DEVELOPMENT OF OVULE AND FEMALE GAMETOPHYTE IN *GINKGO BILOBA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY
XC

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(WITH PLATES V AND VI)

Ovules were collected at Elyria, Ohio, at intervals of two weeks, from the first of May until the latter part of August 1905, and were sent to Chicago packed in wet cotton. In April 1906, additional collections were made. Having been freshened by a stay in a moist chamber, the material was killed and fixed in chromacetic or chrom-acetosmic acid and put up in paraffin.

I wish to acknowledge my indebtedness to Miss A. M. STARR of Elyria, Ohio, for material, and to express my appreciation of the helpful suggestions of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN of the University of Chicago, where the work was begun.

Megasporangium

DEVELOPMENT.—The megasporangia are borne on the short spur shoots, which bear also a few leaves, the longer shoots producing leaves only. By the first of April the terminal buds of the spur shoots have begun to swell slightly, and after removal of the brown bud-scales, the green leaves can be seen, but ovules are not yet distinguishable. Unopened buds collected the middle of April contain ovules which, with the stem bearing them, measure 2^{mm} in length. The ovules alone are only 0.25^{mm} long; they appear singly or in pairs at the end of leafless stems, and are pale cream color, while the leaves in the bud are bright green. Vertical sections (*fig. 1*) show the undifferentiated nucellus not yet wholly enclosed by the integument.

The buds soon open, and the leaves and ovule-bearing stems increase greatly in length. Not all the buds are fertile, but in those which are, the ovule-bearing stems vary in number from one to six. The ovules are still pale cream color, but show a faint green tinge which deepens rapidly as they enlarge. The ovule stalks appear, not at the growing tip of the spur shoot, but around it, as do the

strobili of most other gymnosperms. The succession of structures from without to the center of the bud is (*a*) brown bud-scales, (*b*) green scale-leaves, (*c*) foliage leaves, (*d*) ovules, (*e*) foliage leaves (*fig. 2*). Sections of these ovules show the nucellus enclosed by the integument but still entire, no tissue having broken down to form the pollen chamber.

At the beginning of May the ovules average 0.75^{mm} in length and are little if any greater in diameter than the stems that bear them. In some cases the nucellar beak protrudes as a tiny papilla from the micropyle. Vertical sections (*figs. 3, 4*) show the presence of a large pollen chamber, often containing pollen grains already developing tubes.

SPOROGENOUS TISSUE.—In ovules collected the latter part of April and the first of May, the sporogenous tissue has been differentiated, and all stages from immature mother cells to complete tetrads are to be seen. About at a level with the junction of the nucellus and the integument (*figs. 3, 4*) is an ovoidal mass of cells (*fig. 5*) which differ from those around them in being larger, having granular cytoplasm without vacuoles, and large nuclei with conspicuous nucleoli. All these have the appearance of sporogenous cells, although as a rule there is only one mother cell (*fig. 5*). In fifty ovules only one shows more than a single mother cell (*fig. 6*), that single exception possessing two.

The origin of the mother cell from a hypodermal cell could not be traced. Nor could it be determined whether the differentiation of the mother cell precedes that of the surrounding cells, in which case they would be purely tapetal in morphology as well as in function; or whether the whole mass is differentiated, the picking out of the functional mother cell occurring later. If the latter be the case, the mass is sporogenous, and the presence of such cells around the youngest mother cell observed strengthens the view that such is the case. The origin of the two mother cells and the cells between them from the same row (*fig. 6*) is another point in favor of this probability.

The mother cell is usually slightly below the center of the mass (*fig. 5*), and is distinguishable from the surrounding cells by its greater size, more deeply staining wall, and non-granular cytoplasm. At first it is non-vacuolate and the large nucleus is near the center

(*fig. 7*), but later a large vacuole appears below and sometimes a smaller one above the nucleus, the latter consequently taking a position nearer the top of the cell (*fig. 8*). The mature mother cell shows, in the cytoplasm below the vacuole, a peculiar kinoplasmic mass (*fig. 8*) similar to those already reported in the mother cells of *Thuja* (7), *Taxodium* (1), *Larix* (6), and *Taxus* (2), and in the eggs of *Thuja* and *Taxodium*. The significance of this mass has not been determined.

The nucleus soon goes into synapsis (*fig. 9*), the kinoplasmic mass disappearing about the same time. In the single preparation showing the next stage, the eight chromosomes, which result from synapsis, divide even before the disappearance of the nuclear membrane and entrance of the spindle fibers, although the latter are visible in the surrounding cytoplasm (*fig. 10*). The chromosomes give no indication of their second division, although in some forms such indications are usual. Repeated counting of chromosomes on spindles in various stages through the prothallium gave the constant number eight.

