

Studies in the Development and Morphology of Cycadean Sporangia:

II. The Ovule of *Stangeria paradoxa*.

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With Plates XVII and XVIII.

THE material, upon which the present investigation was made, was obtained from plants of *Stangeria paradoxa* growing in the Royal Botanic Gardens at Kew and Edinburgh. The cones, which served for the study of the development of the ovule, came from the Kew plant, while the fertilized ovules were the result of Mr. Harrow's successful artificial pollination of two cones, borne on a plant in the Edinburgh Gardens, with pollen from Kew. I am indebted to the Director of the Royal Gardens, Kew, and to Professor I. B. Balfour for placing the material at my disposal. Although the result of examination of the ovule and seed in this interesting genus of Cycads has been to show a general agreement with the corresponding structures in the genera already investigated¹, the main stages will be briefly described.

¹ Cf. especially Warming, Oversigter d. k. dan. Vidensk. Selsk. Forhandl. 1877, 1879. Treub, Ann. Jard. Bot. Buitenzorg, 1885.

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This will enable the development of certain parts of the Cycadean ovule, a detailed account of which is wanting, to be dealt with, and will afford a basis for the briefer description and general comparison of the other genera of recent Cycads, in a future number of these studies. The full consideration of the morphology of the Cycadean ovule must be deferred until further materials for this comparison have been accumulated, and the present paper will be restricted to a description of the phenomena observed in *Stangeria*, together with an estimate of the problem as illustrated by this genus.

The female sporophyll of *Stangeria* resembles the male sporophyll in general form. Two ovules are borne on each scale; they are situated one on either side of the short stalk, and their micropyles point towards the axis of the cone and slightly towards one another. The ovules are clearly not marginal, but each is situated slightly within the margin of a small area apparently corresponding to one of the soriferous areas of the lower surface of a male sporophyll. This will be evident on comparing Figs. 1 and 17, which represent sporophylls from the youngest and oldest cones investigated, with Fig. 1 of the first number of these studies¹, representing the distribution of the sori on the young male sporophyll. The intra-marginal position of the ovule in this genus has been verified by the study of sections.

It was found to be impossible to obtain cones young enough to show the first stages of development of the ovule, without risk of injury to the plant. A median longitudinal section of one of the youngest ovules obtained is represented, from a photograph, in Fig. 2. Ovules at this stage of development are borne upon cones just visible among the bud-scales at the apex of the plant. As the figure shows, the rudiments of the free projecting portions of nucellus and integument are just recognizable. At this stage the ovule is only slightly constricted at its region of insertion on the sporophyll. In the median region of this stalk the cells are arranged in short

¹ *Annals of Botany*, vol. xi, p. 421, Pl. XXII.

vertical rows, which are not, however, strictly continuous with the rows of cells composing the nucellus. The latter is marked off from the integument by a series of flattened cells, which can be traced down from the place of separation of the free portions of integument and nucellus. Within this limit the nucellus is composed of vertical rows of cells, from five to seven of which are present in a median section. These rows can be traced to just below the epidermis, which is a definite layer of cells undergoing anticlinal divisions only. Although the continuity of the vertical cell-rows is usually clearly recognizable, a distinction is already apparent in the character of their component cells between the tip of the nucellus and an oval group of larger cells, which in older ovules becomes more sharply defined as the sporogenous tissue. About the centre of this sporogenous group a cell of larger size than the rest can be distinguished (Fig. 2). The nucleus of this cell, which is the mother-cell of the embryo-sac, is somewhat larger than those of its sister-cells. At this stage, however, the embryo-sac mother-cell has not become vacuolated. The position of the vascular bundles supplying the ovule is indicated at the base of the latter by desmogenstrands. As the ovule increases in size its different parts become more clearly defined from one another, and the projecting portions of the integument and nucellus-apex more prominent. The increase of the latter structure is due to periclinal divisions in the tissue just beneath the epidermis. The mother-cell of the embryo-sac, situated in the centre of the now clearly limited sporogenous tissue, has increased considerably in size and become vacuolated; it is still undivided, the large nucleus being situated towards the micropylar end of the cell, the wall of which has become thicker than the walls of the neighbouring cells. Fig. 3 represents the nucellus of an ovule at this stage.

The mother-cell of the embryo-sac in *Stangeria* undergoes considerable increase in size before dividing. The order of the divisions could not be accurately followed, but, as Fig. 4 shows, the mother-cell first divides into a larger lower and

a smaller upper segment ; other ovules have been seen with a row of three uncrushed cells (Fig. 5). From the comparison of these two stages, it would appear to be almost certain that it is the upper cell of the two resulting from the first division that divides again ; only the examination of the cells in the process of division can, however, settle this question definitely. In slightly older ovules the two cells of the row nearest to the micropylar end of the ovule are found crushed and flattened, the lowest cell, which is the young megaspore, being still uni-nucleate (Fig. 6). While these changes have taken place in the embryo-sac mother-cell, the ovule has undergone considerable alteration in size and form. This will be evident from Fig. 7, which represents the median longitudinal section of an ovule with the embryo-sac in the condition last described. As this figure shows, the indication of the division of the integument into three layers is becoming apparent. The margin of the integument has closed in somewhat, narrowing the micropyle, and the bluntly conical apex of the nucellus does not quite reach the level of the integument. The sporogenous mass is very distinct, and exhibits a difference between its more peripheral cells and those immediately around the embryo-sac. The cytological character, upon which this difference mainly depends, is, as the detailed drawing of the sporogenous tissue around the embryo-sac in Fig. 4 shows, the vacuolization of the more central cells. This is most marked in those which immediately surround the embryo-sac. These cells are also noteworthy for containing in their cytoplasm certain spherical bodies, the nature of which it has been impossible to determine with certainty from the material investigated. Since they also occur in the embryo-sac mother-cell it appears to be advisable to record their occurrence. Most frequently two of these spherical bodies are present in each cell ; they are situated in the cytoplasm at a greater or less distance from the nucleus, and stain similarly to the cytoplasm, though more deeply. As the figure shows, one of these bodies remains in each of the first two segments of the mother-cell, but it has not been

possible to demonstrate any connexion between them and the nuclear division.

None of the cones collected supplied the first stages of division within the megaspore, but from the study of the ovules next in age to the one last described (Fig. 7) it was clear that keeping pace with the increase in size of the ovule the embryo-sac enlarges. Its fairly thick wall is lined by a cytoplasmic layer in which numerous nuclei are distributed. Fig. 8 shows the longitudinal, and Fig. 9 the transverse, section of the nucellus of an ovule at this stage. The increase in size of the embryo-sac up to this stage does not appear to involve any considerable destruction of the sporogenous tissue, but to stand in relation to the growth of the ovule as a whole. As this takes place, the histological regions of the integument become more clearly marked out, and the differentiation of the xylem-elements takes place in the vascular bundles.

But the only point which calls for more special description has regard to the changes in form and mutual relations which take place in the nucellus and integument-margin in the region of the micropyle. In the youngest ovules examined the sides of the free apex of the nucellus are vertical, while its flat broad top stands on a level with the margin of the integument or even projects slightly above the latter (Figs. 2, 3, 10). In the general growth of the ovule the projecting portion of the nucellus keeps pace for some time with the free portion of the integument. It becomes more conical, however, but the flat top is retained. In the ovule represented in Fig. 11 this top is just below the level of the micropylar margin which is formed by the edge of the integument. As the latter comes to project further beyond the nucellus-apex, the micropyle is greatly narrowed, though the margin of the canal is still thin. In a slightly more advanced stage the integument becomes greatly thickened so that the micropyle forms a long passage, the external opening of which is narrow, while it widens out in passing inwards. Into this wider portion the conical tip of the nucellus-apex

projects, forming a very distinct pointed structure (Fig. 12). The distinctness of this is due partly to the increase in width of the nucellus-apex below the tip, but in part to the further growth of the tip itself, as is shown by the assumption of the pointed form. The ovule, when this stage of development of the nucellus-apex and micropyle has been reached, has an embryo-sac of considerable size, in which, however, no distinction of separate cells is apparent, the prothallus being still represented by a layer of cytoplasm with numerous nuclei. Little or no destruction of the sporogenous tissue has yet taken place.

