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STANGERIA PARADOXA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 214

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(WITH PLATES XXIV-XXVI AND ONE FIGURE)

Stangeria is the most fernlike of all the cycads; in fact it was described originally as a species of *Lomaria*, one of the Polypodiaceae, and the mistake was not corrected until the cones were discovered. The genus may be monotypic, with *Stangeria paradoxa* as its single polymorphic species. That its general habit, as it appears in the field, is extremely variable is beyond question; and that under cultivation in conservatories and botanical gardens it becomes quite different from the wild form is also apparent.

A plant called *Stangeria eriopus* in the New York Botanical Garden has produced fine crowns of leaves, surpassing anything one is likely to find in the field, and it has had at least one ovulate cone (10). A specimen of *S. paradoxa* in the Botanic Garden at Sydney, Australia, had in 1911 a fine display of leaves and 8 staminate cones; while another plant in the same garden had 5 ovulate cones and an equally fine display of leaves. In the Sydney plants the leaves were not in a single crown, but on subterranean branches with only a few leaves on a branch. In both the Australian and the African gardens *Stangeria* produces leaves and cones more freely than in the field, and both cones and leaves are larger than in field specimens. The cultivated specimens are more beautiful and luxuriant, and differ so much from plants in the field, that some

observers might make new species of them. In the Botanical Garden at Durban, South Africa, Mr. WYLIE showed me a vigorous *Stangeria* with leaves a meter long. The leaflets were 45 cm. long, and so deeply incised that they were almost pinnate. Some taxonomists, doubtless, would regard this plant as a distinct species. In *Dioon* and *Bowenia* the character of the leaf margin is so rigidly fixed and so persistently transmitted from one generation to another that it is entitled to rank as a specific character; but in *Stangeria* the character of the margin is so fluctuating that even the almost bipinnate Durban plant should not be regarded as specifically different from forms with entire leaflets.

Field observations

My field observations on *Stangeria* began at Ngoye, near Mtunzini in Zululand, about 100 miles north of Durban, and extended to East London, about 275 miles south of Durban. *Stangeria* certainly extends farther north, just how much farther I was not able to determine; and its western limit is west of East London. I did not find it at Port Elizabeth, about 150 miles west of East London, or even at Grahamstown, or Trapps Valley, about half-way between East London and Port Elizabeth; but Mr. GEORGE RATTRAY, of Selborn College, East London, has reported it from Port Elizabeth, and he regards this as about the western limit of its range. His extensive knowledge of South African cycads in the field enables him to speak with authority upon their geographical distribution.

In the field, *Stangeria* presents two forms, one growing on the open grass veldt and the other in the shade of bushes or trees, the shaded form being much larger and resembling more nearly the cultivated specimens. PEARSON (9) has contrasted the two forms as *Stangeria paradoxa*, the species originally described from the shaded Natal form, and *Stangeria* sp., the open grass veldt form; but he had seen both forms in the field, and consequently hesitated to give a specific name to the second form. RATTRAY, who lives in a *Stangeria* region, and who has studied the genus throughout its range, believes there is only one species. Before I left South Africa, I had come to the same conclusion. I dug up

several plants from the East London grass veldt and sent them to our greenhouse at the University of Chicago, where after three years of the usual unnatural conditions they are producing leaves and cones as large as those of the bush veldt form.

Near Mtunzini in Zululand the grass veldt stretches for miles, rolling and hilly, broken by huge rocks of granite and gneiss, and



FIG. 1.—*Stangeria paradoxa* growing in the grass veldt at Ngoye, near Mtunzini, Zululand.

occasionally with exposed surfaces covered only with *Selaginella* and lichens. The grass is 30–40 cm. high, and appearing above it are the leaves of *Stangeria* (fig. 1). The leaves are larger and more numerous than in the East London district, three or four leaves being common, and some stems having fine crowns of five or six leaves.

The plants are fairly abundant, as many as 20 being in sight at one time; but the specimens are scattered, with no crowded masses like the thickets of *Macrozamia spiralis* and *Bowenia serrulata* in Australia. Although adult plants are numerous, cones are

rare. At the time of my visit, the middle of January, 1912, the staminate cones had rotted or dried up, and the ovulate cones were falling to pieces. The few seeds which were secured showed embryos in early suspensor stages.

In the Mtunzini bush, which is particularly dense and rich in ferns, only a few plants of *Stangeria* were seen, but they were vigorous, with three or four leaves about twice the size of those in the open grass veldt. Not a single cone or seed was found in the bush. It is said that baboons are very fond of the seeds and carry them away as soon as the cones reach their full size.

