On the Development of the Sporangia and Spores of *Aneimia phyllitidis*.

BY

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With Plates LXXXIV and LXXXV.

IN a contribution to the Botanical Gazette, Binford ('07) has advanced our knowledge of the Schizaeaceae by a careful study of the sporangia of *Lygodium*. He confirms the work of Prantl ('81) in all essential respects, and carries his research on into the development of the tapetum and sporogenous tissue.

Material which I put through Flemming's fixative some years ago in Strasburger's laboratory affords me opportunity to give an account of *Aneimia phyllitidis* also in more detail than Prantl has related it. Prantl begins with the earliest stage of development of the sporangium, and shows that it arises from a single protodermal cell not far from the growing apex of the sorophore. A series of sporangia is thus formed in acropetalar order along the margins of the sorophore; and while this is going on the sorophore grows faster on its upper than on its lower surface, and so shifts the sporangia from their marginal to a dorsal position. This unequal growth at the two surfaces is shown in Pl. LXXXIV, Fig. 6. As the sorophore elongates, new sporangia continue to be started; but not all of these come to maturity, evidently because, in part at least, those first formed divert nutriment to themselves to such an extent that those coming later are starved.

Prantl reports that the protodermal cell that is to produce a sporangium grows out beyond the general level before dividing. My sections do not show this. Rather I would conclude from them that a sporangium arises from several protodermal cells; but I do not have all stages complete enough for a critical discussion of this point. At the early stages shown in Fig. 1, *a* and *c*, we find a few initials of the sporangium wall, and a central cell or initial of the archesporium and tapetum. Anticlinal divisions in the wall initials multiply them many times (Figs. 2, 4, &c.). At the same time the central cell by periclinal divisions cuts off an outer layer

of cells or initial layer of the tapetum, and these surround a single cell, which is proved to be the archesporium.

The tapetal initials now multiply by anticlinal divisions, and finally each undergoes a periclinal division to form a two-layered tapetum (Pl. LXXXIV, Fig. 1, *d*, and Figs. 2, 4, and Pl. LXXXV, Fig. 8). This agrees essentially with the formation of the wall, tapetum, and archesporium of *Lygodium* as described by Binford, excepting that he finds a three-layered tapetum in places. Turning to the Ophioglossaceae for comparison, Bower ('96) has found that in *Helminthostachys* the tapetum is formed by division of the wall-cells alone, and Campbell ('05) reports that in *Botrychium* the tapetum comes partly from the wall-cells and partly from the sporogenous cells.

While the tapetum is thus increasing, the archesporium is undergoing divisions that result in a sporogenous tissue, or group of grandmother-cells of the spores (compare Pl. LXXXIV, Fig. 4, and Pl. LXXXV, Fig. 8). The sporangium is growing rapidly during these stages, the number of tapetal cells increasing step by step with the growth of the wall.

About the time the number of grandmother-cells of the spores, or gonotokonts, to use the term proposed by Lotsy ('04), is complete (approximately 65) the outer tapetal cell layer begins to break down, and this is soon followed by a similar behaviour of the inner layer, leaving the solid mass of gonotokonts suspended in a tapetal cytoplasm (Pl. LXXXIV, Figs. 4, 5, and 6). Prantl found a different behaviour in the sporangia of *Aneimia phyllitidis* studied by him, in that the inner layer of the tapetum formed a plasmodium while the outer layer remained intact (see his Fig. 120); and Binford finds the outer layer persisting in *Lygodium* (his Fig. 30). In some instances I find the outer tapetal layer breaking down even before the archesporial divisions are far advanced (Fig. 3).

There seems to be a consensus of opinion that in all such cases the tapetal cells serve as nurse cells to the gonotokonts through their meiotic divisions, and to the spores through their developmental processes; and there are some considerations which suggest that this function can be carried on better when the entire tapetum is in the condition of a plasmodium, since the nuclei are then free to distribute themselves amongst the gonotokonts after these have separated into relatively small groups or into single free individuals, and also since the tapetal cytoplasm in the form of a plasmodium is free to circulate, and so facilitate the distribution of nutritive materials that in part are held in solution in the cell sap, and in part are suspended in the cytoplasm, or even enter into its composition. The conclusion of de Vries ('85) that, since diffusion has been found inadequately slow, the transportation of materials must be assisted by the rotation and circulation of the protoplasm, is given recent support in the researches of W. Bierberg ('08), who found cytoplasm rotation to more than treble the rate of transportation above that achieved by diffusion alone.

