traced. In the youngest flowers examined, the structures of the embryo-sac were already complete, and in most cases the embryo-sac was ready for fertilization. Hence in the present paper the study of the flower begins at the time when it is ready for pollination.

In the preparations of the material, various reagents were employed in fixing it: one-half to one per cent. chromic acid,

saturated alcoholic solution of picric acid, saturated alcoholic corrosive sublimate, and weak Flemming's solution were employed, but of these the corrosive sublimate, on the whole, proved most satisfactory. The material was imbedded in paraffin and the sections were stained, usually with a double stain of Bismarck-brown and safranine, although in some cases they were stained *in toto* with alum-cochineal before they were imbedded.

I. The Flower.

All of the species of *Sparganium* are monœcious, both staminate and pistillate flowers being aggregated in dense heads which are either sessile or borne on short pedicels. The lower heads are always pistillate and the upper ones staminate. The latter are usually much more numerous. In *S. simplex* and its allies the main axis is unbranched, while in the other species, e. g., *S. eurycarpum* and *S. Greenii*, the stem is branched.

Both sorts of flowers are exceedingly simple in structure. The individual flowers are borne in the axils of small bracts, and are themselves surrounded by a varying number of small membranous leaves which are usually considered to represent a very simple perianth. The male flower is composed of three or more stamens, while the female flower has either a single carpel, as in *S. simplex*, or a compound pistil composed of two completely united carpels, as in *S. ramosum* or *S. Greenii*.

The development of the flower has been studied to some extent in *S. ramosum* (Dietz, 1887), and is briefly as follows: Upon the primary axis are borne elevations which, in the lower part of the axis, where they are produced in axils of leaves, may develop into secondary axes, but in the upper portions of the axis, form at once the young inflorescences. In *S. simplex* and its allies, this axis does not branch, and all of the lateral axes develop into heads of flowers.

In *S. simplex* the lower three or four of these heads develop the pistillate flowers, while the number of staminate heads is usually larger.

The individual flowers, according to Dietz (l. c.), arise as small prominences upon the hemispherical fundament of the inflorescence, and upon these prominences arise secondary outgrowths which develop respectively into stamens or carpels. Dietz does not describe in detail the development of the stamen, and unfortunately my own material was too old to show this, so that it is not now possible to make a comparison with such Monocotyledons as have been investigated.

The scales of the perianth arise early, but Dietz does not describe their development.

To judge from his somewhat brief account and very diagrammatic figures, the development of the carpel and ovule is much like that of the low types like *Zannichellia* and *Lilæa*, which *Sparganium* resembles in these respects more than it does *Typha*, with which it has usually been associated. While further investigations are required before this can be decided, it looks as if in *Sparganium*, also, as in other low Monocotyledons, that the solitary ovule is of axial origin and not a product of the carpel.

The pistillate flower in *S. simplex* (fig. 1) consists of a single carpel surrounded by about six delicate membranaceous scales which form the perianth. Engler (Engler and Prantl, 1889) figures these in this species as having entire margins, but the form studied by me had the end of the narrowly spatulate scale sharply toothed (fig. 2), and the most recent figure (Britton and Brown, 1896) of this species shows the same thing, but with fewer teeth than my specimens. A delicate midrib traverses each scale. The ovary in this species merges gradually into the slender style which terminates in the narrowly sagittate stigma which is covered with minute papillæ.

In *S. longifolium*, which in many respects resembles *S. simplex*, the style and stigma are both shorter. In a second group represented by *S. Greenii* and *S. eurycarpum* the

pistil is composed of two completely united carpels, and the resulting fruit is two-seeded. In both of these the style is very short or quite absent and the two stigmas are very long (fig. 4). The base of the ovary is also less tapering than in *S. simplex* and the perianth scales are heavier and nearly or quite entire at the apex.

S. simplex, which was the species principally studied by me, is a wide-spread form occurring throughout the northern hemisphere. In California it is confined to the higher mountains, the specimens collected by me being found near Lake Tahoe which has an elevation of over 6,000 feet above sea-level.

The Staminate Flower.

In *S. simplex* the staminate flowers form dense heads, each flower consisting of about six stamens. Just before the dehiscence of the anthers, the filaments elongate very much, so that the head becomes much looser, and the pollen is readily dislodged by the swaying of the slender filaments, much as in the grasses and sedges. The anther has four loculi as in most Angiosperms and offers no specially noteworthy peculiarities. The other species seem to agree closely in the structure of the stamen and were not critically examined.

The pollen-spores of *S. simplex* are small globular cells (fig. 6) and at maturity show the usual division of the nucleus into a larger vegetative one, and a smaller generative nucleus, but the latter was not seen to divide again while the pollen remained within the anther. Probably the final division of the generative nucleus takes place within the pollen-tube. Perhaps owing to its small size it was not possible to demonstrate the presence of a definite generative cell, the nucleus apparently lying free in the cytoplasm of the pollen-spore. As the cytoplasm of the ripe spore is very dense and stains strongly, it was not always easy, even in sections, to clearly distinguish the nuclei. The outer spore-membrane is marked with fine granulations but is not very thick. In this species there was almost always present in

the ripe spore a structure which closely resembles the small vegetative prothallial cell in the germinating microspores of such heterosporous Pteridophytes as *Isoetes* and *Selaginella*. Chamberlain (1895) speaks of such a prothallial cell as an occasional occurrence in *Lilium Philadelphicum*, but except for this reference I am not aware of any reference to such a structure in the angiospermous pollen-spore. This prothallial cell, if such it is, in *Sparganium simplex* is lenticular in form (fig. 6), and separated from the body of the spore by a very well defined membrane which stains strongly with Bismarck-brown. While it was not possible to demonstrate positively the presence of a nucleus in this cell, there was usually to be seen a central body which stained more deeply than the rest of the cell with the usual nuclei stains and had much the aspect of a partially disorganized nucleus. While it seems very likely that this really represents a prothallial cell, homologous with the similar one in the microspores of *Isoetes*, for instance, its real nature cannot be positively assumed until its history has been followed.

The stamens of *S. Greenii* closely resemble those of *S. simplex*, but the pollen-grains did not show any trace of the prothallial cell, and the epispore is marked with fine reticulations. At one point (fig. 7) these were absent, and the clear area probably marks the point at which the pollen-tube emerges.

The Pistillate Flower.

The pistil may be composed of a single carpel, as in *S. simplex* and *S. longifolium*, or of two completely united ones, as in *S. Greenii* and *S. eurycarpum*. In *S. simplex* (fig. 3) a longitudinal section of the ovary shows the single anatropous ovule pendent from the upper part of the ovarian cavity. The narrow canal traversing the style opens into the ovary close to the base of the funiculus of the ovule.

The funiculus is rather slender and has a single axial vascular bundle. The nucellus is oval and there are two integuments usually found in the ovules of Monocotyledons.

The outer integument extends above the inner one and its upper margins are somewhat enlarged, so that the micropyle is quite closed.

II. THE EMBRYO-SAC.

The youngest embryo-sacs met with had the egg-apparatus and antipodal cells developed, and nothing can be stated as to their early history. As the ovule at the time of fertilization is of the ordinary type, it is not probable that there are any marked deviations from the type in its early development.

In *S. simplex* (figs. 3, 8) the mature embryo-sac is broadly oval in outline and is covered at the apex by about two layers of nucellar cells, the central ones somewhat elongated, so that the apex of the nucellus is slightly pointed. At the sides there are about four layers of cells in the nucellus, and these remain permanently and are not destroyed by the developing embryo-sac as so often happens. Within the embryo-sac the granular cytoplasm is principally confined to a thin parietal layer, except that surrounding the primary endosperm-nucleus there is a considerable amount of granular cytoplasm.

In *S. Greenii* (fig. 5) the two carpels are completely united. A section of the ovary shows a long central placenta formed by the coherent inner faces of the carpels, and from the upper part of the placenta depend the two ovules. These correspond in structure with those of *S. simplex* but are somewhat longer and more slender, although they are less constant in form than in that species. The embryo-sac is longer and narrower and more pointed at the ends. The part of the nucellus above the apex of the embryo-sac is thicker, there being usually about four layers of cells at this point.

Sparganium longifolium is much like *S. simplex* in the form of the pistil, except for the shorter style and small stigma. The ovule is of about the same size and shape, and the embryo-sac, so far as could be judged from the

small amount of material available, closely resembles that of *S. simplex*, and as in that species there are but two, or at most three, layers of cells at the apex of the nucellus.

The common eastern *S. eurycarpum*, while resembling more nearly *S. Greenii*, differs from it in some details. The ovary is broader, due to an enlargement of the outer cells, and there are numerous enlarged cells present which contain bundles of rhaphides, or needle-shaped crystals, presumably of calcium oxalate. These spicular cells also occur in the placenta, and while not entirely absent from the ovary of *S. Greenii* are much less abundant. There are also found on the upper surface of the placenta papillate cells which are much less marked in *S. Greenii*. These are doubtless connected with the conduction of the pollen-tubes. The structure of the ovule itself, and the embryo-sac are much alike in the two species.

