Spore Formation in *Botrychium virginianum*.

BY

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With Plates XXVIII, XXIX, and XXX.

IN *Botrychium virginianum* the development of the sporangia begins a year previous to their ripening. Plants which are dug up about the time of the shedding of the spores show next year's leaf with sporangia just forming, enclosed by the stipule-like sheath at the base of the leaf of the present year. The youngest sporangia are then in the form of slight elevations. At the apex of each of these is a pyramidal cell which divides by a periclinal wall, and the inner of the two cells thus formed is the archesporium which, by repeated divisions, gives rise to the spore-mother-cells (Campbell, '95).

As soon as the leaves come above the ground in the spring the spore-mother-cells are found in the condition represented in Fig. 1, evidently in the early prophase of their first division. The spore-mother-cells are then seen to be demarked from each other and from the tapetum by a delicate plasmatic membrane. They are not, as a rule, united into a single mass, but are separated into groups of four to thirty-five. It is possible, however, that the separation into groups in this early stage is done in the preparation of the sections, but, at any rate, we may be sure that the tendency to separate exists, since in later stages we find like groups immersed in the plasmodium of the tapetal cells that has flowed in between them. At this stage the nuclear thread is rather loosely-coiled (Pl. XXVIII, Fig. 1), its windings, where applied to the nuclear wall, showing some degree of symmetry (Fig. 2). With the safranin-gentian violet-orange method the nuclear thread is at this stage stained a uniform purple or violet, showing no differentiation into chromatin and linin disks. Usually but one nucleolus is present, but as many as two or three are sometimes found.

The tapetum in this early prophase consists of two layers of cells of varying form and size, each cell being delimited by a plasmatic membrane merely, which, in some instances, is incomplete, so that there is a fusion of the cytoplasm of adjacent cells. This fusion is progressive

and results in a common tapetal plasmodium by the close of the first prophase. The cells of the tapetum are easily distinguished from the spore-mother-cells by their coarser-grained cytoplasm, their irregular size and form, and their smaller nuclei with close-meshed reticulum and numerous nucleoli.

Following now the behaviour of the spore-mother-cells: as the prophase advances the nuclear thread becomes thicker, evidently by shortening through longitudinal contraction, and clearly not by the approximation and apparent fusion of parallel parts of the thread as described by Berghs ('04) for the microspore-formation of *Allium fistulosum*. I have examined my preparations carefully to see if this fusion occurs, and while I can find numerous instances of the close approximation of the parts of the nuclear thread, I do not find these parts fusing, but do find them thickening without fusion until the stage represented in Fig. 3 appears. While the nuclear thread is thickening it is becoming more and more erythrophil, and retains the safranin in preference to the gentian-violet (when the triple stain safranin-gentian violet-orange is used) some time before its separation into chromosomes. Although my preparations show a close succession of stages in the prophase I have not been able to find the gamosomes recently described by Strasburger ('04) for *Thalictrum purpurascens*. It may be that in *Botrychium* their isolation from the linin framework takes place earlier during the long winter rest of the spore-mother-cells.

When the nuclear thread first segments into chromosomes the latter appear from various points of view, as seen in Fig. 4, where *a*, *b*, *c*, and *d* show clefts that have evidently arisen from longitudinal fission; *e*, *f*, *g*, *h*, and *i* show chromosomes bent or doubled in various degrees; and *j* shows an end view of such a figure as *d* or *h*, one arm being longitudinally split and the other not. On comparison with the work of Farmer and Moore ('03), Strasburger ('04), Gregory ('04), and preparations illustrating spermatogenesis in the Acrididae which Dr. McClung has kindly shown me, where the different prophase stages can be seen with remarkable clearness, I am led to the conclusion that these bent chromosomes are or become divided by transverse fission at the apex of the bend. The chromosomes of Fig. 21, culled from stages represented by Figs. 16 and 17, indicate that this fission has taken place, and they are very similar to Strasburger's Fig. 5, where with a better subject he is confident of a transverse fission. My Fig. 18 indicates that in the anaphase the separation of the chromosomes is to take place in the plane of transverse fission. The longitudinal division begun in the early prophase, and clearly seen in Fig. 4, is lost sight of and only slightly indicated in the cross-shaped chromosomes of Fig. 18. In the anaphase (Figs. 22 and 24) all trace of the longitudinal division is lost. It will be seen that *Botrychium* is not a good subject to bring in evidence regarding the vexed question of the manner of chromosome-division. So far

as its evidence is clear, however, it agrees with the recent conclusions of Farmer and Moore, Strasburger, and Gregory.