Pfeffer ('97), however, contrary to de Vries, sets forth that within the cells a sufficiently rapid distribution of materials could take place with the assistance of mechanical bendings, and variation in cell-turgor, tissue tensions and temperature. While all these things must be operative, in the light of Bierberg's results there seems great probability that the plasmodium habit of the tapetal cells may be for the distribution of food by cytoplasmic movements, as well as for setting free the nuclei that they may assemble immediately about the gonotokonts and their descendants (Figs. 5, 6, and 7).

Fig. 7 shows that such movement has actually occurred, for here the cytoplasm has to a large extent forsaken the periphery of the sporangium and accumulated about the gonotokonts. On comparing Pl. LXXXIV, Figs. 6 and 7, and Pl. LXXXV, Figs. 11 and 13, we have evidence that the cytoplasm migrations begin when the tapetum first breaks down and continue through the subsequent stages of sporangial and spore development up to the maturity of the spores. It seems, therefore, highly probable that in this particular instance a distribution of nutrient materials within the sporangium may be aided by a more or less constant plasmodial circulation.

It will be noted, on comparing Pl. LXXXIV, Fig. 2, and Pl. LXXXV, Figs. 8 and 9, that the sporangia and sporogenous cells increase in size up to the time of synapsis in the nuclei of the gonotokonts; then growth is lessened while the meiotic divisions are taking place. After this it appears from the increased size of the vacuoles in which the young spores lie (Fig. 11) that the sporangia resume their growth for a time, while the young spores remain in size about as they were when first formed. In this stage no vestige appears of a wall of the gonotokonts, and the young spores lie widely separated in the vacuole that formerly held their respective gonotokonts. After a time, however, the plasmodium creeps in between the spores and holds each one separately embedded (Figs. 11 and 13). Miss Twiss ('10) finds a similar early disappearance of the gonotokont wall in *Lygodium*, but in *Aneimia hirsuta* she still finds traces of it after the walls of the spores appear in the tetrad.

The separation of the gonotokonts from each other is initiated by their assuming a spherical form (compare Pl. LXXXIV, Figs. 4 and 7). Thereupon it appears that the tapetal plasmodium creeps in between them and shoves them more or less widely apart, while at the same time the nuclei of the plasmodium become dispersed among and around them, as shown by Pl. LXXXIV, Fig. 7, and Pl. LXXXV, Figs. 9 and 10. That the gonotokonts are in a condition of growth at this time is seen on comparing Pl. LXXXIV, Fig. 4, and Pl. LXXXV, Fig. 9, and that it is also a critical nutritive period is attested by the fact that one finds not infrequently whole sporangia now beginning to disintegrate.

When the last division is complete, as shown in Pl. LXXXV, Fig. 10, the plasma membrane of a gonotokont persists as the common outer membrane

of the spores as they lie still connected in the tetrad. It appears that the plasma membranes bounding the inner surfaces of the spores now become dissolved out in a median plane, so that the spores are free to separate. That such disjunction actually soon takes place is shown in Fig. 11.

The processes concerned in the separation of the spores are not entirely evident; some observable facts, however, give basis for a conclusion as to the main events. By comparison of Figs. 10 and 11 it is seen that after the last meiotic division the vacuole in which a gonotokont was lying increases considerably in size, while the young spores just issuing from the tetrad decrease in volume. Evidently it is this shrinkage which initiates the separation of the spores. In some rare instances thin films of the tapetal cytoplasm flow in between the spores before their contraction is far advanced (Fig. 12). Prantl, l. c., considers the clear space around the spores (seen in my Figs. 11 and 12 and his Fig. 77) to be a greatly swollen cell-wall; and the delicate strands extending between the spores, as shown in my Fig. 12 and his Fig. 122, he concludes are planes of differentiation in the swollen wall. Evidently the nature of his preparations led him to the wrong conception.

After the spores have separated the plasmodial cytoplasm creeps in and completely embeds each one, as shown in Fig. 13; and then the spores enter upon the final period of growth in size and formation of cell-wall. Figs. 11, 18, 19, 20, and 21 are drawn to the same scale, and show the progression of these events as the spores advance to maturity. Figs. 18 and 19 were taken from the sporangial stage shown in Fig. 13. Here the wall of the spore has begun to thicken; and, whereas with the triple stain, safranin, gentian-violet, and orange G, it was when first formed stained purple, it now is stained red. At this stage the sculpturings on the surface of the wall characteristic of this species (shown in Fig. 21) have not yet appeared. Fig. 19 is a surface view giving the three flattened surfaces where the other three spores of the tetrad joined it. There is a line of cleavage down each of the three angles, one of which is seen in cross-section in Fig. 18. Even in these immature spores one often finds in his preparations the three flaps parted and revealing the nucleus within.