In all the species examined the cells of the nucellus persist, and the subsequent enlargement of the upper part of the integuments and the apex of the nucellus gives rise to the peculiar cap ("Samen-deckel" of Hegelmaier) which characterizes the ripe seed of all species of *Sparganium*.

The broadly oval embryo-sac of *S. simplex* is scarcely at all narrowed at either end. In all specimens examined the two polar nuclei had already completely fused, and it is evident that the fusion of them to form the primary endosperm-nucleus occurs some time before the fertilization of the egg-cell. The large endosperm-nucleus (fig. 10) is imbedded in a considerable mass of granular cytoplasm which is elsewhere confined to the thin parietal layer. The position of the endosperm-nucleus is not constant, and it was found in nearly all positions in the sac; sometimes it lay close to the egg-apparatus, sometimes it was near the antipodal cells.

The egg-apparatus is of the normal structure, and compared to the size of the embryo-sac is small. A good deal of variation in the size of the egg and synergidæ was noted, and these differences did not appear to be necessarily connected with the age of the egg-apparatus. The

egg-cell was sometimes in the same plane as the synergidæ (fig. 8), and sometimes it was lower down and a good deal elongated (fig. 11). In either case it was nearly hyaline, with only a relatively small amount of granular contents surrounding the nucleus which is usually placed near the free end of the ovum, i. e., the end which projects into the embryo-sac (figs. 8, 11). The synergidæ are alike, somewhat smaller than the egg and filled with densely granular cytoplasm in which lies the small nucleus. All the nuclei of the egg-apparatus have a very distinct nucleolus which is especially conspicuous in material stained with anilin-safranine. The rest of the nucleus, however, does not stain strongly with this reagent, the nuclei preliminary to fertilization appear to have very little chromatin. The nucleus of the egg is somewhat larger than the synergidal nuclei but otherwise closely resembles them.

Above the synergidæ there was seen in many specimens what looked like the remains of the tapetal cells (fig. 8). This mass stained strongly with Bismarck-brown but showed little structure and probably represented the disorganized remnants of one or more tapetal cells. As the younger stages were lacking in my preparations the origin of this body can only be conjectured.

In none of the specimens of *S. simplex* were the separate polar nuclei observed. The large endosperm-nucleus resulting from their fusion is, however, conspicuous. It is much larger than the other nuclei of the embryo-sac, and has a very large nucleolus (figs. 10, 11).

The antipodal end of the sac in this species is almost flat, although there may be a slight depression where the antipodal cells are found. The early history of the latter could not be followed, as the material was all too old; but at the time the egg is mature, these cells are remarkable for their very small size, which is in striking contrast to their later development. Not infrequently where these cells had collapsed in the process of embedding, they could not certainly be distinguished, and may very easily be overlooked. There was nothing, however, to indicate that they are ever

really absent. Where they are uninjured (fig. 12) they may usually be seen to be arranged in the same plane and are very shallow, projecting only very slightly into the cavity of the embryo-sac. The cytoplasm of these cells is finely granular but does not readily stain at this stage. The nuclei are very small and inconspicuous, and it is sometimes difficult to demonstrate them at all. In no case did they stain readily with the usual reagents.

In *S. Greenii* the appearance of the antipodal cells is somewhat different and this resembles more those of other Monocotyledons (fig. 21). The base of the embryo-sac is here prolonged into a narrow cavity within which lie the antipodal cells. These are not usually in the same plane, but one of them lies above the other two, to which it bears much the same relation that the egg-cell does to the synergidæ. The antipodal cells in this species are much larger than in *S. simplex*, and the cell contents denser, and the nuclei are larger and readily demonstrated, as they stain without difficulty.

So far as my observations go upon *S. longifolium* and *S. eurycarpum*, they are more like *S. Greenii* than *S. simplex* in the form of the antipodals, although *S. longifolium* is to some extent intermediate in character.

III. FERTILIZATION.

The small size of the pollen-spore and nuclei is not favorable to a study of the details of fertilization, and although in several instances the pollen-tube was detected within the embryo-sac, nothing was observed which indicated that the fertilization was in any way different from that of other Angiosperms. The pollen-tube after reaching the micropyle pushes down between the cells of the nucellus, which are not injured by its passage. On entering the embryo-sac, it apparently comes into close contact with one synergid, which is probably destroyed in most cases, although this is not necessarily the case, as in one instance, at least, the end of the pollen-tube was seen within the embryo-sac, although both synergidæ were still intact. The very

small size of the generative nucleus makes it extremely difficult to detect, and only a few incomplete observations were made on its behavior after entering the sac. In one case (fig. 9) a small, deeply stained body was observed within one of the synergidæ, and this very probably was one of the generative nuclei on its way to the egg-cell. In another instance (fig. 13) there was visible within the egg a small body looking like a nucleus, but not staining as deeply as might have been expected of the male pronucleus. In the same preparation (fig. 14) there was a small granular mass attached to the end of the pollen-tube, and looking as if it might have been discharged from it into the cavity of the embryo-sac, but there was nothing to indicate the discharge of the second generative nucleus and its fusion with the endosperm-nucleus as described by Nawaschin; however, my observations were too incomplete to warrant any positive statement on this point. One of the synergidæ can usually be detected for a long time after fertilization has been effected, as has been observed in so many other cases.

After fertilization has been effected the egg-cell enlarges very little, and after the membrane is developed about it, it remains unchanged for a long time. At this time the unicellular embryo (fig. 24) is a more or less pear-shaped cell, sometimes in contact with the upper wall of the embryo-sac merely by a very small part of the pointed lower end, sometimes having a much broader base of attachment. In this condition it remains until the sac has increased a good deal in size and the development of the endosperm is well advanced. Owing to this slow development of the embryo the earlier stages between the unicellular condition and that shown in fig. 37 were missed.

IV. The Secondary Growth of the Antipodal Cells.

While the embryo-cell remains unchanged for a long time, the other structures of the embryo-sac undergo marked changes. Almost the first visible result of fertilization is a marked increase in the size of the antipodal cells. In

S. simplex where this was especially studied, and of which a preliminary account has already been published (Campbell, 1899), this is especially noteworthy. Before fertilization (fig. 12) the antipodal cells are extremely inconspicuous, but almost immediately after fertilization has been effected they rapidly increase in bulk, and at the same time show other marked evidences of active growth. The cytoplasm becomes more coarsely and densely granular, and the nuclei enlarge to several times their original size and at the same time take up stains much more readily than before (fig. 26). As the antipodals increase in size they project strongly into the cavity of the embryo-sac and form a conspicuous nearly hemispherical body. The three original cells now rapidly divide until finally a very large mass of cells (fig. 30) results, probably larger than in any other Monocotyledon. The number of antipodal cells may finally exceed 150, a number greater than that yet recorded for any other Angiosperm.

In position and general appearance the group of antipodal cells most nearly recalls that of many Gramineæ (Hofmeister, 1861; Fischer, 1880, etc.). An important difference is that in the latter the development of the antipodal complex is completed previous to the fertilization of the egg. The method of its development, however, is very similar in the two cases.

The embryo-sac in those grasses which have numerous antipodal cells has at first the ordinary number, three, and these subsequently divide to form the larger number ultimately developed. Hofmeister (1861) states that in the Triticeæ the number may be 6–12 and Körnicke (1896) found 36 or more, a large number, but very much less than the normal number finally developed in *Sparganium simplex*.

The first division of the nuclei of the antipodal cells occurs shortly after the first division of the primary endosperm-nucleus. Following the first division, the increase in size of the antipodal cells and the divisions proceed rapidly, while the endosperm at this stage remains slightly

developed. There seems little question, that as Westermaier (1890) pointed out in the grasses, these enlarged antipodal cells replace physiologically for the time being the endosperm, and elaborate food materials for the growth of the developing embryo-sac and embryo.

The first divisions in both endosperm and antipodal cells take place before there has been any marked increase in the size of the embryo-sac; but later there is a rapid increase in the size of the ovule, which probably coincides with the first divisions in the embryo.

Körnicke (1896) in his studies on the antipodal cells of various Gramineæ was unable to detect any karyokinetic figures in these and was inclined to think that the divisions of the nuclei might be direct. This view appeared to be hardly likely, as the nuclei in the developing antipodal cell of *Sparganium* appeared entirely normal, and the cells were actively growing and dividing, not a condition in which one would look for direct nuclear division. Fortunately a preparation was finally secured in which some of the antipodal nuclei were actually in process of division (fig. 29), and it was seen that the division is the typical karyokinesis, which it is safe to say is the normal type of nuclear division, at least in the early stages. In the case under consideration the endosperm-nuclei were also dividing.

In most cases, possibly always, the first division of the nucleus in the antipodal cells in *S. simplex* is not accompanied by the formation of a division-wall, and the enlarged antipodal cells show plainly two conspicuous nuclei (fig. 29). Later, however, all the nuclei become separated by walls and each of the very numerous antipodal cells has evidently but a single nucleus (fig. 30). In some older ones, however, two nuclei were sometimes seen, so that it would seem that the last nuclear division, like the first one, may be independent of cell division. There is thus a certain analogy with the behavior of the normal endosperm-cells which would indicate that the antipodal cells are not markedly different in nature from the endosperm-cells, and may be really homologous with these.