Associated with *Stangeria* in the Ngoye grass veldt is *Encephalartos brachyphyllus*, a species with small cones and tuberous, subterranean stems. I saw one tall specimen of *E. Altensteinii bispinosus*, which is called also *E. Woodii*. It is said that there is not another plant of this species within 50 miles. I did not see a single specimen of any species of *Encephalartos* in the Ngoye bush veldt.

On the grass veldt at East London, *Stangeria* is not nearly so large or so abundant as in Zululand, but its appearance is the same, the leaves projecting a little above the grass, so that most of the plants within a distance of 100 m. can be seen. In this region *Stangeria* is associated with *Encephalartos Altensteinii* and *E. villosus*, while *E. cycadifolius* grows within a few miles.

The stem of *Stangeria* is tuberous and entirely subterranean, with a strong main root and weak branches. It grows in soil so hard and stony that both care and labor must be given to secure an uninjured specimen. The stem is quite smooth, the whole leaf breaking off so cleanly that there is never any armor of leaf bases.

Nearly all the plants dug up in the East London region showed more or less branching, a feature already noted by PEARSON and by RATTRAY. Occasionally the stem is simple, but usually it bears 1-4 branches arising from the lower part of the stem, rarely from the upper part. Often the stem and branches are so nearly alike that it is difficult to distinguish which is the main stem and which is the branch. Sometimes the branching condition is betrayed by the leaves, which may be too scattered to belong to a single crown and too crowded to belong to different plants; but

it may happen that only a single branch will bear a leaf, and in that case the branching is not discovered until the soil is removed.

The stem is monoxyllic, with a single very narrow zone of wood between the large pith and cortex. A stem 9 cm. in diameter had a zone of xylem only 2 mm. wide, and even in this zone the bundles were separated by a large amount of parenchyma. That this plant was an old one was evident, not only from its size, but also from the fact that it had borne several cones. In the field, cones are not abundant, about one coning plant in six being a liberal estimate. No observations have been made to show at what age *Stangeria* begins to bear cones, but some 5-year old seedlings in our greenhouse are only 2 cm. in diameter. We did not see any cones on plants less than 5–6 cm. in diameter, and we should judge that the specimen must have been at least 30 years old, perhaps much older. The display of woody tissue is the scantiest I have ever seen in any cycad of even approximately equal age.

Cone domes are numerous and are of the type already noted for *Dioon*, *Zamia*, and *Ceratozamia* (13). The main stem, therefore, is a sympodium, as are any branches which have borne more than one cone.

Sporangia

Although cones are rather infrequent, material can generally be secured in localities where the plants are abundant.

MEGASPORANGIUM.—The ovulate cone looks like that of *Dioon spinulosum*, only very much smaller, the similarity being due to the densely hairy surface and the thin rounded border of the blade portion of the sporophyll. The ovules, during earlier stages of development, are almost completely inclosed by outgrowths of the sporophyll, suggesting the angiosperm condition; but later the ovules grow rapidly and soon protrude beyond the outgrowths, which do not develop much after the ovules reach a length of 4–5 mm. At maturity the ovules have a rich orange color which is quite characteristic.

Early stages in the development of the ovule have been studied by LANG (5), and its vascular anatomy has been described in detail by WORSDELL (4) and by MATTE (7).

MICROSPORANGIUM.—The staminate cones are long and slender and very symmetrical. They vary greatly in size, those growing on the dry grass veldt sometimes being as short as 8–9 cm. in length, while those in the shaded bush veldt may reach a length of 20 cm., exclusive of the stalk, which may be more than 5 cm. in length. The sporophylls are small and the number of sporangia averages only about 150, which is lower than in any cycad except *Bowenia* and *Zamia*.

We have already stated that *Stangeria* was originally described as a fern, because the leaves so strikingly resemble those of *Lomaria*. The microsporangia are equally fernlike and bear a particularly close resemblance to those of *Angiopteris*. The wall of the microsporangium consists of an outer layer of very thick-walled cells, a very scanty tapetum, and 3–5 intervening wall layers. The thickening of the outer cells is most pronounced at the base, diminishes gradually on the sides, and at the top becomes so thin that it easily breaks, forming a pore through which the dense cell contents escape shortly before the dehiscence of the sporangium.