Soon after the nuclear thread segments into chromosomes the nuclear cavity is seen to be traversed by numerous fine threads which at first take on a grey and later a violet colour by the three-colour-method; the nuclear membrane also becomes fibrillar and is stained violet. These characteristics become progressively more pronounced and the fibrils begin to press out at various points into the cytoplasm in the form of a multipolar spindle (Figs. 7, 8, and 9). In this way the nuclear membrane loses its identity in the spindle (Figs. 10 to 14 inclusive). In the meantime the cytoplasm has become progressively less dense and has evidently contributed of its substance to the growth of the spindle. Later the several cones of the spindle collect at two opposite poles and fuse, forming the usual bipolar spindle (Figs. 15 to 17 inclusive), a polar view of which near the equator shows that the fibrils are about equally distributed throughout the space occupied by the spindle (Fig. 20). No indications of centrosomes can be seen.

Investigations into the formation of the kinoplasmic spindle have revealed two prevailing types. In one type the beginnings of the spindle appear first in the cytoplasm, and in the other first in the nucleus before the breaking down of the nuclear membrane, or in the nuclear region simultaneously with or just after the disappearance of the membrane. To the first type belong *Equisetum* (Osterhout, '97), *Lilium* (Mottier, '97), *Gladiolus* (Lawson, '00), *Osmunda* (Smith, '00), *Agave* (Osterhout, '02), *Iris*, *Disporum*, *Aloe*, *Hesperaloe*, *Hedera* (Lawson, '03). To the second type belong *Cobaea* and *Passiflora* (Williams, '99), *Lavatera* (Byxbee, '00), and *Nymphaea alba* (Strasburger, '00). With this second type *Botrychium virginianum* must now be classed.

There does not, however, appear to be any important significance in the existence of these two types. Strasburger ('00, p. 121) suggests that they may be due to differences in the amount of extranuclear kinoplasm available, and he calls attention to the fact that, as a rule, extranuclear kinoplasm takes an important part in the formation of the first spindle of pollen-mother-cells, and a subordinate part in the formation of the second spindle. It may be also true, it seems to me, that the force which causes the differentiation of the spindle emanates from the nucleus, and that in some cases this force penetrates the cytoplasm with sufficient energy to make itself manifest, and in others it does not. It might well be that the second division, following as a rule close upon the heels of the first, and with the reduced amount of chromosome material, does not have at its disposal sufficient nuclear energy to dominate the cytoplasm, with the result that the second spindle is chiefly intranuclear in its origin.

Evidently cytopolarity (meaning by this term the polarity of the cell

mapped out by the kinoplasmic spindle) is not a constant state, but is engendered at the time of nuclear division. The gradual evolution of the spindle-fibres, in many cases without definite orientation at first, may be interpreted in this way. The force in play during cytopolarity seems to emanate from the nucleus, and particularly from the chromosomes, whether united into the nuclear thread or distinct during the later prophases and thereafter. The evidence for this is found in the facts that the axis of the poles always passes through the nucleus, and the spindle-fibres arise first in the nucleus or in the cytoplasm in definite relation to the nucleus; and in pollen- and spore-mother-cells after the second maturation-division kinoplasmic connecting fibres spring up between and connecting the pairs of granddaughter nuclei preparatory to the formation of the cell-plates (see my Figs. 37 to 40 inclusive, and, without seeking further, Mottier's Fig. 50 in *Cytologische Studien*). That the chromosomes have the dominant part in this appears from the fact that the nucleus as an entirety has in so many instances disappeared, leaving only the chromosomes to represent it, before polarity has become completely established (see my Figs. 9 to 15 inclusive, and Osterhout's and Mottier's Figures in *Cytologische Studien*). This conjecture receives further support from those cases where the second division takes place without the formation of a resting daughter-nucleus.