No cones have been obtained illustrating the intermediate steps between such an ovule and the stage in which the embryo-sac has become completely filled with the tissue of the prothallus. The latter age is of special interest, since it probably is that at which pollination takes place in *Stangeria*. It will therefore be described in some detail, and at the same time the course of the vascular bundles in the ovule may be traced. The general relation and proportion of parts is shown in Fig. 13, which represents a median section of an ovule at this stage. In the integument the three layers are distinct, though the cell walls of the middle one are still unthickened. The long narrow micropylar canal is surrounded by the thick margin of the integument which projects as a short beak round the external opening. In the apex of the nucellus the pollen-chamber has been formed (Figs. 14, 15). The superficial cells of the pointed tip seen in Fig. 12 have their walls thickened, and form a very definite boundary to the sides of the chamber, suggesting a close comparison with the corresponding region of certain fossil Gymnospermous seeds¹. At the actual apex of the conical projection, however, the epidermal cells have broken down, thus leaving an opening into the cavity formed by the disintegration of the internal tissue. From the base of this cavity a strand of more elongated cells

¹ See, for example, Brongniart, *Graines Fossiles Silicifiées*, Pl. III, Figs. 8 and 13 (*Cardiocarpus augustodunensis*). Renault, *Structure comparée de quelques Tiges de la Flore Carbonifère*, Pl. XVII, Figs. 14 and 15 (*Cordaianthus Grand'Euryi*).

can be followed through the middle of the nucellus towards the embryo-sac.

No other disintegration or absorption of cells outside the sporogenous group has taken place. The sporogenous tissue, however, has undergone great changes with the increase in size of the megaspore. The latter is completely filled with the thin-walled parenchymatous tissue of the prothallus, the arrangement of the cells of which points to a gradual filling up of the cavity by divisions having taken place in a peripheral layer of cells. No starch is at this stage stored in the prothallus, which when fresh has a translucent appearance. Archegonia were not present, nor could the cells destined to form them be distinguished. The prothallus readily contracts during fixation, the wall of the megaspore which has become considerably thickened usually remaining more or less adherent to its surface. Around the megaspore a layer of cells was present which is clearly to be traced to the sporogenous group. The thick zone of sporogenous tissue present in earlier stages has, however, become reduced to a single layer. That this has taken place by the absorption of the cells between the outermost layer of sporogenous cells and the embryo-sac is indicated by the presence of smaller cells, in a more or less crushed condition, internal to the definite layer referred to. The latter appears thus to be derived from the most external layer of the sporogenous group, but material to follow the steps of the process was not available. The cells of this persistent layer (Fig. 16) are very large, and stand with the longer axis at right angles to the surface of the megaspore. Sometimes a single nucleus is present; often two are found in a cell. How long this layer of cells, which at this stage shows no signs of crushing or disintegration, persists, cannot at present be determined; in the fertilized seeds all trace of it was gone. Observations on aborting ovules showed that a similar increase in size of the outer layer of sporogenous cells takes place even when a normal embryo-sac is entirely wanting. In the light of the present facts it would appear to be a probable conclusion that, while

the majority of the sporogenous cells surrounding the embryo-sac simply become disintegrated and absorbed, the outermost form a more definite tapetal layer. This tapetum, while persisting longer than the more internal cells, ultimately disappears.

The course of the vascular bundles in the ovule is most easily followed at this stage, and may be briefly described. The general arrangement of the bundles in the sporophyll has been described and figured by Worsdell¹. As he points out, the bundles passing to the ovule arise by division of a single branch from the vascular system of the lateral part of the sporophyll. This branch always divides into two bundles, one of which may again divide before reaching the base of the ovule. Thus two or three bundles of collateral structure enter the ovule. These bundles divide up at the base of the ovule into sixteen small bundles, eight of which bend out at once to form the vascular system of the outer layer of the integument, while the eight bundles alternating with them pass into the innermost layer. The bundles of both the inner and outer series, which thus take their origin at the same level, undergo subdivision though not to any great extent; those of the outer layer extend to the micropyle, while those of the inner series stop at the separation of nucellus from integument.

The remaining observations on the changes taking place in the ovule of *Stangeria* were made on two cones produced in the Edinburgh Botanic Gardens, and pollinated about the middle of February 1899. So far as can be judged, the cones at the date of pollination must have borne ovules of about the age of those last described. Dr. Balfour informs me that they were then erect on stiff peduncles, and considerably smaller than they became after pollination. The increase in size took place shortly after pollination, and was attended by a change in the position of the cones, which became pendulous. When the plant came under my observation in the beginning of July, the cone, excluding the peduncle, measured 14 cm. in

¹ *Annals of Botany*, vol. xii, p. 215, Pl. XVII, Fig. 5.

length, by more than 8 cm. in diameter at the widest part. At first, in the hope of obtaining a series of successive stages, one or two sporophylls were removed at intervals of a week or more. When, however, it was ascertained that even on July 4 embryos were present, this plan was abandoned and all the remaining ovules of one cone removed and fixed. The method is mentioned here, since, though useless in the present case, it would, if adopted early enough, enable all the main stages of fertilization and embryogeny to be studied on a single cone; the careful removal of sporophylls did not appear to injure the remainder of the cone in any way. Besides the seeds a number of aborting ovules were borne on the pollinated cone. As Fig. 17, which represents a scale bearing an aborted and a healthy ovule, shows, the former do not undergo the enlargement which normally succeeds pollination. The prothallus in the aborted ovules was either absent or represented by shrivelled remains, but the examination of these ovules was of use in affording pollen-tubes arrested at an earlier stage of their development than those in the seeds.

The seeds varied somewhat in size, but averaged about 2.5 cm. long by 2 cm. broad: the differences mainly depended on the variable thickness of the fleshy layer of the integument, which is especially thick at the micropylar end of the seed. Within the succulent layer, of a bright purplish pink colour, is the sclerotic layer, which is continued outwards as a pointed projection surrounding the inner fourth of the micropylar canal (Fig. 18). Within this is a brown layer of papery texture including the internal system of vascular bundles. Below the level of separation of nucellus and integument no distinction of these regions can be made in the internal layer. As Fig. 18 shows, the eight vascular bundles of the inner vascular system bifurcate once or twice but no anastomosis takes place. The remains of the nucellus form a thin cap over the micropylar end of the prothallus. In the centre of this, the pollen-chamber with its conical apex is to be seen. The prothallus, now of a white colour owing to its cells being

filled with starch, is still enclosed within the wall of the megaspore. Just beneath the pollen-chamber its surface is depressed, to form a single cylindrical cavity about 2 mm. in diameter with vertical or slightly overhanging sides. At the base of this archegonial depression (Fig. 19) the necks of from two to four archegonia were distinguishable.

Only a single unfertilized archegonium was met with; it is represented in Fig. 20. From this the close agreement between *Stangeria* and other Cycads, as regards the structure of the archegonium, will be evident. The neck consists of two cells projecting above the general level of the surface of the prothallus, and the cap-like ventral canal-cell bears much the same relation to the large ovum as in *Cycas*. The nucleus of the ovum was situated towards the upper end, while in a central position a small irregular area of less granular cytoplasm was to be seen. The thick wall of the ovum is strongly pitted, several pits being present opposite each cell of the layer of the prothallus, forming the wall of the venter. Sometimes the pair of neck-cells was met with, although the lower cell had not developed into an ovum; such a case is represented in Fig. 21. The structure of the prothallus does not call for special remark; its cells, with the exception of those around the archegonia, and the limiting layer, which presents the structure described by Warming¹ in *Zamia*, being filled with starch.