The earlier stages in the development of the microsporangium and in the origin and development of sporogenous tissue have been so thoroughly described and illustrated by LANG (2) that we have not felt it necessary to deal with this phase of the subject; but on account of the peculiar or at least newly described behavior of the chromatin at fertilization, the reduction divisions in the pollen mother cells would have been studied if material in this stage had been available.

Gametophytes

MALE GAMETOPHYTE.—The microspore is formed before any of the wall cells of the microsporangium begin to break down, but before the nucleus of the microspore divides the wall cells begin to disorganize and the tapetum becomes almost indistinguishable. During the two mitoses which result in the formation of prothallial cell, generative cell, and tube cell, the tapetum and the wall cells, with the exception of the hard outer layer, break down and form a dry, homogeneous membrane about the pollen grains, holding them together in one spherical mass. At the time of dehiscence, the

grains are dry and powdery, but are still confined by the thin membrane, which often holds them together even after they fall out from the sporangium. Most of the pollen, however, is in condition for wind pollination. Staminate cones of cycads are very frequently infested by insects, both before and after the pollen has been shed, but I have never seen anything to indicate that pollination was being affected by their agency. Besides, the ovules exude a large pollination drop, as in the case of gymnosperms known to be wind-pollinated.

When I reached Zululand, about the middle of January, 1912, the pollen tubes had already discharged; in fact, embryos were beginning to show suspensors. Although the season is somewhat later farther south, I did not find any cones in which the pollen tubes had not discharged. Accordingly, I am under great obligation to other people for material of pollen tube structures.

On January 6, 1910, Professor W. C. WORSDELL, whose extensive researches have so materially advanced our knowledge of the anatomy of cycads, visited Zululand and fixed for me some material showing pollen tubes with the sperms already formed. Mr. W. T. SAXTON, formerly of the South African College, but now of the Institute of Science at Ahmedabad, India, made several collections in the Transkei, and arranged with Miss SARAH VAN ROOYEN, of Kentani in the Transkei, for a close series of stages. The carefully prepared material furnished by Miss VAN ROOYEN yielded an excellent series of stages from the body cell, through the development of the sperm, and up to early suspensor stages in the embryo. The collections also included ripe seeds with mature embryos. Seedlings have been grown both from Professor WORSDELL's and from Miss VAN ROOYEN's collections.

The date of pollination was not determined definitely, but material collected in the Transkei on July 17, 1907, showed uniu-
cleate pollen grains with exine and intine well developed. This would indicate that pollen is shed late in July. Plants from that region, now growing in our greenhouse, shed the pollen in January 1916. The sporophylls of an ovulate cone loosened at the same time, just as they do normally at the pollination period. This wide difference is due doubtless to greenhouse conditions and not

to the transfer from one hemisphere to another, for *Stangeria* in the Botanic Garden at Sydney was shedding in December 1911. The pollen at the time of shedding shows a prothallial cell, a generative cell, and a tube cell, a condition which I have now observed in all the 9 genera of the family.

Early stages in the development of the pollen tube were not observed, but later stages indicate that they do not differ essentially from the course already described in detail for *Dioon edule* (11). A characteristic view of the pollen tube structures after the pollen chamber has extended entirely through the nucellus is shown in fig. 2. In the tube at the right the body cell has divided, but in the rest the division has not yet taken place. The tube nucleus is regularly near the body cell. The haustorial end of the tube extends in a straight line a few layers of cells below the epidermis, scarcely ever showing any branching, and there are no basal haustoria like those of *Ceratozamia*. While the pollen tube structures of the cycads present many similarities, the differences are probably sufficient for a determination of the genera.

By comparing figs. 2 and 3, which are drawn to the same scale, it is evident that in the later development both the tubes and the sperms increase greatly in size. It is during this rapid increase in size that the spiral band with its immense number of cilia develops from the blepharoplast. The cycad sperm is the largest and most complicated motile cell in either plants or animals. While the series of stages in *Stangeria* is quite satisfactory, the series in *Ceratozamia* is even more complete, thanks to Mr. ALEXANDER M. GAW, of Jalapa, Mexico, who for 10 years has been sending material to supplement my own collections. Besides, the blepharoplast of *Ceratozamia* is larger. Consequently, a more detailed study of the development of the sperm will be reserved for this genus; but some of the more obvious features, as they appear in *Stangeria*, will be presented here.

While the body cell remains elongated during the early stages in the development of the tube, the two blepharoplasts occupy the fore and aft positions; but as the basal end of the tube enlarges and the body cell becomes spherical, the blepharoplasts rotate 90° , so that their axis becomes transverse to the long axis of the tube.