The densely granular cytoplasm of the antipodal cells does not fill them uniformly, but there are usually present large vacuoles (fig. 28). The first division-walls in the antipodals of *S. simplex* are vertical, so that the mass has the form of a disc, but later transverse and oblique divisions may also occur, although the majority of the walls are vertical and the cells become a good deal elongated (fig. 30).

As the seed grows older the antipodal cells begin to show signs of disintegration, but in no cases where sections were made had they entirely disappeared, and traces of them probably persist even in the ripe seed.

The earlier stages of the antipodals were studied also in *S. Greenii* and *S. eurycarpum*, but material was not available for the later development which is probably not entirely like that in *S. simplex*. In *S. Greenii* the antipodals at the time the egg is fertilized are, as we have seen, much more conspicuous than in *S. simplex*. They are also differently arranged, not usually lying in the same plane, but one of them being above the other two (fig. 33). In one instance (fig. 16) there was present above the three antipodal cells a large cell which looked like a fourth antipodal, but as this was the only case seen, there was no clue as to its origin. Otherwise the embryo-sac appeared to be entirely normal. In this species *(S. Greenii)* the polar nuclei remained separate, although often in close contact (fig. 20) until after the fertilization of the egg, and in this respect it differs from *S. simplex* and probably from *S. eurycarpum*. There is also much greater variation in the size of the embryo-sac in *S. Greenii* than in *S. simplex*. As a rule the egg and synergidæ are larger also than in *S. simplex* and sometimes the former show a reticulate appearance in the cytoplasm, due to the presence of numerous vacuoles (fig. 18), an appearance which was not seen in the specimens of the latter species. The embryo-sac in *S. Greenii* is relatively narrower than in *S. simplex*, and there are three or four layers of cells at the apex of the nucellus, which is also larger than in that species. As we have already stated, the antipodal cells, before the fertilization of the egg, are noticeably

larger than in *S. simplex*, and two are placed below the third one, and lie in the depression at the base of the embryo-sac. This is much narrowed, and may account for the fact that sometimes these two lower antipodal cells take no part in the formation of the group of antipodals formed after fertilization (fig. 36). In such cases they remain for a long time quite unchanged, and ultimately show signs of disorganization. The upper of the three original cells, however, enlarges greatly and assumes a vesicular form, projecting strongly into the cavity of the embryo-sac. The nucleus of this cell, as in the older antipodal cells of *S. simplex*, enlarges very much, soon undergoes division, and this is quickly repeated, so that four cells, arranged quadrant-wise, result. Where this was the case division-walls could be seen between the nuclei. Sometimes, however, the lower antipodal cells also enlarge (fig. 35) and the development proceeds much as in *S. simplex*, except for the relative positions of the cells. How far the antipodal cells may finally develop in *S. Greenii* could not be ascertained, as the next stages were absent from my material. So far as could be judged from the few stages examined, the cells show a tendency to become more inflated than in *S. simplex* and the granular contents are less abundant, but these peculiarities, which seem also to characterize *S. eurycarpum*, may not be constant, and a study of the later stages in these species will be necessary before the question can be definitely decided.

V. The Embryo.

The development of the embryo does not, at first, keep pace with the development of the antipodal cells and endosperm, and for some time it remains without any apparent change in appearance. Although a large number of sections were made, it was impossible to find stages between the unicellular embryo and such advanced stages as that shown in fig. 37. It is therefore impossible at present to compare the first divisions in the embryo of *S. simplex* with the corresponding stages of that in *S. ramosum*, the only

species hitherto examined. As the later stages agree quite closely in the two species, it is not likely that there are any very marked differences in the early stages. Nevertheless, it is much to be regretted that these early stages could not have been compared, and it is to be hoped that these may be examined soon in our American species.

According to Hegelmaier (1874) the embryo cell in *S. ramosum* divides into three superimposed cells, but it is not clear whether the second division is in the upper or lower of the two primary cells. Before any longitudinal divisions are formed there may be one or two more transverse divisions, making thus a single row of four or five primary segments in the embryo. A very similar account is given by Nörner (1881) for the young embryo of several grasses, except that he criticizes Hegelmaier's statement as to the time of the fourth and fifth transverse walls, claiming that these are probably not formed until after the first longitudinal divisions. He concludes that three is the regular number of the primary segments in most Monocotyledons. The more recent investigations in the embryo of the lower Monocotyledons (Schaffner, 1896; Chamberlain, 1895; Campbell, 1897, 1898, etc.) indicate that the basal cell, where this becomes enlarged, does not divide further, and that all the further transverse segmentation is in the terminal cells, but it is probable that in *Sparganium* the segments are the product of the division of the original basal cell.

So far as could be judged from an examination of the somewhat advanced embryo of *S. simplex* (figs. 37, 38, 39), it looked as if the first longitudinal divisions in the terminal segment occurred earlier than Hegelmaier gives for *S. ramosum*, and it is doubtful whether here there are more than three primary segments. A comparison of these stages with Hegelmaier's figures 5 and 13 indicated that possibly his segment 2 really belongs to the terminal segment.

The basal segment never becomes enlarged as it does in so many Monocotyledons, but remains usually very small and generally divides early by longitudinal walls into three or four cells, but sometimes a cross-section shows but two

cells. The absence of the large vesicular suspensor cell found in *Naias*, *Alisma* and other Monocotyledons is largely a physiological phenomenon, doubtless connected with the nutrition of the developing embryo, and is intimately associated with the degree of development of the endosperm. Hegelmaier (1874) found that in *S. ramosum* the terminal segment gave origin to all the structures of the embryo except a portion of the root-apex. As he worked entirely with embryos which were simply rendered transparent, but not actually sectioned, he was unable to make out successfully all the details of the arrangement of the tissues in the older embryos. Nevertheless, his results, on the whole, were remarkable accurate, and show a close correspondence to my own preparations of *S. simplex* which were obtained from actual sections. He found that the cotyledon and stem-apex are both products of the terminal segment, and that the plerome of the root is also derived from this segment, and terminates at the wall separating segments I and II.

My own studies of the embryo were necessarily confined almost exclusively to *S. simplex*, as the material collected of the other species did not contain the proper stages of the young seed. The youngest embryos obtained are shown in figs. 37–40. While these agree in general with Hegelmaier's figures of similar stages in *S. ramosum*, there are certain differences which may be noted. The basal, or primary transverse wall (*x*) which corresponds probably to his wall (*i*), is regularly lower down than his figures, indicating that the terminal cell of the young embryo is from the first larger than in *S. ramosum*. It is possible that his segment II may be included in the portion above the basal wall, but this is not probable, as evidences of median vertical walls extending from the apex of the embryo to the basal wall can generally be made out, and from a study of the older stages it is evident that the plerome of the root extends quite to the basal wall, as Hegelmaier describes for *S. ramosum*. The hypobasal portion of the embryo narrows rapidly, and this portion shows ordinarily but two transverse

segments, instead of the three or four found in *S. ramosum*. The small basal segment, which is in contact with the apex of the embryo-sac, varies a good deal in size, this being correlated with the form of the original embryo-cell. If this tapers much, so as to have a small point of attachment, the basal segment remains very small and pointed (fig. 42), but if the embryo-cell is flattened where it is in contact with the wall of the embryo-sac, the basal segment of the embryo is correspondingly broader and shows more divisions.

A median section of such an embryo shows the limits of the three primary segments still visible (fig. 38). This terminal segment is very much larger than the others, and, as already stated, relatively larger than in *S. ramosum*. The first divisions in all the segments are median vertical ones, and it is clear from studies of transverse sections that there are normally intersecting median walls which divide each segment into four equal quadrants. The divisions are less constant in the basal segment, especially when it is small, and transverse sections of this often show only two or three cells, one or both of the second median walls being suppressed. The next divisions are usually vertical also, but there is evidently no absolute rule as to their arrangement. In fig. 40 is shown a series of cross-sections of a young embryo, and it is clear that the divisions are not always entirely alike in the different quadrants of the same section (see fig. 44, *b*). Probably, in most cases, the first division-wall formed in the quadrant is curved, and extends from the quadrant-wall to the periphery. This is then followed by a series of periclinals which cut off the epidermis. Sometimes, however, it looks as if the first walls were periclinals, thus determining at once the separation of the epidermal layer. Hegelmaier shows much the same variation in *S. ramosum* (e. g. his figures 6 and 8).

The variations in the basal segment have already been referred to. It may divide into equal quadrants (fig. 44, *a*), but more commonly there is a suppression of one or both of the second quadrant walls. Where the basal segment

is unusually large, transverse or oblique divisions may also occur (fig. 39). A transverse section of the second segment at this stage shows eight peripheral cells surrounding four central ones.