Having considered the general structure of the pollinated ovule of *Stangeria*, there remain, for more detailed consideration, two classes of facts, those relating to the changes in the pollen-chamber after pollination, the germination of the pollen-grain, and the mode of fertilization, and those relating to the development of the embryo. On both of these questions, although the material did not afford a complete series of stages, it has been found possible to draw sufficient conclusions to bring *Stangeria* into relation with those Cycads in which the phenomena of fertilization and embryogeny have been fully studied.

¹ Warming, loc. cit., 1877. Pl. III, Fig. 30.

When pollination takes place the ovule is probably in the condition represented in Fig. 13. In normal ovules, as growth proceeds, the nucellus becomes reduced to the pollen-chamber in the centre of a thin cap of compressed tissue ; the stages in the absorption of tissue leading to this were not observed, the nucellus of the aborted ovules on the pollinated cone being in the former state, and that of the seeds in the latter. Pollen-grains were present in all the pollen-chambers. While some of the grains presented no alteration or were merely attached to the wall of the chamber by short tubes, others had undergone great changes. The pollen-tubes had penetrated the tissue of the nucellus, and were found radiating on all sides from the pollen-chamber (Figs. 22, 23). Usually their course lay just beneath the surface of the nucellus, but in some of the aborting ovules, in which the more internal tissue of the nucellus was not absorbed, the tube had grown inwards through this ; this latter course was evidently abnormal. The pollen-tubes were of considerable length, and of a much greater diameter than the pollen-grain itself. The free portion of the tube to which the wall of the pollen-grain was attached bent downwards towards the base of the pollen-chamber, which, by the absorption of the internal tissue of the nucellus, offers a free passage towards the megaspore (Fig. 24). The free end of the tube did not appear to project much beyond the lower opening of the pollen-chamber, and was thus in the seed separated from the archegonium-neck by almost the whole depth of the archegonial depression. To what extent this depression is fully formed at the time of fertilization it is impossible to say, but there is sufficient evidence to show that the passage from the pollen-tube to the archegonium-neck is effected by the independent motility of the spermatozoid. The details of the development of the latter from the generative cell of the pollen-grain could not be followed, but in the free ends of pollen-tubes in the aborted ovules two fully developed spermatozoids were present. These had apparently died in the unopened pollen-tubes, and consequently stained very badly, but the spiral cilium-bearing

band was rendered prominent by the use of Heidenhain's Haematoxylin (Fig. 25). In the pollen-chambers of the seeds the free ends of the pollen-tubes were always open and empty (Fig. 23). As the vertical section of the chamber shows (Fig. 24), the spermatozoids could easily find their way into the archegonial depression, and in fact remains of dead spermatozoids were not unfrequently met with against the wall of the latter; they were usually entangled in an amorphous substance which could be traced into the necks of fertilized archegonia. Analogy would appear to justify the conclusion that the archegonial depression had for a time been filled with fluid, in which the motion of the spermatozoids had taken place; this had, however, disappeared at the time of collecting the material. More or less disorganized spermatozoids, which had failed to effect fertilization, were also found just within the archegonium.

In the cytoplasm of the ovum, at the end adjoining the archegonium-neck, remains of the spermatozoid, by which fertilization had been effected, were frequently visible. The persistent part was the spiral cilia-bearing band; it was usually so altered in form as to give no idea of the shape of the spermatozoid, though the cilia attached to it were visible. Fig. 26, however, shows one of the sections through such a spiral band, the form of which has been retained; reconstruction of the series showed that it formed a conical spiral of five turns.

These observations taken together show clearly that the behaviour of the pollen-grain in the pollen-chamber of *Stangeria*, the production of spermatozoids, and the mode of entrance of the latter into the archegonium, agree with what has been described for *Cycas*¹ and *Zamia*². Further, although well-fixed spermatozoids were not available for study, the form of the spiral band (blepharoplast) leaves no room for doubt that they were of the same type as those of the genera mentioned.

¹ Ikono, Jahrb. für wiss. Bot. xxxii, p. 557.

² Webber, Botanical Gazette, xxiv, p. 16.

II. The Ovule of *Stangeria paradoxa*. 293

The actual process of fertilization was not observed, the youngest fertilized archegonium showing several nuclei in its cytoplasm. These were situated in the central area of more finely granular protoplasm referred to in the description of the archegonium, and, in the only embryo of this age found, were undergoing karyokinetic division. Slightly older embryos (Fig. 27) had numerous small nuclei in the cytoplasm; these tended to take up their position at the periphery, and were most numerous at the lower end of the embryo. In the next stage, though the embryo is still enclosed in the wall of the archegonium, it no longer forms a continuous mass filling the venter of the latter, but appears as a hollow sac enclosing a large cavity (Fig. 28). The occurrence of this change has been described by Treub¹ and Ikeno² in the similar embryo of *Cycas*; in this genus also it would appear to take place before the embryo has undergone any increase in size. As in *Cycas*, the embryo with its suspensor is developed entirely from the lower end of the hollow sac of tissue. The upper portion remains as a thin layer of cytoplasm with free nuclei in it, lining the thin cell-wall, with which the embryo surrounds itself. The cells forming the thicker lower end of the sac become enclosed by cell-walls, and, as Fig. 29 shows, become differentiated into the suspensor and embryo. The latter has broken through the wall of the archegonium, and projects into the tissue of the prothallus. At this stage the embryos from the different archegonia of a prothallus, though inclined towards one another, are separated by tissue of the prothallus. The short suspensor is composed of vacuolated cells; those forming the embryo are meristematic, and can be distinguished into an epidermal layer and the internal smaller-celled tissue. As the suspensors of the several embryos increase in length, the latter come to occupy a single cavity, formed by the progressive destruction of the surrounding tissue of the prothallus. In the upper part of this common space the suspensors, which become considerably coiled and folded, are found, while at the base, embedded in the still

¹ Ann. Jard. Bot. Buitenzorg, iv, p. 5.

² loc. cit.

coherent tissue, are the embryos (Fig. 30). The embryo is usually wedge-shaped, but exhibits no greater differentiation of its tissues than in the earlier stage. One of the embryos has, as Fig. 30 shows, by this time become larger than the others, and penetrates more deeply into the prothallus. This isolation of the successful embryo is more apparent in Fig. 31, which represents the largest embryo found in an unsown seed. In the process of elongation of the suspensor its base is often forced upwards towards the neck of the archegonium, the contents of which have, as a rule, by this time entirely disappeared.

The embryo in one seed, which had been placed under suitable conditions for germination in the Edinburgh Gardens, was examined after some weeks. As Fig. 32 shows, it was not firmly embedded in the prothallus, but hung free on the suspensor into the cavity formed by the destruction of the surrounding tissue. The embryo had increased somewhat in size, but showed no advance in morphological differentiation, no indication of the cotyledons being apparent: the tissue composing it resembles an apical meristem more closely than it did at earlier stages.

In the next stage obtained the primary root of the seedling was already over an inch in length. The seedling resembled those of other Cycads at this stage. The anatomy agreed generally with the description by Worsdell¹, founded on a somewhat more advanced seedling of *Stangeria*. It would appear to be a justifiable inference from a comparison of the embryos figured in Figs. 31 and 32 with the seedling, that the intimate connexion, which ultimately obtains between cotyledons and prothallus, is effected by the origin of the former on an embryo hanging free in the absorption cavity. This explains the way in which the stem-apex is freed from contact with the prothallus, and is in a position to be carried outside the seed by intercalary growth of the cotyledons when germination takes place.

One or two deviations from the normal embryogeny may

¹ Journ. Linn. Soc., vol. xxxiii, p. 447, 1898.

be mentioned in conclusion. Evidence was obtained that small accessory embryos¹ may be formed on suspensors which terminate in normal embryos, but the material did not permit of their detailed study. In one instance an embryo was found to have originated at the upper end of the archegonium. The suspensor, which was of considerable length, projected from the archegonium-neck into the depression at the micropylar end of the prothallus (Fig. 33), but the embryo borne on it had apparently disappeared. Probably the disappearance of the fluid filling the archegonial depression at the time of fertilization had arrested its growth. A small accessory embryo was present within the archegonium.