At first, the blepharoplast is a solid, homogeneous body, but as it increases in diameter it becomes vacuolate and soon appears to be little more than a shell containing droplets of liquid (fig. 4). This stage is reached before the body cell divides. During the division of the body cell, the vacuolate blepharoplast breaks up into hundreds of small granules which, for a short time, occupy the space previously occupied by the blepharoplast (fig. 5). The daughter nuclei immediately after the division of the body cell are smaller than the blepharoplasts; but the nuclei increase rapidly in size, while the group of granules remains stationary until it begins to form the ciliated band. The beginning of the band takes place during a rapid increase in the size of the sperm (fig. 6). Many of the granules become elongated and have a distinct tendency to arrange themselves in rows, forming a band one layer of granules in thickness (fig. 7). From the entire surface of one side of this band, stiff bristle-like cilia grow out. The young band is variously twisted and curved, so that the bristles point in every direction, some of them even toward the nucleus, but the band soon turns so that all the bristles face outward (fig. 8). As the band nears the surface, the bristles prick through the cytoplasm and the growth of the cytoplasm at this point is checked, so that a spiral groove results. After piercing the cytoplasm, the bristles grow out into long, slender cilia, which at first are compressed by the wall of the sperm mother cell, but later extend outward and vibrate vigorously when this wall breaks down. The mature sperm varies from 150 to 190 μ in diameter, and its dense nucleus constitutes most of its mass, the diameter of the nucleus being usually only about 20 μ less than that of the entire sperm, so that the cytoplasm containing the spiral band is only a thin sheath. The number of turns in the spiral varies from 5 to 7, but one peculiar sperm was found with a long, narrow apex and 10 turns of the band (fig. 10).

FEMALE GAMETOPHYTE.—In the young nucellus LANG (5) found an axial row of 3 cells, the lowest of which became the functioning megaspore. In my own material the earliest stages showed archegonia shortly before the division which gives rise to the ventral canal nucleus and the egg. The figure for this division was observed only once, but it was not difficult to determine the

number of chromosomes as 12. Several preparations show that no wall is formed between the two nuclei, and that the ventral canal nucleus soon disorganizes, while the egg nucleus increases rapidly in size as it moves toward the center of the egg.

Some irregularities were noted. In two cases there were 4 neck cells instead of 2, in one of the two cases the two original cells having divided longitudinally, and in the other transversely. In several cases the central cell of the archegonium had not continued its development and was no larger than the tissue cells of the gametophyte, but the two neck cells were as large and turgid as those of a normal archegonium.

Fertilization

The archegonial chamber at the time of fertilization is moist, but no droplets of fluid can be seen, even with a 16 mm. objective. On the other hand, the pollen tubes are turgid, and as they discharge furnish enough fluid for a limited movement of the sperms. After the nucellus has been removed from the ovule, it is easy to see the sperms swimming in the pollen tube, but practically impossible to observe them, under natural conditions, in the archegonial chamber. The entrance of the sperm into the egg is probably effected as already suggested for *Dioon edule* (12). The liquid discharged from the pollen tube is of high osmotic pressure, and consequently draws some liquid from the turgid neck cells, and the lowered turgidity of these cells allows a portion of the upper part of the egg to escape, thus forming a vacuole which draws the sperm into the egg. The careful experiments of MIYAKE (8) have shown that the cycad sperm does not respond to chemotactic stimuli, being indifferent even to the material of the egg.

It is quite common for more than one sperm to pass through the neck of the archegonium, but rare for more than one to enter the egg itself (figs. 10, 11, 13). In one case 7 extra sperms had passed through the neck; in another case 4; in several cases 3; and in many cases 2 or 1. The apex of the extra sperm is usually directed toward the main body of the egg, and in such cases the sperm becomes more or less imbedded, doubtless on account of the movements of the cilia; but when the axis is not directed in this way,

the sperm is not likely to cause so deep a depression (figs. 10, 11, 13). The ciliated band is so conspicuous and persists so long that a complete series of sections will show, even to the close of the free nuclear period, whether more than one sperm has entered the egg. The photomicrograph (fig. 24) shows a part of the spiral band of the sperm whose nucleus has fused with that of the egg; and also shows two more sperms which have passed through the neck, one of which has become almost completely imbedded in the egg.