The wall now thickens, heaping up ridges at its outer surface and adding an inner layer which comes out almost colourless from the triple stain (Fig. 20). The inner coat (endospore, or intine) is not continuous at the three angles, as shown in cross-section through one of the angles in Fig. 20, neither does it follow the protruding lips of the outer coat (exospore, or extine) at this place, as Beer ('06) finds to be the case in *Helminthostachys*.

When the spore is of the age shown in Fig. 18 a thin film of cytoplasm can be followed out from the mass which holds the nucleus embedded, around the inside of the wall; but when the spores have arrived at the stage shown in Fig. 20 the entire protoplast seems to have receded to one corner

of the spore, either from plasmolysis caused by the reagents or from natural causes. The balled-up condition of the nucleus in Fig. 20 indicates that the former alternative is the more probable.

We find in the literature speculations as to how the growth in thickness of spore coats comes about. In *Helminthostachys*, Beer, l. c., found that the spore protoplast, at the time when the exospore first appeared, was so poor in substance as to make it unlikely that this could be employed in the growth of the wall. The protoplasmic substance of *Aneimia* spores also is very little, even relatively less than in the spores of *Helminthostachys* (compare my Figs. 18 and 20 with Beer's Fig. 11), and entirely inadequate, it seems to me, to supply materials for the increase in wall thickness that takes place between the stages represented in Figs. 18 and 20. As Beer suggests, it appears that the substance for this increase must come from, or through the agency of, the tapetal cytoplasm. Fitting ('00) comes to a similar conclusion for the spores of *Isoëtes* and *Selaginella*. Beer holds it impossible to decide whether this material is applied to the wall by the plasmodium or by the spore protoplast. He suggests that in the latter event the nutrient substance would have to enter the spore protoplast in liquid form before it could be applied to wall construction. As was stated above, in *Aneimia* the exospore takes the safranin stain strongly while it is yet quite thin. This reaction, according to Thomson ('05), indicates that the exospore has undergone the cutin or suberin modification; and this would make difficult the passage of materials to and from the spore protoplast. This fact adds plausibility to the theory that the plasmodium, working from without, supplies the materials, and possibly the stimuli also, for the characteristic growth of the wall.

In his studies of various species of *Isoëtes* and *Selaginella*, Fitting, l. c., finds the exospore widely separated from the mesospore and the spore protoplast withdrawn from contact with the latter, excepting at the spore apex, before these coats have attained their characteristic thickness, and before the endospore has been laid down. Although he finds no indication of protoplasm inhabiting the spore coats, he nevertheless comes to the conclusion that these have the power of constructing their own substance from the materials that come to them from the tapetum. He says: 'Ich glaube also, dass gewichtige Gründe zu der Annahme vorliegen, dass die neuen Hauttheilchen erst innerhalb der wachsenden Membranen entstehen oder, mit anderen Worten, dass diese Membranen die Fähigkeit besitzen, ihre constituirenden Verbindungen selbst aus einer Nährlösung aufzubauen.' And again: 'Ich halte es für denkbar, ja für wahrscheinlich, dass die Sporenhäute von *Isoetes* und *Selaginella* nicht von dem Sporenplasma aus, sondern direct von den Tapetenzellen ernährt werden.'

A more recent study of *Selaginella* by Florence Lyon ('05) suggests, however, that where gaps occurred between the membranes and spore

protoplast in the sections studied by Fitting, Campbell ('05), and herself they were due to shrinkage during the preparation of the sections. In sections which showed no signs of shrinkage anywhere, Miss Lyon found the region of the gaps filled with a substance that seemed to be a part of the spore membrane.

In *Aneimia* the spore protoplast is found in contact with the wall all around after the thickening and cutinization of the wall are well advanced (Fig. 18). In older spores the recedence of the protoplast away from most of the wall (Fig. 20) may be due to plasmolysis during the processes of fixing and embedding; or it may be that the thin film of cytoplasm shown in Fig. 18 has been used up in nourishing the spore coats, as Strasburger ('82, '89) has suggested for similar appearances in many pollen-grains.