The above description will have made evident the close general agreement in development of ovule, pollination, fertilization, and embryogeny, which *Stangeria* presents with other genera of Cycads. The phenomena observed in *Stangeria* may be briefly summarized:—

1. Two ovules are developed on each sporophyll; they are situated one on either side of the short stalk and are not marginal, but borne on the morphologically lower surface.

2. The development of the ovule is similar to that of *Ceratosamia*, save that the mother-cell of the embryo-sac appears to attain a greater size before it undergoes division into three. The parts of the ovule (stalk, integument, nucellus, sporogenous group and megaspore) bear the same relations to one another as in *Ceratosamia*.

3. At the time of pollination the prothallus fills the megaspore, but archegonia are probably not present; the sporogenous tissue is represented by a single persistent layer, possibly tapetal in nature; the pollen-chamber is fully formed, but absorption of the tissue of the nucellus between it and the megaspore has not commenced.

4. The pollen-tubes penetrate the nucellus as in *Cycas* and *Zamia*; in the free end of each two spermatozoids are formed.

¹ Such accessory embryos are described in *Macrozamia* by Miquel, Nouveaux Matériaux pour la Connaissance des Cycadées, p. 18, Pl. II, figs. 4 and 8 (Arch. Néerland., t. iii, 1868).

The spermatozoid is of large size and possesses cilia attached to a blepharoplast which forms a spiral of five turns.

5. By the absorption of the intervening tissue of the nucellus a free passage is formed between the pollen-chamber and the prothallus. The spermatozoids, on their escape from the pollen-tube, which only bends down for a short distance, probably reach the archegonium by their independent motility.

6. The embryos, which are formed singly at the lower ends of the archegonia as in *Cycas*, possess long suspensors and come to occupy a common cavity formed by absorption of the tissue of the prothallus; ultimately one embryo becomes dominant in each seed.

7. The embryo frees itself from the prothallus before the cotyledons are formed; these become in turn intimately attached to the prothallus.

THE MATURE MICROSPORIUM OF *STANGERIA*.

A CORRECTION.

In the first number of these studies too close a comparison was drawn between the microsporangia of *Stangeria* and the sporangia of *Angiopteris*. Since the resemblance between the microsporangium and ovule may be of importance in discussing the results obtained from the study of *Stangeria*, this opportunity may be taken of correcting the erroneous impression conveyed in my earlier paper; at the same time some additional facts ascertained regarding the microsporangium may be stated.

The error referred to does not concern any of the points illustrated in the figures of my former paper, but is contained in the reference to the sporangium of *Angiopteris* on p. 432. The view there expressed was that bands of thicker-walled epidermal cells on either side the line of dehiscence in the microsporangium of *Stangeria* might be regarded as corresponding to the distinct lateral bands of indurated cells in a corresponding position in *Angiopteris*. Further observa-

tions have led to the conclusion that the recognition of these as definite bands in *Stangeria* was due to the sporangial wall not being quite mature, and that, although the epidermis in this position and extending down from the apex of the sporangium towards the lower surface has somewhat thicker walls than that on the sides of the sporangium, the differences do not warrant the close comparison made with any particular sporangium among Ferns, such as that of *Angiopteris*.

With regard to the group of small isodiametrical cells situated at the apex of the sporangium, these further observations have shown a considerable range of variation in *Stangeria*. As a rule the group consists of only a few cells with fairly thick colourless walls, surrounded by the brown thick-walled cells, which present no distinctive characters when they border on the apical group. It is this apical group which has usually been compared to the annulus of *Osmunda* and *Angiopteris*. But some of the microsporangia of *Stangeria* were found to present an apical group of larger size composed of fairly thin-walled cells. Around such a group as this the thicker walled brown cells form a definite and regular margin. Further, the series of cells immediately adjoining the apical group in these cases is characterized by its definiteness, and suggests a comparison with the annulus of the *Schizaeaceae*, in its less defined form, as seen in *Mohria*. It is not intended to imply by this comparison any actual relationship with the *Schizaeaceae*, but to suggest that, until more evidence is forthcoming as to the type of sporangium from which the Cycad microsporangia are derived, the nature of the apical group must be regarded as an open question. On the one hand, as is usually assumed, it might correspond to the annulus, on the other it may be more nearly comparable to the region of the sporangial wall above the annulus in the Schizaeaceous sporangium. It may be hoped that a comparative study of the other genera of Cycadaceae will throw further light on its true nature.

THEORETICAL DISCUSSION OF RESULTS.

In this and the preceding number of these studies, the development and structure of the microsporangium and ovule of *Stangeria* have been examined, so far as was possible on material obtained from cultivated plants. The general agreement of the results attained for this genus with those previously recorded by Warming and Treub, renders a similarly detailed investigation of the remaining genera unnecessary. It therefore appears advisable to discuss briefly the general conclusions indicated by the facts in connexion with *Stangeria* and to reserve for a future number of these studies a comparative treatment of the sporangia of the other Cycads; this will further enable the general view here advanced to be tested in detail. The equally important data supplied by the vegetative organs of the *Cycadaceae*, and the evidence regarding the phylogeny of the group obtainable from fossil plants, will also be most suitably considered after the series of existing genera has been comparatively treated. In this place the consideration of the general phylogenetic problem will be left on one side and only the morphology of the sporangia discussed. It is only necessary to point out here the justification, which the geological history of the seed-bearing plants affords, for considering the nature of the Cycadean ovule and its relation to the microsporangium, without extending the comparison to the other Gymnosperms or to the Angiosperms. So far as is at present known, Cycadean plants can be traced back to the Permo-Carboniferous flora. In rocks of this age well-preserved seeds are also found, some of which are probably Cycadean, while others may belong to the Cordaiteae. The remains of the latter group carry the first origin of the Cycadeo-Cordaitean type of ovule to beyond the earliest period of which adequate fossils remains are preserved. It is unnecessary for our present purpose to consider the probable degree and kind of relationship between Cordaiteae and Cycadaceae.

II. The Ovule of *Stangeria paradoxa*. 299

The general type of ovule is the same and may fairly be regarded as the most primitive preserved among existing plants, a conclusion that has received the strongest support from the discovery in recent years of the peculiar behaviour of the pollen-grain, and the free passage of motile spermatozooids from the pollen-chamber to the archegonium in Cycads. It thus appears to be a legitimate course to pursue, to seek for indications of the probable mode of origin of the Cycadean ovule from the living forms, in which alone the ontogeny is accessible, since the group has preserved so many primitive characters apparently little altered.

The inquiry, as limited to the sporangia of *Cycadaceae*, has two aspects, the question as to the group of Pteridophyta from which the Cycads were derived, and that of the morphology of the Cycadean ovule. The former of these questions, if capable of a satisfactory answer, would afford a starting-point for the solution of the latter, by indicating the type of sporangium, from which both microsporangium and ovule were derived, though it would remain a question whether the ovule is to be regarded simply as a modified sporangium, or as a sporangium together with part of the sporophyll. Looking to the habit, structure, and floral morphology of both *Cordaiteae* and *Cycadaceae* it cannot be said that their relationship to any particular group of Vascular Cryptogams is at present satisfactorily proved, but as regards the *Cycadaceae* a number of facts are known pointing to an origin from a Fern-like group of plants. This (which may be taken as the most probable hypothesis open to us at present) would accord well with the position and arrangement of the microsporangia.

Even if this question is regarded as in some degree an open one, it is possible to inquire into the morphology of the Cycadean ovule, since we may assume with some probability that the microsporangium, with its general resemblance to the more bulky sporangia found among the Ferns, has not come to differ very widely from the sporangia of the unknown ancestral group.