In the upper segment, the next divisions are transverse, and sometimes this gives the appearance of a complete transverse division across, and it is possible that the segment numbered II by Hegelmaier may in some of his figures really refer to a secondary transverse division in the terminal segment. The separation of the epidermis is brought about as in the second segment, and soon after another series of similar divisions separates the central group of plerome cells from the periblem. The plerome extends to the basal wall where it ends abruptly, not being found at all in the two lower segments. In cross-section (fig. 44, *c*) the young plerome shows four cells, one belonging to each quadrant, but there are soon found other longitudinal walls which increase the number of plerome cells.

The older embryo is not perfectly cylindrical in form, but is somewhat broader in the plane of the future cotyledon. Sections made in this direction have the upper segment nearly circular in outline, but the median wall is often somewhat oblique, so that the segment is not always perfectly symmetrical. In this view the small basal segment usually shows a vertical division into two nearly equal cells. In the cotyledon, which constitutes the major part of the terminal segment, the epidermis is already clearly differentiated, but this is not always so evident in the second segment, where the interlayer of cells usually undergoes another periclinal division, which is never the case, so far as I could determine, in the epidermis of the cotyledon. If the section is made at right angles to the face of the cotyledon (fig. 42) the embryo appears more pear-shaped in outline, and the basal segment may appear undivided. Cross-sections of similar embryos show that in such cases the basal segment is composed of but two or three cells. In a number of instances the second transverse wall was very oblique (fig. 42) and might even intersect the basal

wall, and such embryos resemble very much the embryo of such grasses as *Triticum* and *Hordeum*, figured by Nörner (1881). Hegelmaier does not show these oblique walls in his figures of *S. ramosum*, and in this respect *S. simplex* is more like the grasses than is *S. ramosum*.

The primary tissues are now pretty well defined. The central plerome-strand extends to the basal wall and is separated from the epidermis by one layer of periblem cells (fig. 46). In the segment below, the superficial cells, as we have seen, divide again by periclinal walls, so that there are often two well defined layers of cells, evidently derived from a periclinal division of the primary epidermis. In the basal segment there are seldom more than four cells, and in case there is another segment between the basal one and the second segment (fig. 51) its divisions are quite irregular.

Hegelmaier's account of the origin of the different members of the embryo in *S. ramosum* corresponds closely to what was seen in *S. simplex*. Much the greater part of the embryo is taken up by the cotyledon which elongates rapidly, so that the embryo soon becomes several times longer than broad, this growth in length being mainly in the cotyledon, the stem and root remaining short. The stem-apex originates from the terminal segment, but on one side well toward the base. Its position and origin correspond closely to those in the grass-embryo, to judge from Nörner's account and figures (Nörner, 1881). In its origin from the terminal segment it differs from such Monocotyledons as *Alisma* and *Naias*, where the stem-apex belongs to the second segment. *Zannichellia*, however, has a terminal stem-apex, and Solms-Laubach (1878) describes several Monocotyledons with terminal stem-apices. In *Lilæa* (Campbell, 1898) the stem-apex is probably derived from the terminal segment as in *Sparganium*. *Sparganium* is, therefore, in regard to the origin of the stem-apex, intermediate between such extreme forms as *Zannichellia* and the majority of Monocotyledons investigated, in which the stem-apex arises from the second segment.

Cross-sections of the older embryo are usually more or less oval in outline, the longer axis coinciding with the face of the cotyledon. In sections made just above the basal wall (fig. 47), the first indication of the stem-apex (*st*) is evident in a more rapid division in the epidermis where the cells are decidedly narrower. At this time, the section of the plerome shows about nine cells, and the original quadrant divisions are very evident. The stem-apex appears to be the product of one only of the quadrants, and soon becomes sunk in a depression formed by the excessive growth of the basal cells of the cotyledon immediately adjacent to it (fig. 44). The stem-apex later becomes deeply sunk in the narrow cavity formed by the excessive growth of the base of the cotyledon, and this forms a sheath, such as is so frequently met with among the lower Monocotyledons (figs. 49 and 53).

The plerome-strand is continued upward into the cotyledon and downward into the root, but there is no trace of a cauline bundle. The other tissues of the root arise from the cells of the second segment of the embryo. This also divides at first by quadrant walls, and later a central growth of four cells (fig. 44, *b*) is separated from an outer row of cells. The latter again divides into two by periclinal walls. The initial group of cells, which contributes to the growth of the periblem, dermatogen and calyptrogen, is derived from the four central cells which may be said to constitute the initial for all the tissues of the root except the central plerome-cylinder.

Before the embryo is mature, the second leaf makes its appearance on the side of the stem opposite the cotyledon. The nearly flat stem-apex develops a protuberance on its outer side, and this quickly assumes the form of a short cylindrical body whose tissues are continuous with those of the stem, except that the plerome-cylinder connects with that of the root.

The embryo rapidly increases in size as the seed ripens, and finally occupies the whole axial part of the embryo-sac. The space between it and the wall of the sac has in the

meantime become completely filled with the endosperm. The full-grown embryo (fig. 52) is a spindle-shaped body, of which the cotyledon constitutes much the greater portion. The outer edges of the sheath at the base of the cotyledon overlap so as to completely conceal the stem-apex and second leaf (fig. 54).

A longitudinal section through the base of the full-grown embryo (fig. 53) shows that the root is very broad and short. Occupying its axis is the conical plerome which in section shows about six rows of cells; this ends at the basal wall whose limits are still discernible. No single initial cell could be made out at the apex of the plerome, nor could any absolute regularity in the segmentation of the terminal cells be recognized. Connected with the main plerome-strand are smaller branches extending into the young leaves, but, as already stated, none is given off to the stem. Between the plerome of the root and the epidermis there are five or six layers of cells.

The origin of the other tissues of the root can be traced back to the central group of cells lying next the apex of the plerome and derived from the central cells of the second segment of the embryo. There are probably four of these initial cells, and two can usually be seen in longitudinal sections. Periclinal segments are cut off from those which add to the root-cap, and lateral segments are also formed which, dividing again, form the initials for the dermatogen and periblem. The primary root of the embryo of *Sparganium* therefore conforms closely to the type described by De Bary (1884), as found most commonly in Monocotyledons. The root-cap is well developed in the older embryo and has the form of a biconvex lens, as the central part of the root-apex is strongly concave.

A noticeable difference between the embryo of *Sparganium* and that of most other aquatic Monocotyledons hitherto examined, e. g. *Alisma*, *Naias*, *Lilæa*, etc., is the absence of the larger vesicular suspensor cell, so conspicuous in those forms. This cell is evidently of great importance in these plants, and directly concerned with the food supply

of the young embryo. Its absence in *Sparganium*, the Gramineæ, and such Aroids as have been examined is no doubt to be sought in the better development of the endosperm in these forms, and possibly also in the presence of numerous antipodal cells, which assume temporarily the functions of the endosperm.

The first division of the primary endosperm-nucleus occurs almost immediately after fertilization. At this time the endosperm-nucleus is, usually at least, near the antipodal end of the sac. The divisions are repeated until there are formed in the usual manner many free nuclei arranged in a single layer in the parietal cytoplasm (fig. 30). These nuclei are more or less flattened and possess a single large nucleolus. At the apex of the sac they are more numerous, and the cytoplasm is more abundant and quite surrounds the young embryo. As usual, the first cell-division in the endosperm takes place at the apex and proceeds toward the antipodal region.

While the endosperm-nuclei have been dividing, and the peripheral layer of protoplasm is still very thin, the antipodals have increased enormously in bulk and divided rapidly to form the large mass of cells found at the antipodal end of the sac. We have already spoken of the karyokinetic division of the antipodal nuclei and it is very doubtful whether even in the binucleate cells sometimes found in the later stages this division is ever direct, as it has been found to be in the enlarged antipodal cells of a number of plants (e. g. in Ranunculaceæ, Mottier, 1895). As the embryo-sac enlarges it changes its form somewhat in *S. simplex*, becoming relatively much narrower at the upper end which early becomes completely filled with endosperm (fig. 37).

In the micropylar end of the sac the protoplasm early becomes much denser, the nuclei are larger than at the sides of the sac and not confined to a single layer. The nuclei increase a good deal in size before the cell-walls are formed between them (fig. 57).

As *Sparganium simplex* shows very clearly the endosperm formation, this was studied in some detail. Before the division-walls are evident, the nuclei are arranged at nearly equal

distances. The delicate radiating lines connecting them are clearly evident (fig. 57), and in these the cell-plates are soon visible, and the nuclei are thus enclosed in polygonal areas which become cells by this transformation of the cell-plates into cellulose membranes (fig. 58). The cells at the apex of the sac are quite irregular in form and completely surround the embryo (fig. 37). At the sides of the sac these cells seen from the surface are often regularly hexagonal. The endosperm-cells at the apex have much denser contents than those at the sides, which are almost transparent. Figure 56 illustrates the way in which the development of the endosperm proceeds centripetally. This section is a nearly median one and shows how the nuclear divisions are mainly in a plane parallel to the periphery of the sac. The first-formed endosperm-cells are at first open on their inner face, and when the nucleus divides a division-wall is found closing up the outer cell, but leaving the inner one also open. This continues, one layer of new cells after another being added to the endosperm, until finally the whole upper part of the sac is filled up, but the lower portion remains open in the center up to a late enlarged period. Free nuclei can always be seen in this open part of the sac, lying in the layer of protoplasm covering the free walls of the inner endosperm-cells.