The behavior of the chromatin during fertilization has not yet been described for any cycad, but WEBBER (6) figured and described the sperm nucleus of *Zamia* imbedded in the egg nucleus, and IKENO (3) found the same condition in *Cycas*. In *Stangeria* the behavior is the same (fig. 9). Both nuclei, at this stage, are in the resting condition. Whether they continue in this condition as fusion proceeds, or break up into two groups of chromosomes, as in *Pinus* and several other conifers, has not been observed as yet. However, the structure of the first mitosis of the fertilized egg indicates that the resting condition is soon followed by the formation of chromosomes by each of the fusing nuclei. The number of chromosomes, as counted at the equatorial plate stage of the first mitosis, is 12; but later free nuclear divisions and also mitoses in root tips show that 24 is the diploid number; further, the mitosis at the formation of the ventral canal nucleus and egg shows 12 chromosomes, proving that this is the haploid number. With the conspicuous spiral band indicating the entrance of a sperm, parthenogenesis is out of the question. HUTCHINSON'S (14) recent study of fertilization in *Abies*, from an abundance of material in critical stages, which it has been my privilege to examine, furnishes an explanation of this apparently haploid, but really diploid condition. HUTCHINSON (14) finds that the spirems of the two conjugating nuclei segment into chromosomes, as has been described for various conifers; but, after the segmentation, the chromosomes unite in pairs, just as in the prophase of the heterotypic mitosis. Each pair then segments transversely and the two longitudinal parts separate, so that 4 chromosomes are formed from each pair and the diploid number is established. In *Stangeria*, 4 eggs were found which showed the first mitosis, all of them in the early

equatorial plate stage. In all of these the number of chromosomes is 12, but the double character of the chromosomes is evident; and since 12 and 24 are the haploid and diploid numbers, I believe that there is a pairing of chromosomes at fertilization, as described by HUTCHINSON (14) for *Abies*.

Embryogeny

FIRST MITOSIS.—At the first division in the fertilized egg, the mitotic figure is surrounded by a remarkable display of achromatic structures, arranged in irregular nets or grouped into cones resembling half-spindles (figs. 10, 11, 25). This achromatic area easily identifies the first mitosis, even when a second sperm has entered and divided, since the achromatic area surrounding the dividing sperm is much smaller (fig. 11). The division of an extra sperm was observed in two cases, and in both the mitotic figure showed 12 chromosomes. After the first mitosis the achromatic display gradually diminishes, but is still quite conspicuous as late as the fourth or fifth division. After the first mitosis, however, the display consists almost entirely of slender threads like the spindle fibers (fig. 26).

FIRST FREE NUCLEAR PERIOD.—According to previous accounts of the embryogeny of cycads and *Ginkgo*, there is a regular series of free nuclear divisions, so that the nuclei at the successive divisions number 2, 4, 8, 16, 32, 64, 128, 256, 512, and in some cases 1024, with only such variations as might be expected from the occasional failure of a nucleus to divide. In *Dioon edule* (12) it was noted that the 8th, 9th, and 10th mitoses were irregular, especially in the upper part of the proembryo, so that the number of nuclei beyond the 256-nucleate stage was likely to vary widely from the theoretical estimate.

In *Stangeria*, irregularities begin to appear earlier and are more pronounced. The first 4 mitoses, giving rise to 2, 4, 8, and 16 nuclei, are likely to be strictly simultaneous and regular, and the 32-nucleate stage was observed in two cases; but after the 16-nucleate stage (fig. 12), the number of nuclei not only varies from the anticipated 32, 64, 128, etc., but the discrepancies are great. An examination of a large number of cases showed that the

free nuclei in the upper half of the proembryo were often smaller and more numerous than those in the lower half. The explanation was soon found, for the nuclei in the upper half may divide simultaneously, while those in the lower half remain in the resting condition (fig. 14). This would double the number of nuclei in the upper part, and naturally the nuclei would be smaller. A counting of nuclei in several complete series of sections indicated that such a division had taken place. When the lower nuclei divide, the upper ones divide also, even if they are already much more numerous (fig. 13). There is one important exception to this statement which will be considered later.

While such nuclear conditions are frequent, they are not universal, for two series showed approximately 512 nuclei of uniform size and uniformly distributed throughout the egg, thus indicating that there had been 9 simultaneous divisions. With the 9th or sometimes the 10th nuclear division the free nuclear period comes to a close.

At any time after the 7th division there may be an evanescent segmentation of the protoplasm, especially in the upper part of the proembryo (fig. 15). We have already described such a condition in *Dioon edule* (12), and while we have not yet published the details, we may state that in *Macrozamia*, *Encephalartos*, and *Cycas* the segmentation is much more complete than in *Stangeria* or *Dioon*, and is more permanent, since cellulose walls are formed.