Before, however, considering the facts regarding *Stangeria*

from this point of view, the main views recently expressed on the morphology of the Cycadean ovule by investigators who have studied the group must be shortly summarized. Since 1880, when the sporangium was definitely recognized by Goebel as an organ *sui generis*, the comparison between the ovule and a bud has been practically abandoned. The nucellus has been clearly recognized as corresponding to a sporangium; the integument being regarded either as homologous with such an indusium as that of the *Hymenophyllaceae*¹, or simply as a new formation. In his earlier work Strasburger regarded the whole ovule as corresponding to a sporangium, but in his latest statement² on the Gymnospermous ovule he definitely compares the nucellus with the sporangium, and adopts the view that the integument is a new formation. One point common to the estimate of the Cycadean ovule formed by both these observers and to the conclusion of Treub³, is the comparison of the ovule in this group with a sporangium enclosed within the tissues of the sporophyll, and not projecting freely from it, such, for instance, as the individual sporangia in the *Ophioglossum* spike. In *Ceratozamia*, the ovule of which he investigated, Treub recognized the appearance of the sporogenous group of cells *within the sporangiferous lobe of the sporophyll*, and regards as a new formation the free upgrowth of nucellus and integument from the summit of the sporangiferous lobe turned towards the axis of the cone.

In these views of the nature of the Cycadean ovule and the type of sporangium from which it may be derived, there is, so far as I am aware, no comparison instituted between the microsporangium and ovule, except as regards the sporogenous group and the layers immediately surrounding it. Indeed the microsporangia and the ovules of Cycads have usually been compared with such very different sporangia as those of *Angiopteris* and *Ophioglossum* respectively. It is probable that this absence of direct comparison between the

¹ Warming, *Syst. Bot.*, p. 169.

² Bot. Practicum, 3rd ed., p. 514.

³ loc. cit., 1885, p. 48.

Cycadean ovules and the microsporangia has been due to the ovules investigated having a marginal position on the sporophyll, while the microsporangia are usually borne on the under surface. In *Stangeria* (and the case is the same with some other genera) the ovule is clearly intramarginal, and careful comparison of the male and female sporophylls shows that as regards position the ovule clearly corresponds to a microsporangium or to a sorus of microsporangia. In seeking for evidence as to the homology of any structure, relative position, although important, must be confirmed by the study of the mode of development and by the structure of the organ when mature. With regard to the development considerable correspondence between the ovule and the sorus can be recognized in the early stages. The first stages of the ovules of *Ceratosamia* described by Treub, from which there is no reason to assume *Stangeria* to differ, show clearly that the sporogenous group of tissue has the same origin as in the microsporangium, though probably in the case of the ovule derived from more numerous hypodermal cells. Even in the ovule represented in Fig. 2 the continuity of the rows from the hypodermal layer to the base of the sporogenous group may be traced. The differences between the development of the sorus of microsporangia and the ovule only become pronounced when the arrest of growth in the region between the individual sporangia takes place, or (a mode of expressing the same fact which is probably more correct) when active growth becomes localized around each archesporial group. The comparison at later stages must be made between the individual sporangium and the ovule; in both a large oval mass of sporogenous tissue is developed, which is marked off more clearly by the flattening of the layers of cells immediately around, and readily separates at this region. Further, the comparison is perhaps justifiable between the persistent tapetal layer, derived from the outer layer of the sporogenous tissue in the microsporangium, and the layer which has the same place of origin in the ovule. The facts of development then would support the view that the ovule

of *Stangeria* is homologous with a sorus of microsporangia, in which only one sporangium is present, so that the sorus as a whole had kept pace with the developing sporogenous mass. When the origin of the microsporangium in the sorus is taken into account, it is evident that there is little, if any, difference between this conclusion and the statement that the ovule is homologous with a microsporangium. It is not difficult to recognize the equivalent of the projecting tip of the nucellus in the divisions which take place in the wall of the microsporangium above the sporogenous tissue. These divisions are better represented in the microsporangium of *Zamia* than in that of *Stangeria*; in the former case, as Treub's figures¹ show, they lead to the development of a pointed tip to the sporangium, which presents a striking resemblance to the apex of the nucellus of the ovule. There remains only the integument to be considered. The projecting portion of this is clearly unrepresented in the microsporangium, and, on the view here suggested, would be regarded as an annular upgrowth, around the apex of the nucellus, of the bulky sporangial wall, or, which comes to the same thing, of the edge of the receptacle which had kept pace with the single sporangium. It will be evident from this comparison of the ovule with the microsporangium, and from the mode of origin of the integument suggested as probable, that the ovule is not regarded as a sporangium sunken in a lobe of the sporophyll. The facts appear to the author more naturally explained, when we compare the ovule to a sorus consisting of a single sporangium, which develops on the whole similarly to a microsporangium, save that it is bulkier, and the wall from the first thicker.

If the more advanced ovule, say at the period of pollination, be compared with the mature microsporangium, it is not surprising to find nearly all traces of the structure of the epidermal layer, adapted for dehiscence in the microsporangium, absent in the ovule. It may, however, be pointed out that in the Cycadean type of ovule we have a mega-

¹ Treub, loc cit., 1885, Pl. II, Fig. 4.

sporangium which still opens to the outside, so that free access is given to the spermatozoids to reach the megaspore though the latter is not liberated. As regards the structure of the opening, it can only be tentatively suggested that the pollen-chamber, with its thick-walled cells, may correspond to the ring of thick-walled cells around the apical cap of the microsporangium.

Whatever the modifications were that led to the origin of the Cycadean type of ovule, no direct evidence of their nature is available at present, and it is thus necessary to connect the facts by as reasonable an hypothesis as possible. It may be pointed out, however, that the view above expressed, which rests primarily on the similar position held by the ovule of *Stangeria* to that of one of the sori of microsporangia, derives support from an examination of the relation borne by the individual sporangium to the sorus in some groups of Vascular Cryptogams. The reduction of the number of sporangia in the sorus to a single one occurs not infrequently in some species of *Gleichenia* and is the normal state of affairs in the majority of the *Schizaeaceae*, in which the monangial sorus has been definitely recognized. In these cases the variation is unconnected with heterospory, but its connexion with this is strikingly illustrated by *Azolla* among the *Hydropterideae*. It will be obvious that the reduction in number of the sporangia in a sorus, and along with this of sori on a sporophyll, would be still more necessary when the completion of the changes within the megaspore and the production of the embryo were all to go on at the expense of the parent plant. There would thus appear to be, on *a priori* grounds, good reason to assume that the need of such a reduction in number of sporangia in the sorus would have been even greater in the evolution of the ovule of Cycads than in the group of *Hydropterideae*, which are here cited as an unrelated but somewhat parallel case.

A provisional examination of material of a number of other genera of Cycads has not disclosed anything inconsistent with the view of the nature of the Cycadean ovule here put

forward, while indications are not wanting that the comparative study of these genera will afford it further support. As, however, the material collected does not as yet allow of a sufficiently wide comparison, this will be deferred for the present. Until this is done, however, the view that the Cycadean ovule is the equivalent of the male sorus can only be put forward as a provisional statement, which will have to be carefully tested by a comparative examination of the whole series of living Cycads, in the light of the evidence obtainable from extinct forms.

EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Dr. Lang's paper on the ovule of *Stangeria paradoxa*.

PLATE XVII.

Fig. 1. Young sporophyll, bearing two ovules, viewed from its point of attachment to the axis; the ovules are situated within the margin on the abaxial surface. *st* = cut surface of stalk of sporophyll. ($\times 25$.)

Fig. 2. Median longitudinal section of a young ovule; the free portions of nucellus and integument are just recognizable, and the mother-cell of the embryo-sac can be distinguished in the centre of the sporogenous tissue. ($\times 110$.)

Fig. 3. Similar section of a slightly older ovule, the integument being omitted; the mother-cell of the embryo-sac has increased in size and become thicker-walled but is still undivided; the sporogenous tissue is shaded. ($\times 375$.)

Fig. 4. Mother-cell of embryo-sac divided into two segments, with the adjoining sporogenous tissue; in the cells of the latter the spherical bodies described in the text are visible. ($\times 375$.)

Fig. 5. Mother-cell of embryo-sac divided into three segments, the lowest of which is destined to become the embryo-sac. ($\times 375$.)

Fig. 6. Young embryo-sac, still uni-nucleate, with the two sister-cells in a crushed condition at its upper end. ($\times 375$.)