In *Botrychium*, as the chromosomes of the first division approach the poles the spindle-fibres form a dense cone, as shown in Fig. 24. Apparently the chromosomes are being drawn toward the pole by a contraction of the fibres. I see nothing in my preparations to indicate that the anaphase translocation of the chromosomes is brought about in any other way. (For a summation of the evidence on which he grounds his opinion of chromosome translocation by a contraction of the spindle-fibres see Strasburger, '00, pp. 140-2; and for a *résumé* of the different theories regarding this subject see Häcker's *Praxis und Theorie der Zellen- und Befruchtungslehre*, pp. 73-8).

Arrived at the poles the chromosomes fuse together, and the daughter-nuclei enter upon a resting-stage in which the chromatin becomes again cyanophil, forming a fine-meshed reticulum, and one or more nucleoli appear (Figs. 25-8).

While the daughter-nuclei are entering into the resting-stage the connecting fibres are spreading out along the equatorial plane (Fig. 26), and in the equatorial zone, deposits of granules are accumulating which are stained from grey to brownish by the three-colour method (Figs. 26-8). The connecting fibres disappear soon after they have traversed the equatorial plane, and the resting-stage then appears as in Fig. 28. It is seen that no cell-plate of the usual kind is formed, but in its place is a broad and dense zone of cytoplasm.

In the fact that the daughter-nuclei resulting from the first division pass through a resting-stage, *Botrychium* differs from *Pteris* as reported by Calkins ('97), and *Scolopendrium* as described by myself ('98). According to Calkins, in *Pteris*, when the chromosomes of the first division have arrived at the poles they again divide and pass through the stages of the second division without having fused with each other. In *Scolopendrium* I found that the chromosomes of the first division fuse together after they have arrived at the poles, and form a thick thread, but that no other steps towards the formation of a resting-nucleus are taken.

The processes of the second division do not seem to differ essentially from those of the first so far as concerns the formation of the spindle. The thread of the reticulum thickens and the reticulum loses its netted character (Fig. 29); the chromatin gathers together in clumps (Fig. 30); the nucleoli disappear, and the nascent spindle becomes visible within the nuclear cavity, and finally presses outwards in the form of a multipolar spindle (Figs. 31-2). These prophases evidently come rapidly to completion, for they are not frequently found, even in preparations in which all other phases abound. The manner of the separation of the chromosomes in the second division I have found it impossible to determine (see Figs. 30 to 36 inclusive).

In the second division the daughter-nuclei may divide in the same plane or in planes at right angles to each other (Figs. 33 to 36 inclusive). In either case, soon after the chromosomes have arrived at the poles, and before the resting granddaughter-nuclei have been formed, the thick plate of cytoplasm which was laid down in the equator of the first division becomes gradually transformed into kinoplasmic connecting fibres (Figs. 37 to 41 inclusive). If the planes of division are at right angles to each other the fibres connect the granddaughter-nuclei as shown in Figs. 37 to 40; but if both nuclei divide in the same plane the granddaughter-nuclei become joined by the connecting fibres as shown in Fig. 41. Fig. 37 represents a slightly later stage than Fig. 36. At *a* in Fig. 37 is the bundle of connecting fibres remaining at the close of the anaphase of the second division, while at *b* and *b'* are nascent connecting fibres arising out of the dense cytoplasm laid down in the equatorial region at the close of the first division, as in Fig. 28. These may be called secondary connecting fibres to distinguish them from the primary connecting fibres at *a*. Both primary and secondary connecting fibres take part in the formation of the cell-plates that are to demark the spores, as is clearly shown in Fig. 41, where both of the last nuclear divisions took place in the same plane. Figs. 40 to 44 inclusive show a progression of events in the formation of the cell-plates, with attendant changes in the condition of the cytoplasm. Reviewing the stages thus far described we find a striking confirmation of the opinion of Strasburger ('97) that the trophoplasm and kinoplasm are mutually interdependent, one increasing at the expense of the other as the require-