As already stated, the haploid and diploid numbers of chromosomes are 12 and 24. The number 24 is fairly stable in the lower part of the proembryo; but in the upper part there are frequent and extreme variations, the numbers ranging from the theoretical 24 down to a single chromosome. If many of the figures should show just 12 chromosomes, the number could be explained by the continued division of an extra sperm, the first division of which has already been noted; but no such cases were observed. The cause of the irregularity was not determined.

POLARITY.—A striking feature of the free nuclear period is the frequency of a definite polarity. During the earlier divisions, the nuclei may be fairly evenly distributed, but still in two recognizable groups (fig. 12). Very often, however, the grouping is conspicuous, with a considerable space separating the upper and lower

groups (fig. 13). Sometimes the middle third of the proembryo is entirely free from nuclei; sometimes the nuclei are distributed throughout, but are smaller and more numerous above; sometimes the nuclei are dividing above but resting below, and later they divide below while the nuclei above are resting; there may be evanescent segmentation above, but no trace of it below; later, there is a migration of nuclei toward the base, followed by segmentation below, with none above; the embryo is formed below, while the upper region furnishes nutrition. In fact, there is a constant manifestation of polarity from the appearance of the archegonium initial throughout the life of the plant. The source of nutrition is probably the cause of a part of these phenomena, but much is still to be explained.

CAUSE OF FREE NUCLEAR PERIOD.—Why the cycad embryogeny should begin with a period of free nuclear division is not hard to imagine. It would seem a physical impossibility for the small mitotic figure of the first division to segment the comparatively immense mass of the egg. Further, the large amount of nutritive material doubtless causes the nuclear divisions to follow in rapid succession. That they do follow in rapid succession is shown by the fact that the 2-, 4-, and 8-nucleate stages are very rarely found; and also by the fact that nuclei which have reached the resting condition are not likely to be found before the 16-nucleate stage. Further, a glance at the illustrations accompanying this paper will show that the mitotic figures, nearly 100, are all in the equatorial plate stage, a fact which indicates that other phases are comparatively rapid.

The small size of the mitotic figure, the great mass of the egg, and the rapid succession of mitoses doubtless cause the free nuclear period which characterizes not only cycads, but nearly all living gymnosperms. As the divisions proceed and the mass of the nucleus approaches that of the surrounding cytoplasm, segmentation, which may be more or less evanescent, appears; and later permanent segmentation with the formation of cellulose walls takes place. In the Bennettitales and earlier cycadophytes the seeds were much smaller than in any of the living cycads yet studied. It would be interesting to know the embryogeny of these forms.

An examination of the tiny *Zamia pygmaea* might show a comparatively short free nuclear period, but I have not yet been able to secure it. *Ginkgo*, with its comparatively small eggs, has only 7 free nuclear divisions, the walls appearing with the 8th division. *Selaginella* and *Isoetes* have a short period of free nuclear division at the germination of the megaspore, but none of the living heterosporous pteridophytes has any free nuclear division in the embryogeny. These stages have not yet been described in any fossil heterosporous pteridophyte, but some of the paleozoic forms had megaspores much larger than those of *Isoetes* or *Selaginella*. Doubtless, with the increase in the size of the egg, a free nuclear period became established, becoming more and more extensive as the egg became larger, until it reached its maximum in some of the living cycads, which have eggs 5 or 6 mm. in length, and which may have more than 1000 free nuclei before wall formation begins. Among the coniferophytes, *Ginkgo* has the most extended free nuclear period yet known. While we believe that the *Ginkgo* condition represents a culmination, it is not so clear that genera with less and less extended free nuclear periods in the embryogeny represent a reduction series, ending in forms like *Sequoia*, in which a cell wall follows the first mitosis in the fertilized egg. This may seem rather speculative, but we believe enough has been observed to warrant our theory that the origin and development of the free nuclear period has been due to a progressive increase in the size of the egg.

MIGRATION OF NUCLEI.—As soon as the first free nuclear period has come to a close, many of the nuclei in the upper and middle portions of the proembryo migrate to the bottom (figs. 16, 17, 18, 27). That nuclei actually move to the basal region is shown by the structure of the cytoplasm and arrangement of the nuclei, and also by the relative numbers of nuclei in the upper and lower parts of the proembryo. The cytoplasm becomes vacuolated in the lower portion, before any movement of the nuclei begins (fig. 15); but during the movement and in succeeding stages the vacuolated condition extends throughout. As the nuclei move to the base of the proembryo, the cytoplasm at that point becomes denser and denser, until it contrasts rather sharply with the vacuolated structure above (fig. 18).