II. The Ovule of *Stangeria paradoxa*. 305

Fig. 7. General view, in median longitudinal section, of the whole ovule at the stage with uni-nucleate embryo-sac. ($\times 50$.)

Fig. 8. Longitudinal section of nucellus of older ovule, showing the embryo-sac with numerous free nuclei in its cytoplasm. ($\times 70$.)

Fig. 9. Transverse section of ovule of similar age passing through the sporogenous tissue and megaspore. ($\times 70$.)

Figs. 10-12. Series of photographs of the apex of the nucellus and micropyle from ovules of increasing age; in Fig. 12 the pointed projection of the nucellus destined to form the pollen-chamber is just apparent. ($\times 110$.)

Fig. 13. Median longitudinal section of older ovule; the sporogenous tissue is reduced to a single layer around the megaspore, which is filled with the prothallus; the pollen-chamber is fully formed. ($\times 7$.)

Fig. 14. Micropylar region, from a similar section, showing the relation of the pollen-chamber to the micropyle. ($\times 25$.)

Fig. 15. Pollen-chamber from a similar section of an aborting ovule, showing the large thick-walled cells forming the wall. ($\times 70$.)

Fig. 16. Part of a section through an ovule of the same age as that in Fig. 14. From left to right are seen the edge of the prothallus, the wall of the megaspore, the persistent layer of the sporogenous tissue, and the tissue of the integument. ($\times 110$.)

PLATE XVIII.

Fig. 17. Sporophyll from the fertilized cone bearing a seed and an aborted ovule; seen from above. (Natural size.)

Fig. 18. Dissected seed, showing the succulent and woody layer of the seed-coat cut through, the inner layer with its system of vascular bundles covering the prothallus, and the remains of the nucellus on the micropylar end of the latter. (Natural size.)

Fig. 19. View of the micropylar end of the prothallus from above, showing the apical depression with the necks of four archegonia at its base. ($\times 2$.)

Fig. 20. Longitudinal section through an unfertilized archegonium. ($\times 25$.)

Fig. 21. Archegonium-neck from the base of the archegonial depression, probably representing an arrested archegonium. ($\times 100$.)

Fig. 22. Surface view of the pollen-chamber and remains of the nucellus from a seed, showing the pollen tubes radiating out from the chamber. ($\times 7$.)

Fig. 23. One half of a similar specimen viewed from the inside, showing the blind ends of the pollen-tubes. ($\times 7$.)

Fig. 24. Median vertical section through the pollen-chamber and nucellus of a seed. ($\times 35$.)

Fig. 25. Part of a vertical section through the pollen-chamber of an absorbed ovule showing a dead spermatozoid (spm) in a pollen-tube which has been cut obliquely. ($\times 110$.)

Fig. 26. Spiral body of spermatozoid embedded in the upper part of the cytoplasm of a young embryo. ($\times 110$.)

Fig. 27. Young embryo showing commencement of vacuolization. ($\times 25$.)

Fig. 28. Older embryo still enclosed in the archegonium-wall, showing the large central cavity. ($\times 25$.)

306 *Lang.—Development of Cycadean Sporangia.*

Fig. 29. Longitudinal section of embryo borne on a short suspensor in the tissue of the prothallus; the archegonium-wall is broken through at its lower end. ($\times 70$.)

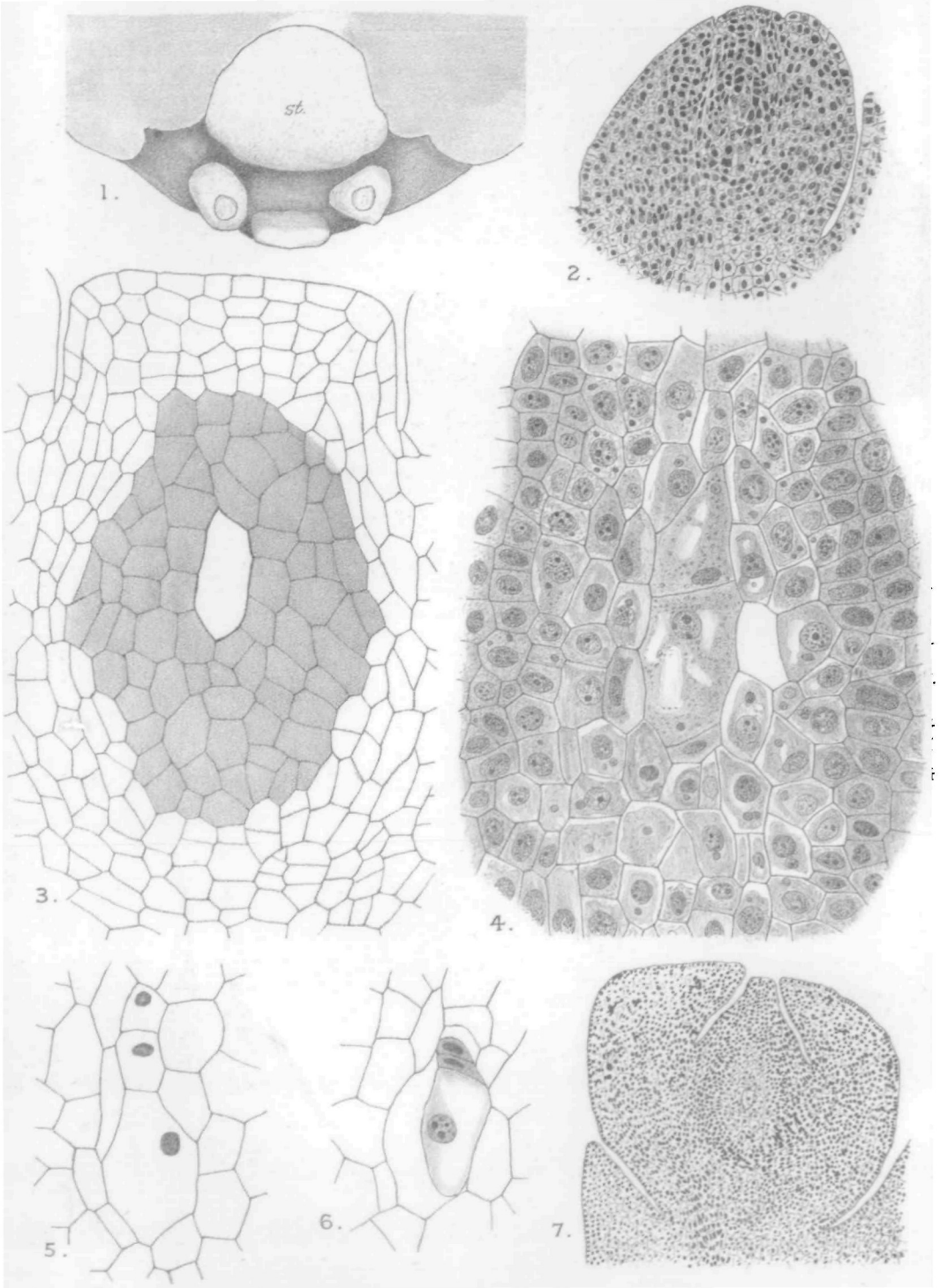
Fig. 30. Older embryo on long suspensor, the embryo still firmly fixed in the prothallus-tissue. ($\times 70$.)

Fig. 31. Similar section of the largest embryo found in a seed still borne on the cone; the embryo shown has outstripped its competitors more completely than that in Fig. 30. ($\times 70$.)

Fig. 32. Similar section of an embryo from a seed sown for some weeks, the embryo is at this stage suspended freely in the cavity formed by the destruction of the tissue of the prothallus. ($\times 70$.)

Fig. 33. Abnormal embryo, the suspensor having grown through the archegonium-neck into the archegonial depression; the remains of a small accessory embryo are seen below the base of the suspensor. ($\times 50$.)

Figs. 1, 3-6, 13, 18, 19, 21-24 and 28 from drawings, the rest from photographs.