ments of the cell demand. The evidence which *Botrychium* affords for this may be summarized as follows:—In the prophases of the first division the density of the trophoplasm diminishes as the kinoplasmic spindle increases in size (Figs. 9 to 18). There is a gradual transition, both in structure and reaction to stains, from the kinoplasmic connecting fibres to the dense granular trophoplasm occupying the equatorial zone in the first telophase (Figs. 25 to 28). The trophoplasm as a whole becomes regenerated after the daughter-nuclei have entered into the resting-stage, and all kinoplasmic differentiations then disappear (Fig. 28). Later, the dense equatorial zone of trophoplasm gradually disappears as the kinoplasmic secondary connecting fibres are generated between the pairs of granddaughter-nuclei (Figs. 37 to 41). And, finally, after the cell-plates demarking the granddaughter-cells or spores have been laid down, we find a gradual transition from the filar kinoplasm to the alveolar trophoplasm (compare Figs. 41 to 44 inclusive).

The plasmodium formed by the fusion of the tapetal cells continues to flow in between the groups of mother-cells while the division phases are progressing, until only a very thin layer of the plasmodium remains at the exterior. The plasmodium appears to be quite fluid, for very minute rifts between the mother-cells, sometimes not more than one micron in diameter, become filled by it. The nuclei of the plasmodium are unable to pass into the smaller crevices and so accumulate in large numbers where the spaces are larger, as shown in Fig. 47.

After the disappearance of the connecting fibres of the second division the cell-plates thicken, nucleoli appear in the nuclei and the mother-cells become more or less dissociated from their previous grouping (Fig. 44). The middle lamella of the cell-plates separating the granddaughter-cells now becomes soluble and the granddaughter-cells or young spores become detached from each other while still enclosed within the membrane of the mother-cell (Fig. 45). Later, the granddaughter-cells become separated from the membrane of the mother-cell also and lie free within it (Fig. 46). The membrane of the mother-cells soon disappears and the granddaughter-cells then lie embedded in the tapetal plasmodium. The young spores now enlarge and build a nodulated wall about themselves which is differentiated into a relatively thick exosporium stained red by the safranin, and a thin endosporium stained violet by the gentian-violet of the three-colour-method (Fig. 48). As the spores advance in their development the tapetal plasmodium becomes strikingly depleted. Instead of the dense cytoplasm of the earlier plasmodium shown in Fig. 47 we find the vacuolate and attenuate structure of Fig. 48. The nuclei of the plasmodium possess at the same time a reticulum which also is depleted, the threads being thinner and taking the stain with less intensity. Their nucleoli appear, however, as in the earlier stages.

The massing of the plasmodial nuclei in the lakes of cytoplasm which occupy the larger clefts between the groups of mother-cells may have an important significance in the nutrition of the developing spores. Loeb ('99) came to the conclusion that enucleated cells lose their capacity for regeneration because the power of oxidation departs with the nucleus, and the synthesis which must depend upon the preliminary exercise of this power can no longer take place. From Lillie's observations ('02) it seems clear that 'the oxidative activities of the organs must be largely a function of their extent of nuclear surface,' and 'the same conclusion applies also to synthetic processes in so far as they depend on oxidations.' In the light of these conclusions the massing of the nuclei of the plasmodium may not be merely an incident following the inflow of the cytoplasm. It may well be that there, where they occur associated in large numbers (see Fig. 47), they exert a powerful influence in the nutrition of the developing spores at the cost of the substance of the plasmodium itself.

METHODS.

Small pieces of the sporophyll bearing only a few sporangia were fixed in the field as soon as they were cut off in the following manner:—The pieces of sporophyll were placed in a phial containing a 0.5% solution of chromic acid, from which the air was then removed by a portable air pump. When the atmospheric pressure was again turned on the infiltration of the pieces by the fixative was shown by their immediately sinking in it. The material was then transferred at once to Flemming's fixative. Both the stronger and weaker solutions were employed, and both seemed to give equally good results. The material was embedded in paraffin, and thin sections, which were cut 5 microns thick, were stained by Flemming's three-colour method.

NOTE.