The endosperm-nuclei become a good deal enlarged before the division-walls are formed, and these enlarged nuclei generally show several nucleoli. The younger stages have only a single nucleolus and the outline of the nucleus is round and oval. As they increase in size, it was found that the nucleolus became elongated and constricted (fig. 58), finally resulting in a division into two, generally unequal, portions. This is probably repeated in the case of those which have more than two nucleoli. Each nucleolus is surrounded by a clear area, while the rest of the nucleus presents a granular appearance. As the nucleus itself becomes more or less distorted in outline with the division of the nucleolus, and often appears lobed, it was at first supposed that we had to do with a case of nuclear

fusion, but in reality it is more nearly a case of fragmentation. Hegelmaier (1885) figures a number of similar cases, and they have been often observed, but so far as I know the origin of the secondary nucleoli has not been studied.

As the seed ripens, numerous very large crystalloids are formed, at least in *S. simplex*, in the endosperm-cells surrounding the embryo (figs. 63, 64). These are beautifully fixed by the action of alcoholic corrosive-sublimate, and stain readily with anilin-safranine. They vary in shape, the most perfect ones being rhombic in outline. Often they were aggregated in large masses, but usually it was evident that these masses were composed of separate crystals. *Sparganium simplex* may be recommended as an admirable subject for demonstrating these bodies. The material collected of the other species was either too young or had not been treated with the proper reagents, so that I cannot speak as to the occurrence of these crystalloids in the endosperm of the other species. They have, however, been noted in *S. ramosum* Sachs (1887).

No hard testa is formed about the seed of *Sparganium*, although this outer part of the nucellus and the integuments persist until a late stage, and probably permanently. Engler's statement (1889) that the endosperm is only separated from the pericarp by the integuments needs confirmation. In the oldest stages examined by me, there were several layers of nucellus cells still evident. The development of the hard inner pericarp is readily followed. The cells early have thickening layers deposited upon their walls, and these later become very thick and have deep pits developed in them. This sclerenchyma is rather better developed in *S. Greenii* than in *S. simplex*, and sometimes in the former the thickening is so great as to almost obliterate the lumen of the cell.

Hegelmaier (1874) has studied in *S. ramosum* the development of the curious structure to which he applies the name "Samen-deckel." The structure of this in *S. simplex* agrees closely with his account. It is formed from an enlargement of the micropylar part of the integuments,

which become very much swollen, and form two caps, one above the other. The upper part of the nucellus is also included, but the cap is principally derived from the integuments (fig. 59). The margins of the inner integument are prolonged upward into a conical beak which fits closely into the opening in the outer integument. The whole structure is enclosed in the space at the top of the ovary which remains as a conical cavity in the pericarp.

The fruit of *Sparganium* is not very unlike the caryopsis of the grasses, but differs in the persistence of the integuments and probably also of a portion of the nucellus. By a further compression and disorganization of these a typical caryopsis would be formed.

VI. Abnormal Embryo-sacs.

In *Sparganium*, as in most other low Monocotyledons hitherto examined, there are occasional deviations from the normal development in the embryo-sac. Some of these have already been noted by the writer (Campbell, 1897) in the case of *S. eurycarpum;* indeed, it was these abnormalities which directed attention to *Sparganium*. In both *S. simplex* and *S. Greenii* similar deviations from the type were noted.

The most usual form is a multiplication of the nuclei within the embryo-sac without any noticeable increase in its size. As in such cases (fig. 68) the embryo cannot be detected, and the egg-apparatus has either not been formed, or has disappeared, the most probable explanation is that the sac had not been fertilized, but that the vegetative tissue of the gametophyte, i. e., the antipodal cells and the endosperm, are capable of a limited growth. This is quite comprehensible if *Sparganium* is, as it seems to be, a very low type of Angiosperm, and the case might be very well compared to the limited growth of the prothallium in the heterosporous Pteridophytes when fertilization is not effected.

Other abnormal embryo-sacs are shown in figs. 65 and 69. In the first case, taken from an ovule of *S. simplex*, the embryo-sac was very broad and divided longitudinally

by a membrane—whether of cellulose or not was not determined. Three antipodal cells having the appearance of the normal ones in a recently fertilized sac could be distinguished. The structures at the micropylar end of the sac were not clearly distinguishable. The extremely broad form of the sac suggested the possibility of there having been two embryo-sacs formed in the ovule, but if this was the case, they had become entirely confluent.

Another puzzling form in *S. Greenii* is shown in fig. 69. Here the antipodal cells were apparently normal, but the polar nuclei, which were in close contact, were separated from the cavity of the embryo-sac by an evident membrane, and the upper portion of the sac was similarly shut off. Occupying the extreme upper part of this was a body looking like a very large nucleus, but the nucleolus (?) did not appear homogeneous, and it is possible that this apparent nucleus was really a cell, as the nucleolus-like body was quite similar in size and appearance to the nuclei of the surrounding nucellar cells. No other nuclei could be certainly made out in this region, except a deeply staining small body close to the wall of a vesicle which lay below the large apical nucleus. If the latter is really a nucleus it probably means that one nuclear division in the upper part of the young embryo-sac had been suppressed, and no egg-apparatus developed.

In another specimen of *S. Greenii* the embryo-sac was seen to be completely filled with granular cytoplasm, and a similar condition has also been seen in *S. eurycarpum*.

VII. Recapitulation.

1. The stamen in *Sparganium* is of the usual type: the ripe pollen-spore shows but one generative nucleus, but in *S. simplex* there is regularly a structure present which probably represents a vegetative or prothallial cell which was not observed, however, in *S. Greenii*.

2. The early development of the embryo-sac follows the normal course; the egg-apparatus is small in *S. simplex* and the polar nuclei fuse completely before the egg is

fertilized; in *S. Greenii* they remain separate until after fertilization.

3. The antipodal cells are three in number and in *S. simplex* are very inconspicuous; after fertilization there is a remarkable secondary growth in the antipodal cells, resulting finally in a large cell-mass, containing in *S. simplex* over 150 cells.

4. The process of fertilization, so far as it was studied, offered no anomalies.

5. The development of the embryo in *S. simplex* agrees closely with that of *S. ramosum*, studied by Hegelmaier. There are regularly three primary transverse segments, of which the terminal one gives rise to cotyledon, stem-apex, and part of the root. The suspensor remains undeveloped, and in this respect the embryo is like that of the Gramineæ. The slight development of the suspensor is associated with the complete investment of the young embryo by the endosperm.

6. The stem-apex is lateral in origin like that of most Monocotyledons, but is not developed from the middle of the three primary segments.

7. The primary tissues of the embryo are very early developed, especially in the terminal embryonal segment. The plerome of the root is derived entirely from the terminal segment; the initials for the other tissues of the root arise from the middle segment.

8. The development of the endosperm follows the usual course, but is rather late in forming the first division-walls. The enlarged antipodal cells doubtless function at first as endosperm.

9. The large endosperm-nuclei have often several nucleoli which are formed by a fragmentation of the original nucleolus.

10. Large crystalloids are abundantly developed in the older endosperm-cells of *S. simplex*.

11. The inner pericarp develops into sclerenchyma with thick, deeply pitted walls. The development of the "Samen-deckel" is the same as described by Hegelmaier for *S. ramosum*.

VIII. The Affinities of Sparganium.

The genus *Sparganium* has until recently been associated with *Typha* in the family Typhaceæ, but Engler (1889) has pointed out that the differences between *Typha* and *Sparganium* are so great as to make such a union unwarranted, and he proposes placing *Sparganium* in a separate family, following the Pandanaceæ. Until more is known about the development of the Pandanaceæ, however, it will be impossible to decide how closely the two families are related. A thorough study of some species of *Pandanus* would be of great value in solving this question.

Hegelmaier (1874) has referred to certain resemblances in the embryo of *Sparganium* to that of the Gramineæ, and my own study of *S. simplex* confirms this. The extraordinary character of the antipodal cells also is strongly suggestive of the grasses. The probably terminal origin of the single ovule is also like that of the grasses, and this it also shares with most other low Monocotyledons. Indeed the solitary uni-ovulate carpel is probably the most primitive type among the Monocotyledons, and is not the result of a reduction. In this particular *S. simplex* is probably more primitive than the forms like *S. Greenii* and *S. eurycarpum*, in which the ovary is compound.

The type of fruit in *Sparganium* is not unlike that of the Gramineæ. While the majority of the latter have a caryopsis, there are others in which the fruit is nut-like, corresponding to that of *Sparganium* (see Hackel, 1890). A comparison of some of these forms with *Sparganium* would be interesting.