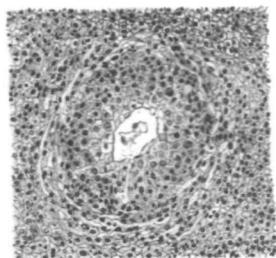


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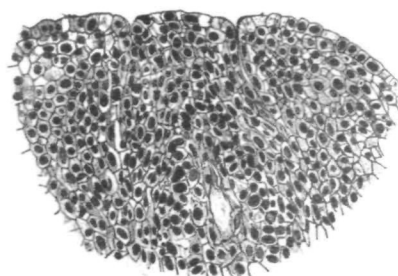
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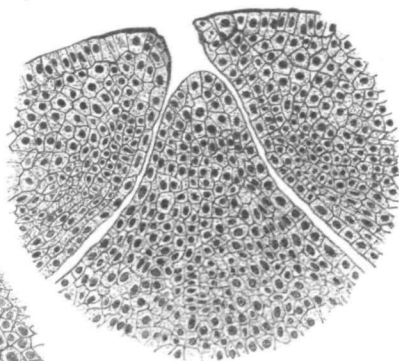
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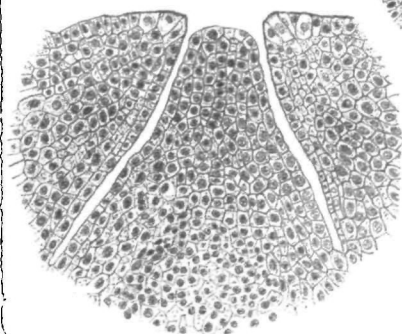
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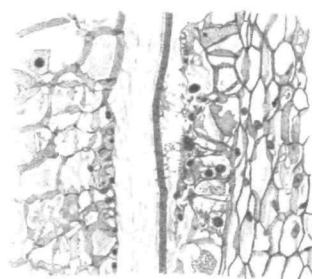
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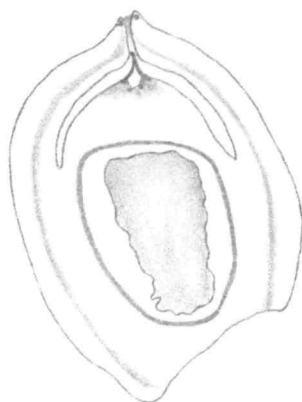
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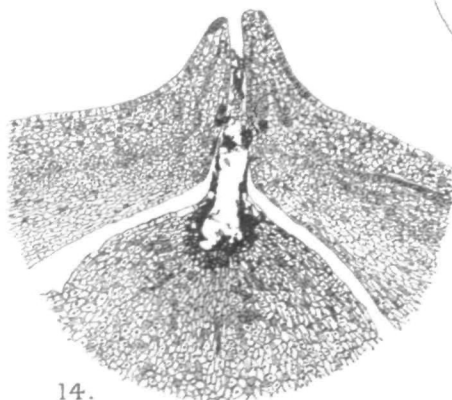
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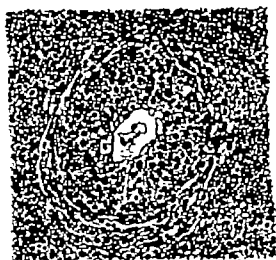
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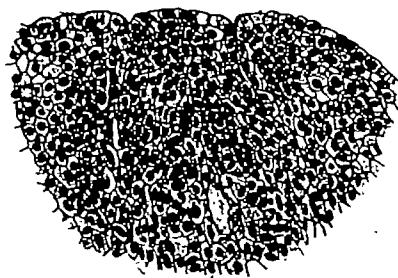
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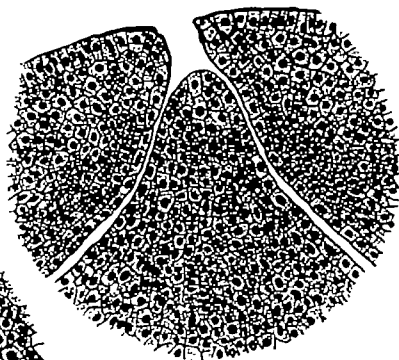
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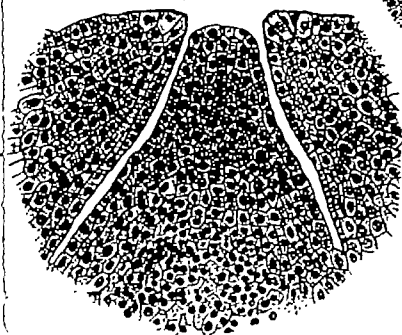
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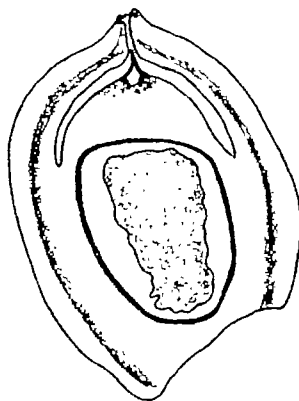
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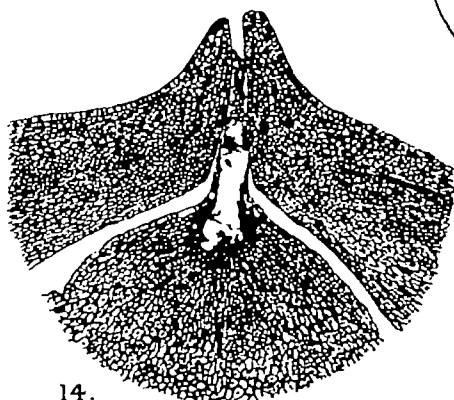
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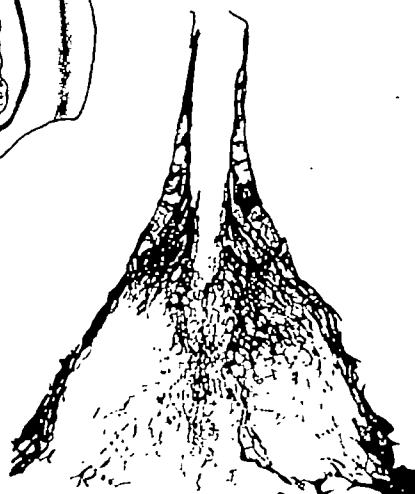
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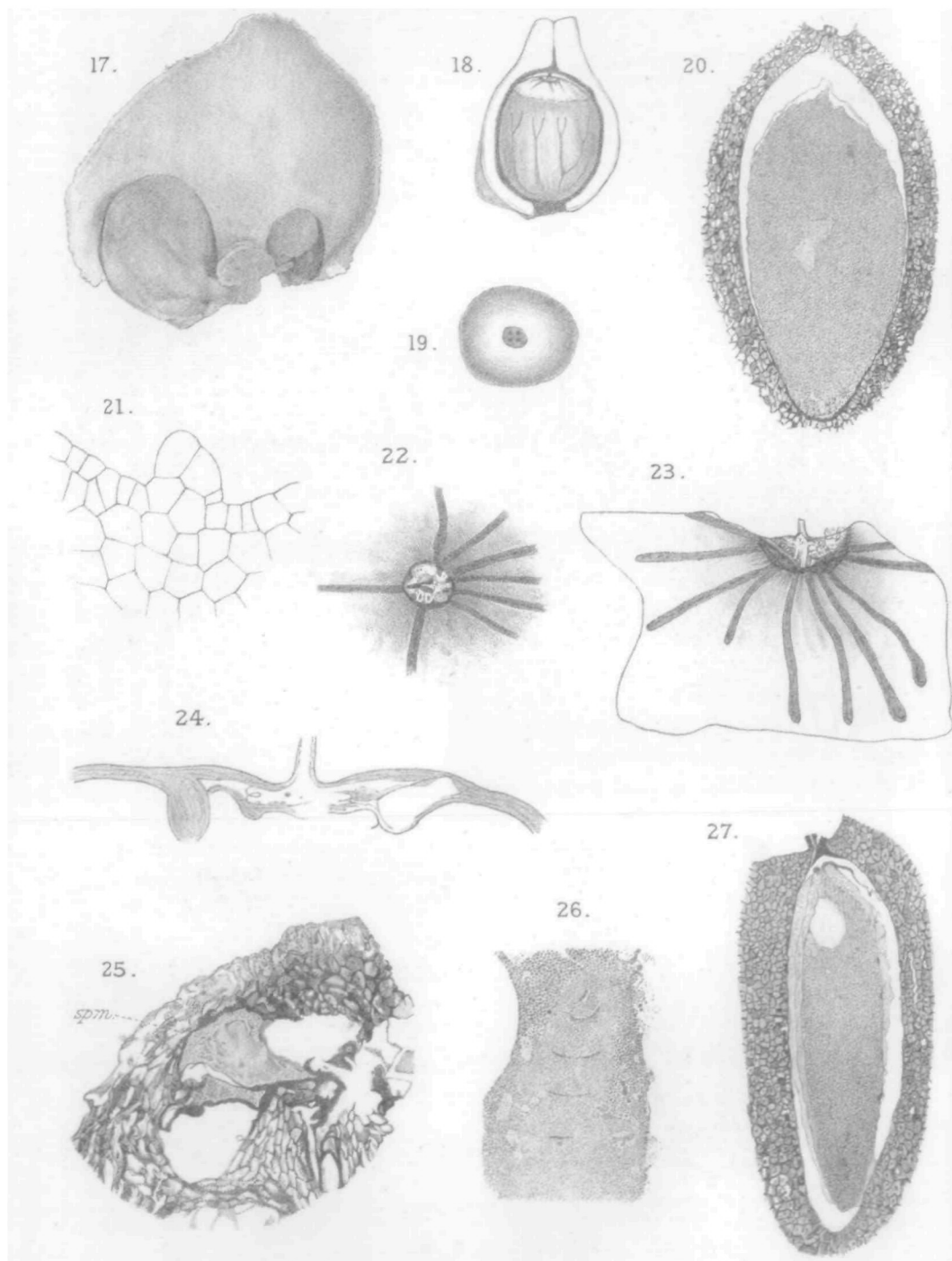