After this paper was sent to the publishers a contribution by Ira D. Cardiff on 'The Development of the Sporangium in *Botrychium*' appeared in the Botanical Gazette, xxxix, 5, p. 340. As the title indicates, Cardiff has made a study of the development of the sporangium rather than of the spores, and our papers cross the same ground chiefly as regards the tapetum. Cardiff worked with two species of *Botrychium* (*B. virginianum* and *B. ternatum*), and he states that the two are found to be essentially the same in their sporangial development. The details of the paper and drawings do not refer to either species in particular, and I assume that they are intended to apply equally well to either. On this assumption it is interesting to note the differences that occur in members of the same species growing in different localities (Cardiff's *B. virginianum* was taken in the vicinity of Woodville, Ind., and mine near Lawrence, Kans.). He finds that the tapetal

layer becomes as much as four or five cells in thickness, while I find two cells in thickness the rule. His material shows an excessive growth of the tapetum about the time of the first prophase and after, while mine does not. I have measured the volume of the tapetum before it begins to flow in between the groups of spore-mother-cells, and find it more than two-thirds the volume of the sporogenous tissue; and since the sporangia do not essentially increase in size while the inflow is taking place, this amount is evidently sufficient to fill the gaps between the groups and still leave the thin layer that remains at the exterior. He finds frequent amitotic nuclear division in the tapetum, while my material does not show this. Our subjects agree in showing that none of the spore-mother-cells is restrained from forming spores.

LITERATURE CITED.

- BERGS, JULES ('04): Formation des chromosomes hétérotypiques dans la sporogénèse végétale. *La Cellule*, tome XXI, 2^e fascicule.
- BYXBEE, E. S. ('00): The Development of the Karyokinetic Spindle in *Lavatera*. *Proc. Cal. Acad. Sci.*, III. Bot., ii, p. 63.
- CALKENS, G. N. ('97): Chromatin-reduction and Tetrad-formation in Pteridophytes. *Bull. Torr. Bot. Club*, xxiv, No. 3.
- CAMPBELL, D. H. ('95): Mosses and Ferns, p. 242.
- FARMER, J. B., and MOORE, J. E. S. ('03): New Investigations into the Reduction of Animals and Plants. *Proc. Royal Soc.*, lxxii, No. 478.
- GREGORY, R. P. ('04): Spore-Formation in Leptosporangiate Ferns. *Ann. of Bot.*, xviii, No. 71.
- HAECKER, V. ('99): Praxis und Theorie der Zellen- und Befruchtungslehre.
- LAWSON, A. A. ('00): Origin of the Cones of the Multipolar Spindle in *Gladiolus*. *Bot. Gaz.*, xxx, p. 145.
- ('03): Studies in Spindle-Formation. *Bot. Gaz.*, xxxvii, p. 81.
- LILLIE, R. S. ('02): On the Oxidative Properties of the Cell-Nucleus. *Am. Journ. Physiol.*, vii, p. 412.
- LOEB, J. ('97): Warum ist die Regeneration kernloser Protoplasmastücke unmöglich oder erschwert? *Arch. f. Entwicklungsmechanik*, viii, p. 689.
- MOTTIER, D. M. ('97): Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. *Jahrb. f. wiss. Bot.*, Bd. xxx, p. 169.
- OSTERHOUT, W. J. V. ('97): Ueber Entstehung der karyokineticischen Spindel bei *Equisetum*. *Jahrb. f. wiss. Bot.*, Bd. xxx, p. 125.
- ('02): Cell Studies: 1. Spindle-Formation in *Agave*. *Proc. Cal. Acad.*, III. Bot., ii, p. 255.
- SMITH, R. W. ('00): The Acromatic Spindle in the Spore-Mother-Cells of *Osmunda regalis*. *Bot. Gaz.*, xxx, p. 361.
- STEVENS, W. C. ('98): Ueber Chromosomentheilung bei der Sporenbildung der Farne. *Ber. d. d. bot. Gesell.*, Bd. XVI, Heft 8.
- STRASBURGER, E. ('97): Ueber Cytoplasmastructuren, Kern- und Zelltheilung. *Jahrb. f. wiss. Bot.*, Bd. xxx, p. 375.
- ('00): Ueber Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. *Hist. Beiträge*, VI.
- ('04): Ueber Reduktionstheilung. *Sitzber. der k. Preuss. Ak. der Wiss.*, xviii.
- WILLIAMS, C. L. ('99): The Origin of the Karyokinetic Spindle in *Passiflora coerulea*. *Proc. Cal. Acad. Sci.*, III. Bot., i, p. 189.

EXPLANATION OF PLATES XXVIII-XXX.