The monœcious flowers of *Sparganium* also are shared by some grasses, e. g. *Zea*, so that the possibility of an actual relation between these two puzzling families may very well be considered. Of course the resemblances may be purely fortuitous, but it is more likely that they indicate a real, even if remote, affinity, and any further study of the flower and fruit of the Gramineæ should consider this. In short, so far as a comparative study has been made, *Sparganium* shows a closer affinity with the Gramineæ than with any other family, unless possibly the Pandanaceæ.

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EXPLANATION OF PLATE XLVI.

All figures except 1 and 4 were studied with Leitz lenses and drawn with a Zeiss camera. All figures slightly reduced.

Figs. 4, 5, 7, 15 and 23 refer to *Sparganium Greenii* Morong, the others to *S. simplex* Huds.

Fig. 1. Two female flowers of *S. simplex* enlarged about eight times; the one at the right with the perianth removed.

Fig. 2. A single scale of the perianth more enlarged.

Fig. 3. Longitudinal section of the ovary showing the single ovule; *ma*, embryo-sac. Oc. 1, obj. 3.

Fig. 4. Pistil of *S. Greenii;* × about 8.

Fig. 5. Section of the ovary of *S. Greenii.* Oc. 1, obj. 3.

Fig. 6. Sections of ripe pollen-spores of *S. simplex*, showing the large vegetative, and smaller generative, nucleus; *pr*, prothallial cell. Oc. 1, im. $\frac{1}{16}$.

Fig. 7. Sections of younger pollen-spores of *S. Greenii;* the nucleus is still undivided, and no prothallial cell is visible. The clear space on the surface probably marks the place of exit of the pollen-tube.

Fig. 8. Upper part of the embryo-sac of *S. simplex* just after the entrance of the pollen-tube; *o*, the egg-cell; *sy*, one of the synergidæ; *pt*, pollen-tube. Oc. 1, im. $\frac{1}{16}$.

Fig. 9. Another section of the same embryo-sac; the small dark body within the synergid is probably one of the generative nuclei from the pollen-tube.

Fig. 10. The antipodal end of the same embryo-sac, showing the single large endosperm-nucleus and two of the antipodal cells.

Fig. 11. The egg-apparatus and endosperm-nucleus from a mature embryo-sac. Oc. 1, im. $\frac{1}{16}$.

Fig. 12. (*a*) Two antipodal cells from a mature embryo-sac; (*b*) outline showing the position of the three small antipodals. Oc. 1, im. $\frac{1}{16}$.

Fig. 13. Section of the egg-apparatus showing the entrance of the pollen-tube, *pt*. A small body (the generative nucleus ?) is visible within the egg; both synergidæ are still intact. Oc. 1, im. $\frac{1}{16}$.

Fig. 14. Another section of the same; the pollen-tube has apparently discharged a small granular mass into the embryo-sac.

Fig. 15. Section of the nucellus and mature embryo-sac of *S. Greenii.* Oc. 1, obj. 7. A large cell (an extra antipodal ?), lies above the three antipodal cells.

Fig. 16. The antipodal region of fig. 16 more highly magnified.

Figs. 17 and 18. Two sections of the egg-apparatus of *S. Greenii.* Oc. 1, im. $\frac{1}{16}$.

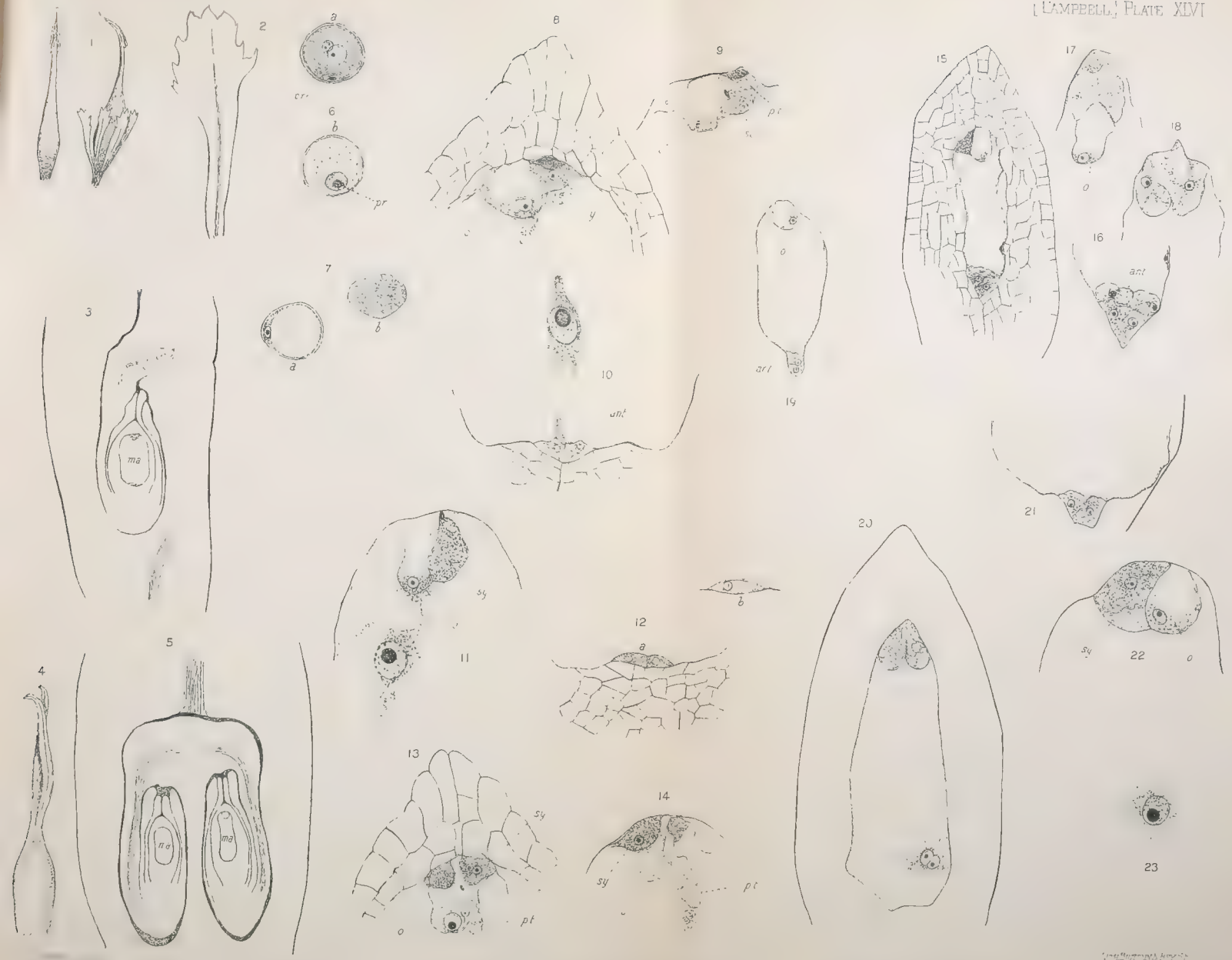
Fig. 19. Embryo-sac of *S. Greenii*, showing egg, *o*, and two antipodals, *ant.* Oc. 1, obj. 7.

Fig. 20. Unusually large sac of the same species; the section shows the two synergidæ and the still separate polar nuclei. Oc. 1, obj. 7.

Fig. 21. Two of the antipodal cells from the same sac more highly magnified.

Fig. 22. Section of egg-apparatus of *S. Greenii.* Oc. 1, im. $\frac{1}{16}$.

Fig. 23. Endosperm-nucleus from a similar sac.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Sparganium

EXPLANATION OF PLATE XLVII.

Figs. 33–36 refer to *S. Greenii*, the others to *S. simplex*.

Fig. 24. Apex of fertilized embryo-sac with one-celled embryo. Oc. 1, im. $\frac{1}{16}$.

Fig. 25. An embryo of the same age, but with a broader base of attachment. One synergid could be still made out.

Fig. 26. A recently fertilized embryo-sac; one of the enlarged antipodals, *ant.*, is shown. The endosperm-nucleus has divided once. Oc. 1, obj. 7.

Fig. 27. The antipodal region of the same more highly magnified; the three enlarged antipodal cells lie in the same plane; one of the two endosperm-nuclei is shown.

Fig. 28. Enlarged antipodal cells from an older sac; each of the cells shown has two nuclei; the whole number of nuclei was eight or nine. Oc. 1, im. $\frac{1}{16}$.

Fig. 29. Two sections of the antipodal group from a still older sac; some of the nuclei are dividing; twelve antipodal nuclei could be distinguished. Oc. 1, im. $\frac{1}{16}$.

Fig. 30. Nearly median section of the antipodal group from a much older sac. Oc. 1, obj. 7. Divisions have formed between all the nuclei; numerous free endosperm-nuclei, *en.*, are present.

Fig. 31. Part of the lining layer of protoplasm with a single endosperm-nucleus. Oc. 1, im. $\frac{1}{16}$.

Fig. 32. Surface view of a portion of the nucleated protoplasmic layer lining the wall of the embryo-sac.