Miss Twiss, l. c., finds for *Lygodium circinatum* and *Aneimia hirsuta* that soon after the spore wall has been laid down, and before its thickening has set in, it begins to stain red with the safranin-gentian-violet combination; then as the wall thickens its outer portion stains yellow, indicating pectin, while the inner part reacts to safranin as before. While this differentiation of the exospore is going on, or before, an endospore layer of cellulose is laid down which remains thenceforth chemically unaltered. This differentiation of the exospore into a pectinized and a cutinized or suberized layer does not appear in my preparations.

When the spore walls of *Aneimia phyllitidis* are first formed, we find the cytoplasm and nuclei of the tapetal plasmodium in a well-nourished condition, while at a later stage when the spores are more fully matured, and with the growth of the walls essentially completed, the tapetal cytoplasm is very noticeably impoverished and its nuclei are found in various stages of disintegration.

As might be expected, many of the phenomena of sporangial development have to do especially with the nutrition of the spores. Mention has already been made of the advantages due to the surrounding of the gonotokonts and spores by the tapetal plasmodium, and attention should now be called to the provision for the storing of food and water. I find that at the stage shown in Pl. LXXXIV, Fig. 7, the wall-cells of the sporangium are usually loaded with starch. The cells of the sorophore also, adjacent to the sporangium, contain starch, although in less amount. The very large cells on the marginal upper surface of the sorophore doubtless serve for the storage of water as well as food. Their reaction to the stains shows them to be filled with a very finely granular substance that presumably is some form of food. Within the tapetal and sporogenous cells, however, stored food is not evident. Beer, l. c., finds the wall-cells and tapetal plasma of *Helminthostachys* to contain reserve starch. Burlingame ('09) finds an abundance of starch in the plasmodium, and 'food granules' in the sporogenous cells of *Ophioglossum reticulatum*; and Binford, l. c., finds the

wall-cells filled with reserve food in those sporangia of *Lygodium* whose sporogenous cells have failed to produce spores. This is true of *Aneimia* also, but not of their sterile sporangia alone. I find, rather, that there is on the whole less starch in the sterile than in the fertile sporangia.

The fact that there is such an abundance of stored food in the sporangia in all stages of their development, and that sterile sporangia contain reserve starch, suggests that sterilization may not be due to insufficient nutriment alone.

SUMMARY.

1. Starting from one, or possibly more than one protoderm cell, a few initials of the sporangium wall are soon formed surrounding a central cell, which proves to be the initial of the archesporium and tapetum.

2. This initial by periclinal walls cuts off a layer of tapetal cells, each of which in its turn undergoes one periclinal division, and forms a two-layered tapetum. The single cell surrounded by these is the archesporium.

3. The archesporium gives rise to approximately sixty-five gonotokonts or grandmother-cells of the spores.

4. Before the archesporial divisions are complete the outer tapetal layer begins to break down, and this is soon followed by the similar behaviour of the inner layer, and the mass of gonotokonts thus becomes suspended in the common tapetal cytoplasm now forming a plasmodium.

5. There is evidence that circulation of the tapetal cytoplasm takes place, which probably assists in the distribution of nutrient materials.

6. The sporangium increases in size up to the time of synapsis in the gonotokonts; then apparently growth is retarded during the progress of the meiotic divisions.

7. The mature gonotokonts separate from each other, and the tapetal plasmodium flows in and entirely surrounds each one.

8. From the time of synapsis to the completion of the meiotic divisions seems to be a critical nutritive period, for then it not infrequently happens that sporangia cease their growth and begin to disintegrate.

9. No wall is seen around a gonotokont as it lies separately embedded in the plasmodium, and when the last meiotic division is finished the plasma membrane of the gonotokont persists as the external membrane of the tetrad.

10. The separation of the young spores of a tetrad from each other is initiated by their expelling water and shrinking apart, and thereupon the tapetal plasmodium flows in between them.

11. At a very early stage in its thickening the spore coat reacts as cutin does to the triple stain. Down the three angles of a spore this coat shows lines of fission. Very often in the preparations the exospore breaks apart down these lines into three distinct flaps.

12. The early cutinization of the exospore, which would make difficult the passage of materials through it, is in favour of the theory that the increase in thickness of this coat is the work of the tapetal plasmodium.