SECOND FREE NUCLEAR PERIOD.—After the migration of nuclei, there is a second period of simultaneous free nuclear division at the base of the proembryo, but the nuclei above do not divide (fig. 19). The mitotic figures of this division were observed in only two cases, but in two others there was evidence that such a division had recently occurred. Since so few cases were observed, the extent of this free nuclear period could not be determined, but early stages in the cellular period would indicate that there are probably only two or three simultaneous mitoses before wall formation begins. The cause of this second period is probably the dense basal accumulation of cytoplasm during the migration of nuclei. The embryo, the suspensor, and some cells which remain within the limits of the egg are all formed from cells resulting from the second free nuclear period, the nuclei and cytoplasm above being resorbed by the growing embryo.

IKENO (3), in his classic account of *Cycas revoluta*, described free nuclear mitoses at the base of the proembryo, with amitotic divisions above, but did not give any further description of the embryogeny. His investigation dealt chiefly with the pollen tube structures and oogenesis. TREUB (1), who had previously made a study of *Cycas circinalis*, did not mention such a stage.

FORMATION OF WALLS.—At the close of the second free nuclear period, with the last simultaneous mitosis, there is a simultaneous formation of cell walls (fig. 20). At the middle of the cellular region there are about three layers of cells with cellulose walls, and at the edges only one layer. Above these cells with cellulose walls there is a segmentation of the cytoplasm, but the walls do not reach the cellulose stage and are weak and evanescent. Within the cellular region, mitoses are no longer simultaneous, and each nuclear division is followed by the formation of a wall. The region soon becomes sharply marked off from the cytoplasm above (fig. 21). Later, the peripheral walls of the cells bordering upon the cytoplasm become thickened, so that the cellular region is still more sharply defined (fig. 23). The thickening is mucilaginous. The free nuclei in the cytoplasm may undergo occasional divisions, giving rise to numerous small nuclei (fig. 21), while the cytoplasm bordering upon the cellular region becomes denser, losing more or

less completely its vacuolated appearance as it is resorbed by the rapidly developing embryo. Before the appearance of cotyledons, the young embryo has resorbed all the cytoplasm and nuclei of the free nuclear region, which becomes an empty shell, maintaining its contour only by the rigidity of the tough egg membrane. All the structures of this region, however, are finally crushed by the backward thrust of the suspensor, which is stopped only by the stony coat of the seed.

DIFFERENTIATION OF CELLULAR REGION.—Differentiation of the cellular region begins almost as soon as it is marked off from the free nuclear region above it. At first there is seen a differentiation into two general regions: the upper, in contact with the free nuclear region, consisting of larger cells; while the cells of the lower are much smaller, but denser and more numerous (fig. 21). Some of the upper cells, especially in the center, increase greatly in size and become actively haustorial; it is through these that the contents of the free nuclear region pass down to the developing embryo (fig. 23).

The smaller, denser cells then become differentiated into two regions, the lower consisting of a single layer of large cells which looks like a dermatogen, and above it several layers of small, dense cells (fig. 22). Most of the outer cells become haustorial, especially at the center. These cells partly digest and partly crush the egg membrane, and the young embryo begins to advance into the endosperm (fig. 23). Only a small portion of the egg membrane is ruptured, and the advancing embryo is extremely narrow in comparison with those of cycads previously described. The region of small, dense cells contributes most of the suspensor and all of the periblem and plerome of the embryo.

Stages in the development of the body regions of the embryo are still lacking in my material, but will doubtless be found in collections now being made in South Africa. From the mature embryo up to seedlings with several leaves the series is fairly complete, and some account will be given at another time in connection with a study of seedlings of other genera.

This paper, like several earlier papers on cycads, is largely descriptive. Since material is in hand for a fairly complete study

of all the genera, it seems wiser to reserve theoretical considerations until the available evidence has been examined.

Summary

1. *Stangeria* is probably monotypic, with *S. paradoxa* as its single polymorphic species.

2. At fertilization there is a pairing of chromosomes resembling the pairing in the heterotypic mitosis, so that the number during the metaphase of the first division is apparently haploid, although really diploid.