Illustrating Professor W. C. Stevens's paper on *Botrychium*.

PLATE XXVIII.

- Fig. 1. Spore-mother-cell in early prophase. $\times 1800$.
Fig. 2. The same, showing spireme applied to nuclear wall. $\times 1800$.
Fig. 3. Later prophase-stage showing greatly thickened nuclear thread. $\times 1800$.
Fig. 4. Chromosomes from various points of view immediately after the segmentation of the nuclear thread. $\times 1800$.
Figs. 5, 6. Later prophases than Fig. 4, showing chromosomes contracted and thickened and lines of cleavage no longer recognizable. $\times 1800$.
Figs. 7, 8. Prophases showing the beginning of the formation of the spindle within the nuclear cavity. $\times 1800$.
Figs. 9-12. Various stages in the development of the multipolar spindle. $\times 1800$.
Figs. 13-15. Stages in the fusion of the multipolar spindles to form bipolar spindles. $\times 1800$.
Figs. 16, 17. Late prophases with bipolar spindles. $\times 1800$.
Fig. 18. First metaphase. $\times 1800$.
Fig. 19. Polar view of nuclear plate. $\times 1800$.
Fig. 20. Polar view above nuclear plate showing trans-section of the spindle-fibres. $\times 1800$.
Fig. 21. Chromosomes culled from stages represented in Figs. 16 and 17. These indicate that transverse fission has taken place following the stage shown in Fig. 4. $\times 1800$.
Fig. 22. Anaphase of the first division. $\times 1800$.
Fig. 23. Late anaphase of the first division. $\times 1800$.
Fig. 24. Polar view of anaphase of the first division. $\times 1800$.

PLATE XXIX.

- Fig. 25. Two spore-mother-cells in telophase of the first division. $\times 1800$.
Fig. 26. Later stage showing daughter-nuclei demarked by nuclear membrane, and beginning of dense equatorial zone of cytoplasm. $\times 1800$.
Fig. 27. A stage still later than Fig. 26. The nucleoli have appeared in the daughter-nuclei, and the connecting fibres have nearly disappeared. $\times 1800$.
Fig. 28. A group of spore-mother-cells after the close of the first division. The daughter-nuclei are in the resting-stage. The cytoplasm has become entirely regenerated and the dense equatorial region is very apparent. $\times 1800$.
Fig. 29. Early prophase of the second division. $\times 1800$.
Figs. 30, 31. Later prophase stages of the second division. In Fig. 31 the spindle-fibres are becoming evolved within the nuclear cavity. $\times 1800$.
Fig. 32. A succeeding stage showing a multipolar spindle. $\times 1800$.
Figs. 33, 34. Metaphases of the second division. In Fig. 33 the nuclei are dividing in the same plane, and in Fig. 34 in planes at right angles to each other. $\times 1800$.
Figs. 35, 36. Anaphases of the second division.
Figs. 37, 38. Telophases of the second division, showing the origin of the secondary connecting fibres from the dense equatorial cytoplasm of Fig. 28. $\times 1800$.
Fig. 39. Completion of formation of granddaughter-nuclei. $\times 1800$.
Fig. 40. Beginning of formation of cell-plate. $\times 1800$.
Figs. 41-43. Completion of cell-plates of second division, and gradual transition from the fibrillar condition of the connecting fibres to the alveolar condition of the general cytoplasm. $\times 1800$.

PLATE XXX.

Fig. 44. Group of mother-cells after the completion of the second division, showing the grand-daughter-cells or young spores still connected with each other and closely invested by the membrane of the mother-cell. $\times 1800$.

Fig. 45. Granddaughter-cells separating from each other, but still adhering to the membrane of the mother-cell. $\times 1800$.

Fig. 46. Granddaughter-cells lying free within the membrane of the mother-cell. $\times 1800$.

Fig. 47. Portion of tapetal plasmodium that has flowed in between the groups of spore-mother-cells, as it appears at the close of the last division. $\times 900$.

Fig. 48. Mature spore embedded in the plasmodium which is now much depleted. One nucleus of the plasmodium also shown. $\times 900$.

Fig. 49. Nucleus of the plasmodium on a larger scale embedded in the depleted cytoplasm of the plasmodium. $\times 1800$.

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