Fig. 33. Antipodal cells from the unfertilized sac of *S. Greenii*. Oc. 1, im. $\frac{1}{16}$.

Fig. 34. Antipodal group shortly after fertilization; the upper cell has enlarged and divided; the two lower ones show indications of disintegration.

Fig. 35. Antipodal group of *S. Greenii* in which all the cells appear active.

Fig. 36. A somewhat older group of the same species; growth is mainly confined to the upper cell.

Fig. 37. Upper end of embryo-sac of *S. simplex* showing the young embryo surrounded by the endosperm-cells; the central nuclei, *en*, are still free. Oc. 1, obj. 7.

Fig. 38. The embryo from fig. 37 more enlarged.

Fig. 39. Median section of a similar embryo. Oc. 1, im. $\frac{1}{16}$; *xx*, the basal wall.

Fig. 40. Series of three cross-sections of an embryo of about the same age; *a*, the lowest segment.

Fig. 41. Two sections of an older embryo, cut in the plane of the cotyledon. Oc. 1, obj. 7.

Figs. 42, 43. Longitudinal sections cut at right angles to the plane of the cotyledon; the basal wall is decidedly oblique. Oc. 1, obj. 7.

Fig. 44. Three cross-sections of an embryo of about the same age as 42; the lowest segment is divided into four cells. Oc. 1, obj. 7.

Fig. 45. Upper part of embryo-sac showing the embryo surrounded by the densely granular endosperm-cells; the granular cell-contents of the embryo are not shown. Oc. 1, obj. 7.

Fig. 46. The same embryo shown in fig. 45, showing the differentiation of the primary tissues, the plerome ends at the basal wall, *xx*.

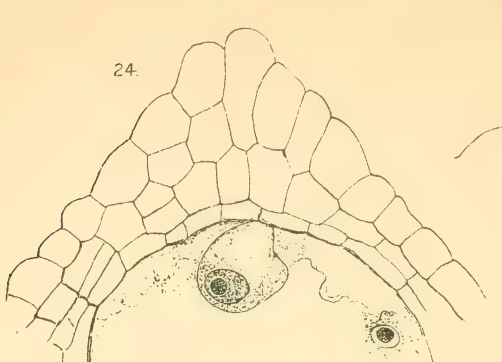
Fig. 47. A transverse section of an embryo of about the same age. Oc. 1, obj. 7.

Fig. 48. Section in the plane of the cotyledon of a similar embryo, with very regular arrangement of the tissues. Oc. 1, obj. 7.

Fig. 49. Median section of an older embryo, showing the stem-groove, *st.* Oc. 1, obj. 7.

Fig. 50. Embryo of about the same age, cut in the plane of the cotyledon.

Fig. 51. Median section of the base of the same embryo, more highly magnified; *pl.*, the plerome.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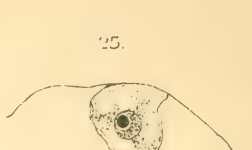
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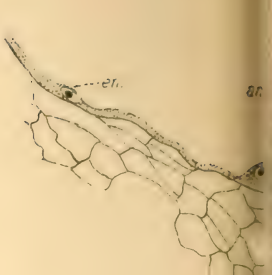
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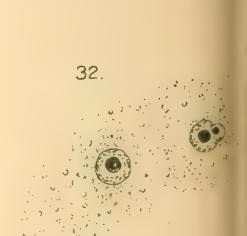
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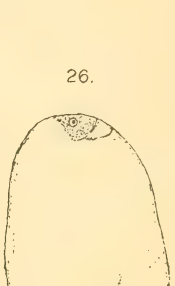
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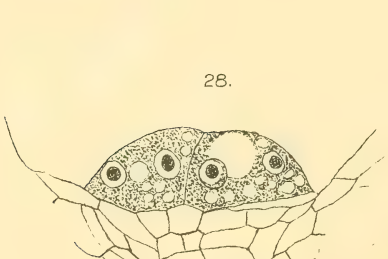
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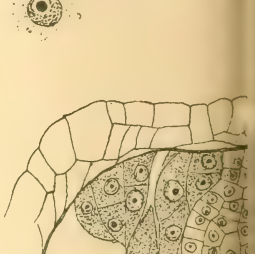
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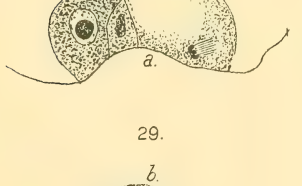
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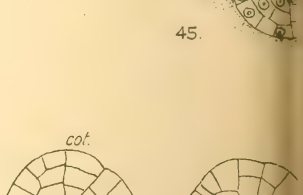
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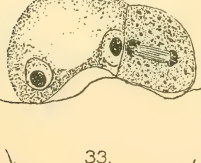
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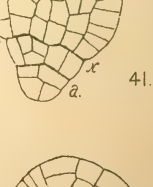
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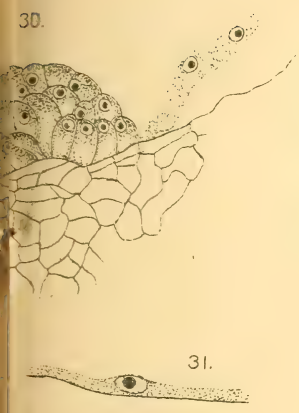
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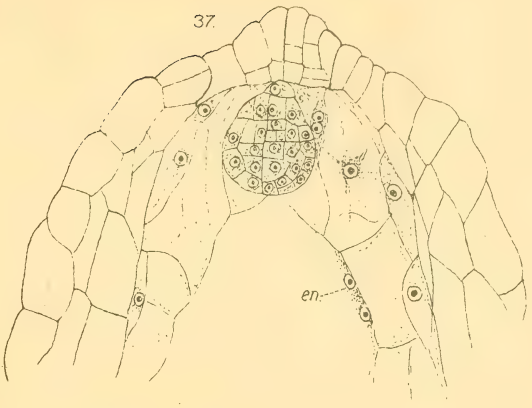
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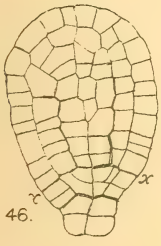
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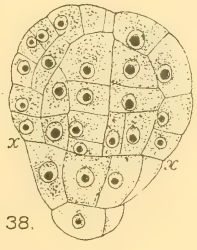
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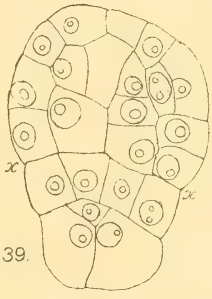
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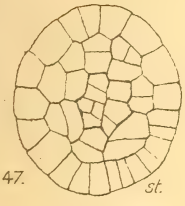
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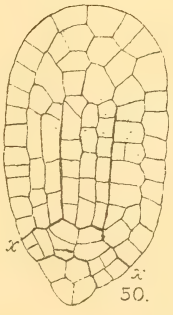
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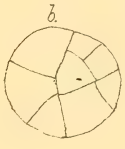
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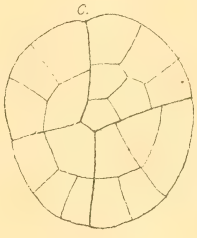
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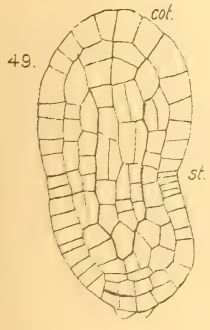
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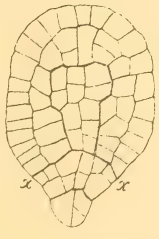
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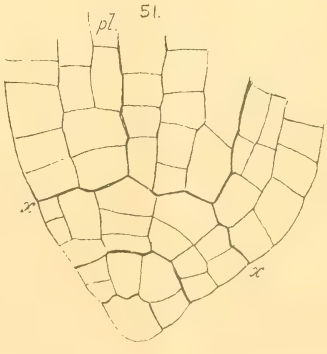
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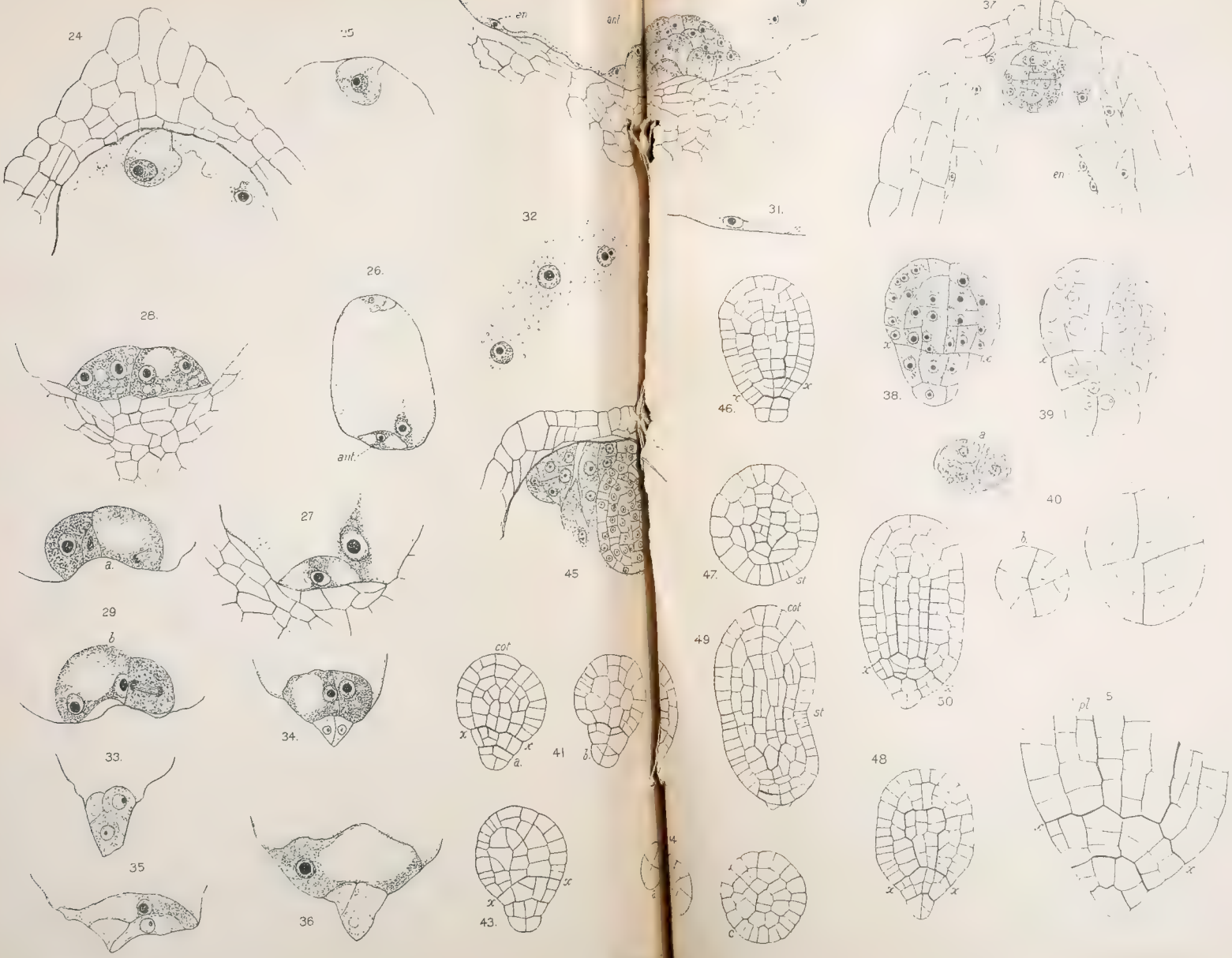
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EXPLANATION OF PLATE XLVIII.