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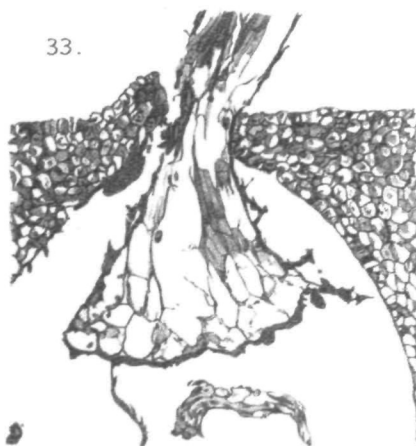
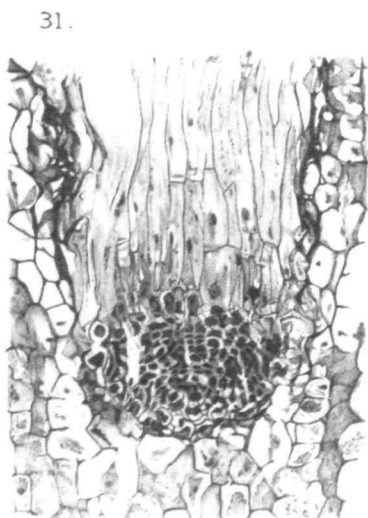
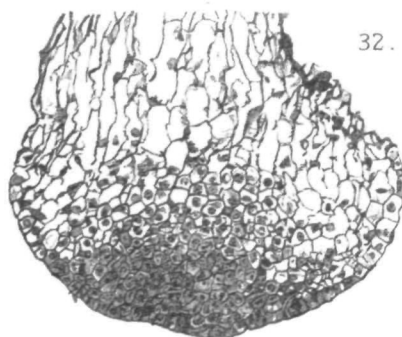
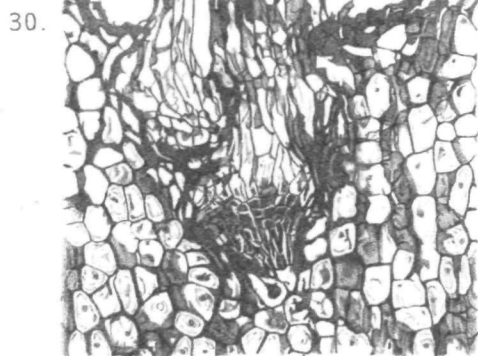
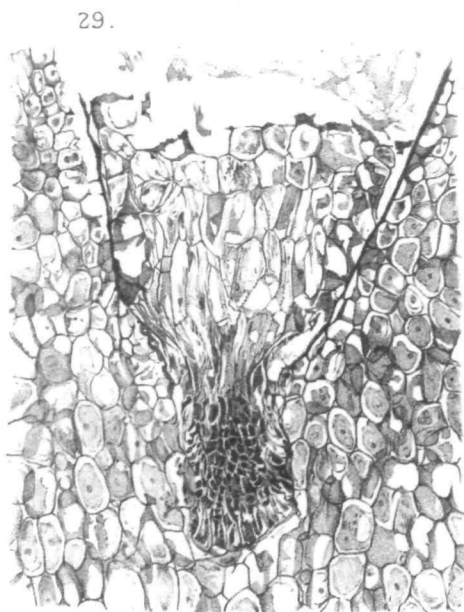
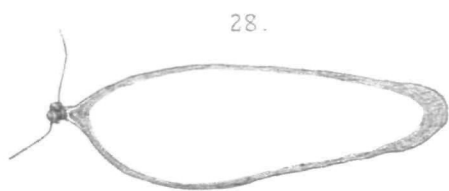


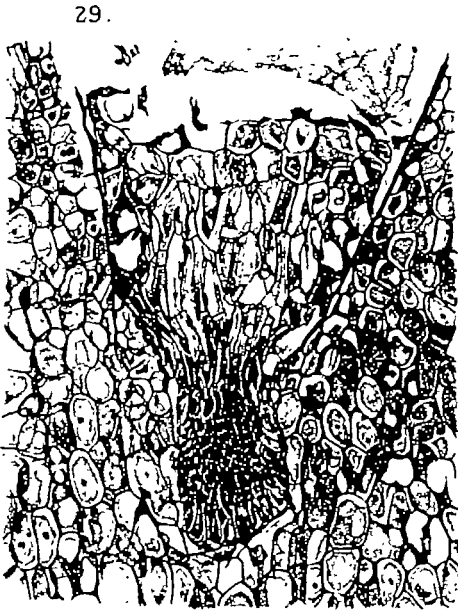
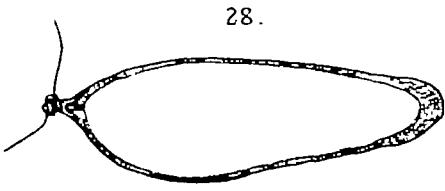
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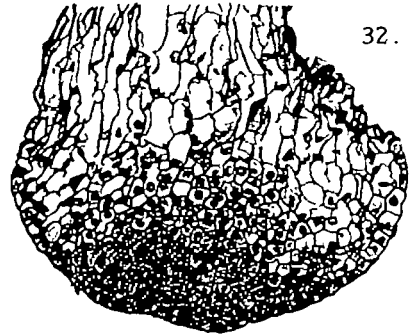




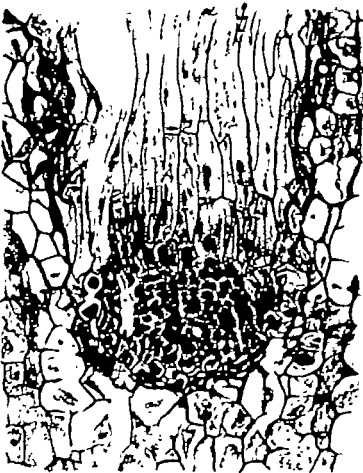
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