3. There are two free nuclear periods in the embryogeny, the first comprising 9 or 10 simultaneous mitoses and extending throughout the proembryo, and the second with only 2 or 3 mitoses and confined to the lower part of the proembryo. The embryo and suspensor are formed from the second series.

4. There is an evanescent segmentation of the entire egg, as in *Dioon*.

5. The young embryo is very narrow and its haustorial structures are more conspicuous than in any other cycad yet described.

6. Polarity, which may appear even at the beginning of embryogeny, becomes more and more marked as development proceeds.

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EXPLANATION OF PLATES XXIV-XXVI

Most of the sections were cut at 5 μ ; none was thicker than 10 μ . With the exception of figs. 4-8, the drawings were reconstructed from two or more sections. Figs. 12 and 13 are reconstructed to show all the nuclei of these proembryos.

FIG. 1.—Text cut, showing *Stangeria* in the field.

FIG. 2.—Nucellus with pollen tubes showing prothallial cell, stalk cell, body cell with blepharoplasts, and tube nucleus; in one tube (at the right) the body cell has divided; $\times 50$.

FIG. 3.—Later stage: mature sperms are about to be shed; $\times 50$.

FIG. 4.—Blepharoplast, much vacuolated, shortly before breaking up into granules; the body cell has not yet divided; $\times 365$.

FIG. 5.—The blepharoplast has broken up into an immense number of small granules; the nucleus of the young sperm is smaller than the blepharoplast of the preceding figure; the median portion of the spindle can still be seen; $\times 365$.

FIG. 6.—The granules have increased in size and number and are becoming arranged into a flat band; note the great increase in the size of the nucleus; $\times 365$.

FIG. 7.—Transverse section of a band at the stage shown in fig. 6; $\times 365$.

FIG. 8.—The bristle-like cilia beginning to develop from the band; some are directed toward the nucleus; $\times 365$.

FIG. 9.—Fertilization: the sperm nucleus entering the egg nucleus; the ciliated band remains at the top of the egg; $\times 42$.

FIG. 10.—First mitosis in the fertilized egg: at the top, 3 sperms which have passed through the neck, but have not entered the egg; the ciliated band of the sperm which effected fertilization is easily seen; $\times 42$.

FIG. 11.—First division of fertilized egg: a second sperm has entered and is dividing in the upper part of the egg; two more sperms which have almost entered the egg are seen at the top; $\times 42$.

FIG. 12.—The fourth simultaneous free nuclear division; $\times 42$.

FIG. 13.—At the bottom, 8 approximately equal nuclei are dividing; at the top, the division is also simultaneous, but the number of nuclei is large and they are irregular in size; there is a very marked polarity; $\times 42$.

FIG. 14.—The nuclei of the lower half are large and are in the resting condition, while those of the upper half are smaller and are dividing simultaneously; the polarity is evident; $\times 42$.

FIG. 15.—Evanescent segmentation: the nuclei of the lower part are larger and the cytoplasm is becoming vacuolated; $\times 42$.

FIG. 16.—Many nuclei have migrated to the bottom and the cytoplasm of the entire proembryo is vacuolated; the ciliated band is still evident at the top; $\times 42$.

FIG. 17.—Slightly later stage: many nuclei have amoeboid shapes; $\times 42$.

FIG. 18.—Nuclei with dense cytoplasm at the base of the proembryo, just before the second free nuclear period; $\times 140$.

FIG. 19.—Free nuclear division of the second free nuclear period; $\times 140$.

FIG. 20.—Segmentation at the close of the second free nuclear period; $\times 140$.

FIG. 21.—The cellular region is differentiated from the free nuclear region and there is some differentiation within the cellular region itself; $\times 100$.

FIG. 22.—An outer layer of dermatogen-like cells has been differentiated; the dense cells just above are to produce the periblem, the plerome, and most of the suspensor; $\times 100$.

FIG. 23.—The upper cells of the cellular region are distinctly haustorial, and the slender tip, which has just broken through the egg membrane, is composed largely of haustorial cells; $\times 100$.

FIG. 24.—Photomicrograph: upper part of fertilized egg, showing a part of the ciliated band and two sperms which have almost entered the egg; $\times 80$.

FIG. 25.—Photomicrograph: first mitosis in fertilized egg, showing the mototic figure and the conspicuous kinoplasmic area about it; $\times 86$.

FIG. 26.—Photomicrograph: one of the mototic figures of the fourth simultaneous free nuclear division; $\times 517$.

FIG. 27.—Proembryo, showing the migration of nuclei toward the bottom; $\times 33$.



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