13. While the exospore is thickening an endospore is laid down in contact with it, which gives the cellulose reaction with the triple stain. This coat is continuous excepting in front of the lines of cleavage in the exospore, so that the endospore would offer no resistance to the breaking apart of the spore-wall into the three flaps above spoken of.

14. At first the spore protoplast lines the wall throughout; but after the endospore has been laid down, either its substance has been used up over the larger area of the wall, or it shrinks away from the wall and is found balled up at one end of the spore; or the alternative remains that this condition is due to plasmolysis during the preparation of the materials.

15. As the growth of the spores progresses the gradual depletion of the tapetal cytoplasm and nuclei is evidence that the substance of these has been diverted to the nutrition of the spores.

16. Storage of food, largely in the form of starch, takes place in the sporangium wall and in the cells of the sorophore at the base of the sporangium.

17. The tapetal plasmodium and the gonotokonts do not appear to contain stored food.

18. Even sterile sporangia contain an abundance of stored food in their walls, and this suggests that their sterilization has been due to other causes than insufficient nutritive materials alone.

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EXPLANATION OF PLATES LXXXIV, and LXXXV.

Illustrating Professor Stevens's paper on *Aneimia*.

PLATE LXXXIV.

Fig. 1. Longitudinal section through the apex of a young sorophore, showing sporangia in successive stages of development. $\times 393$.

Fig. 2. Median longitudinal section through young sporangium, showing wall-cells, initial cells of tapetum, and archesporium. $\times 860$.

Fig. 3. Median longitudinal section through young sporangium, slightly older than in Fig. 2. The initial cells of the tapetum have divided, producing a two-layered tapetum, and the archesporium has undergone division. Even at this early stage the walls of the tapetum have begun to be absorbed. $\times 308$.

Fig. 4. Still older stage than in Fig. 3, showing young gonotokonts or grandmother-cells of the spores surrounded by the two-layered tapetum. $\times 860$.

Fig. 5. Section remote from the centre through a sporangium, showing tapetal cells completely broken down and tapetal nuclei migrated from the periphery to cluster about the gonotokonts, two of which are here shown. $\times 308$.

Fig. 6. Longitudinal section through young sporangium and sorophore, showing the epidermal cells of the upper surface greatly enlarged, resulting in the turning of the sporangia, which are at first marginal, to the under side of the sorophore. $\times 393$.

Fig. 7. Section through sporangium, showing mature gonotokonts previous to the synaptic stage. At this stage the wall-cells are filled with starch grains. $\times 540$.

PLATE LXXXV.

Fig. 8. Section through young sporangium. All of the mother-cells of the tapetum have divided but one, and this is in the late prophase of division. Even at this early stage the meshes of the cytoplasm of the wall-cells are being filled with reserve starch. $\times 860$.

Fig. 9. Portion of tapetal plasmodium and embedded gonotokonts in the synaptic stage. One of the gonotokonts disintegrating. $\times 860$.

Fig. 10. After second meiotic division, showing young spores still in contact. $\times 860$.

Fig. 11. A later stage. Young spores separated and dispersed within the cavities previously occupied by their respective grandmother-cells. $\times 860$.

Fig. 12. The same as Fig. 11, but showing thin strands of cytoplasm that evidently had flowed in between the spores while they were yet in close proximity. $\times 860$.

Fig. 13. Longitudinal section through a sporangium, showing young spores after the tapetal plasmodium has surrounded each one separately. The wall-cells, excepting the cap-cells, are loaded with reserve starch. $\times 308$.

Fig. 14. One of the wall-cells of Fig. 13, showing reserve starch assembled about the nucleus. $\times 860$.

Fig. 15. Polar view of late prophase of the first meiotic division. $\times 2,000$.

Fig. 16. Early anaphase of the first meiotic division. $\times 2,000$.

Fig. 17. Telophase of second meiotic division. Nuclear membrane not yet formed. $\times 2,000$.

Fig. 18. Section of immature spore through its apex, showing the spore coat thinner there than elsewhere. Endospore not yet laid down. $\times 860$.

Fig. 19. Surface view of immature spore of the age shown in Fig. 18. $\times 860$.

Fig. 20. Section through mature spore, showing exospore and endospore, the former with a line of cleavage at the angle and the latter not lining the exospore at this place. The protoplast has receded entirely to one end of the spore. $\times 860$.

Fig. 21. Surface view of mature spore, showing wall sculpturings and lines of fission through the spore coats down the three angles. $\times 860$.

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