All figures except 69, refer to *S. simplex*.

Fig. 52. Two sections of a nearly mature embryo. Oc. 1, obj. 3.

Fig. 53. Root-end of a somewhat younger embryo. The position of the original transverse, or basal wall (x) is still evident, l^2, the second leaf; *st.* the stem-apex. Oc. 1, obj. 7.

Fig. 54. Three cross-sections of a mature embryo. Oc. 3, obj. 3.

Fig. 55. Sections of the young seed before the endosperm has filled the embryo-sac; *e*, embryo; in^1, in^2, integuments.

Fig. 56. Section through the layer of developing endosperm lining the embryo-sac; many of the nuclei are dividing. Oc. 1, obj. 7.

Fig. 57. Larger endosperm-nuclei from the base of the sac at the time the walls are first beginning to form; some of the nuclei have more than one nucleolus; the fine fibres connecting them are plainly visible. Oc. 1, im. $\frac{1}{16}$.

Fig. 58. Transverse section of recently formed endosperm-cells showing the nucleolus fragmenting. Oc. 1, im. $\frac{1}{16}$.

Fig. 59. Upper part of the young seed showing the enlargement of the micropylar portion of the integuments. Oc. 3, obj. 3.

Fig. 60. Young sclerenchyma-cells from the pericarp. Oc. 1, obj. 7.

Fig. 61. Fully developed sclerenchyma-cell showing the deeply pitted walls. Oc. 1, obj. 7.

Fig. 62. Surface view of the pits more highly magnified.

Fig. 63. Endosperm-cells from nearly ripe seed, showing large crystalloids. Oc. 1, obj. 7.

Fig. 64. Separate crystalloids more highly magnified.

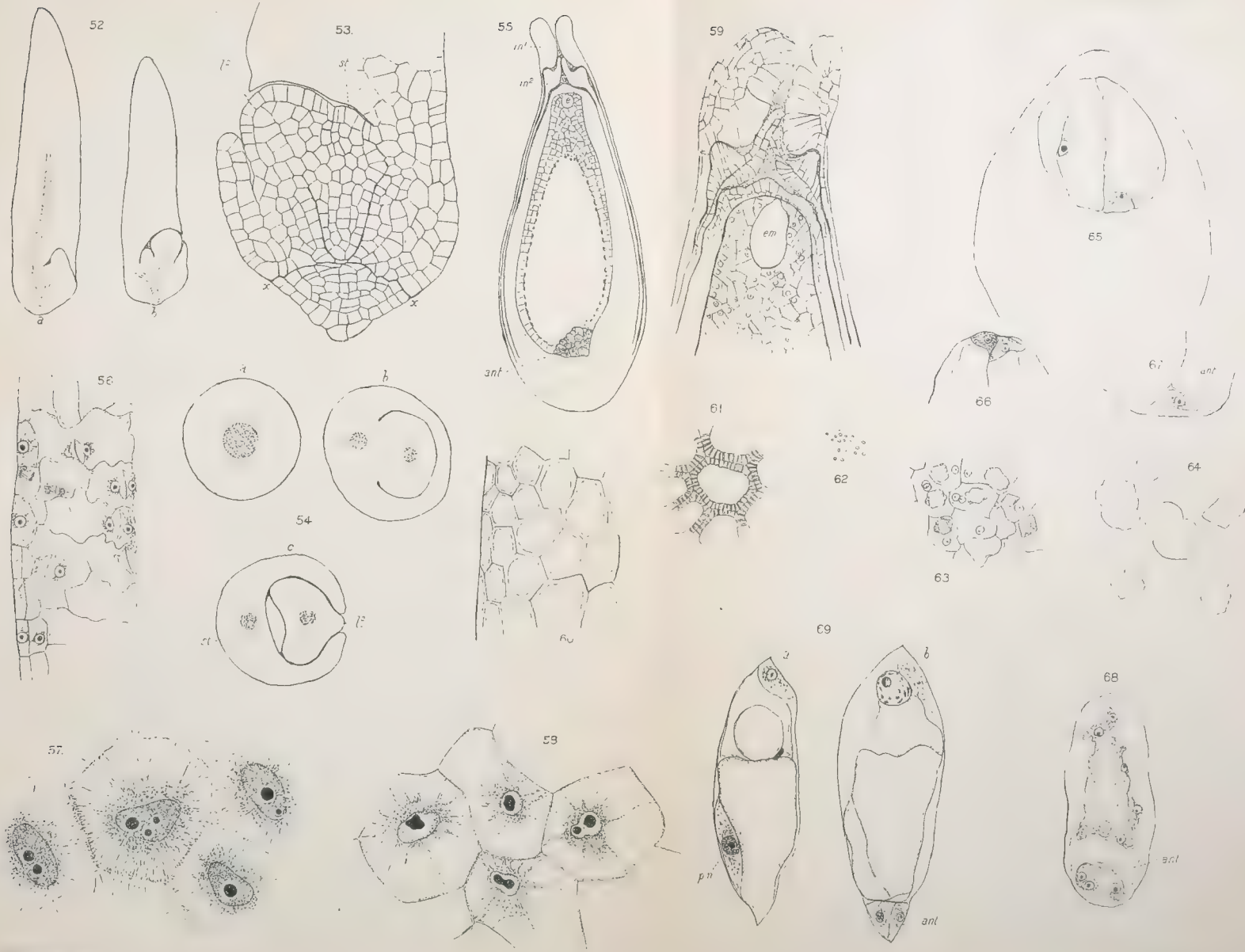
Fig. 65. Abnormal embryo-sac of *S. simplex;* the sac divided longtudinally with the endosperm-nucleus on one side. Three antipodals could be distinguished. Oc. 1, obj. 7.

Fig. 66. Micropylar end of the same sac.

Fig. 67. One of the antipodal cells of the same.

Fig. 68. Abnomal embryo-sac (perhaps unfertilized?); there has been little increase in size of the sac, but numerous endosperm-nuclei are present, and the antipodals have enlarged and divided. Oc. 1, obj. 7.

Fig. 69. Two sections of an abnormal embryo-sac of *S. Greenii;* three antipodals could be seen, and the polar-nuclei (*p.n.*), in close contact, were separated from the body of the sac. The upper part was also shut off by a membrane, and no definite egg-apparatus could be distinguished. Oc. 1, im. $\frac{1}{16}$.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