TETRAD.—The spindle of the first division is peculiar in being obliquely placed (*figs. 11, 12*), possibly as a result of the vacuole in the lower part of the cell, the kinoplasmic fibers extending only in the cytoplasm next the wall. The lower of the two cells resulting from this division is slightly larger (*fig. 6*) than its sister. It soon markedly exceeds the other cell, however, the dividing wall arching up into the latter (*fig. 13*). The division of the lower cell (*figs. 14, 15*) precedes that of the upper, which in some cases does not divide at all (*fig. 14*). Usually, however, a linear tetrad is formed (*fig. 16*), the lowest spore of which enlarges rapidly, absorbing the contents of the other three. Although the linear arrangement of the four spores is most common, other forms of tetrads were observed. One case of a bilateral tetrad (*fig. 18*), and two instances of the transverse division of the lower cell followed by the vertical division of the upper cell were found. In one of the latter the nucleus of the upper cell has divided, but the separating wall has failed to appear (*fig. 9*). In all cases the lowest spore, the one nearest the chalaza, enlarges before the upper ones do, and by absorbing material from them and monopolizing most of the material coming into the ovule prevents their further development. Similar to this earlier development of the lowest spore of the tetrad is

the development of the lower of the two mother cells in a single ovule (*fig. 6*). COULTER (3) suggests that this early development of the lowest cell may be the result of a favorable position in regard to nutriment coming up through the chalazal tissue.

The functional spore enlarges rapidly, its nucleus being held near the upper end of the cell by a large vacuole below. The presence of this vacuole in the spore suggests the probability of the parietal position of the free nuclei of the embryo sac from the very beginning. The appearance of a vacuole and the consequent parietal placement of the two nuclei resulting from the first division of the spore nucleus is reported by LAND in *Ephedra trijurca* (8); but in Ginkgo the vacuole is present even before the formation of the first gametophytic spindle, being in fact the same vacuole that was in the mother cell.

PROTHALLIUM.—*Free nuclear division*.—Ovules of two weeks later are somewhat larger, and the embryo sacs contain free nuclei (*fig. 20*), the number ranging from 16 to 64. At this stage the sac is very thin, staining no more deeply than does an ordinary cell wall, and the plasmic layer lining it is very delicate. The nuclei are well separated and each has a conspicuous nucleolus. They are lenticular in side view and circular in face view, and average 13.5μ in long diameter by 8.14μ in short diameter. In these early stages the division of the free nuclei is simultaneous, although each of several sacs with 64 spindles shows the latter in several different phases (*fig. 21*). This is of interest, inasmuch as later free nuclear division proceeds irregularly, nuclei in a single sac showing all conditions through the various stages of karyokinesis and resting (*fig. 24*.) At first the nuclei of such sacs are definitely placed, so that although all stages are to be seen in the sac, the nuclei in any given portion are in the same stage. Later the sacs show nuclei in all stages grouped without regard to condition.

Free nuclear division continues from the second week in May until the first week in July, the whole ovule meanwhile enlarging and the embryo sac growing rapidly in both size and thickness. The protoplasm becomes granular and the free nuclei divide so rapidly that they decrease in size (*figs. 22, 24*). By the first of July the cytoplasm has become very granular (*fig. 23*), and the number of nuclei

large, the numerous divisions and their irregularity making the number larger than 256, which is so common a number among the gymnosperms. It is not impossible that the irregularity in division of the free nuclei, varying as it does from the simultaneous division said to obtain in other forms, may have been due to the unnatural conditions—growth after separation from the tree—although an effort was made to render conditions as natural as possible.

Wall formation.—About this time there appears on the outer surface of the plasmic sac a delicate membrane (*fig. 24*). This is not a *Hautschicht*, nor is it in any way attached to the original spore wall or embryo sac. It is a true cell wall of the type known as walls of deposit, and is formed by the protoplasm which lines it. Then walls appear in the cytoplasm, perpendicular to this enclosing membrane and with their outer edges fastened to it (*fig. 25*). The current accounts of wall formation following free nuclear division in the embryo sac, beginning with Miss SOKOLOWA'S (10) description and continuing through subsequent papers by other investigators, state that the walls appear at right angles to the embryo sac or spore wall, and with their outer edges fastened to it. This is distinctly not the case in Ginkgo; and the ease with which the spore membrane may be peeled away from the prothallia of many other gymnosperms, even when the latter are but partly developed, suggests the probability that it is not the case in them.

It has long been known that the walls of microspores are quite separate and distinct from the wall of the mother cell in which they lie, and that the spores when mature escape from the mother cell membrane through ruptures or by its solution. In sectioned microsporangia showing developing tetrads, these separate surrounding walls of the mother cells may be readily seen. It is known also that the outer portion of the microspore wall, that next the wall of the mother cell, is formed by deposit, and not upon spindle fibers, as are the walls between the spores themselves. The megaspore wall, as can be seen in the figures showing the tetrad formation, with the exception of that comparatively small portion which divides it from the non-functional spores, is the old membrane of the mother cell and corresponds to the mother cell wall enclosing the microspore tetrad. The formation of this separate membrane upon the outer surface of

the sac by deposit, therefore, corresponds to the formation of the walls of the microspore, and the fastening to it of the outer ends of the radial walls corresponds to the similar attachment of the walls cutting off the prothallial cells in the male gametophyte.¹

These walls, at right angles to the enclosing membrane and fastened to it, separate the nuclei, probably each one being enclosed in a cell of its own. In other forms the first radial walls are said to come in on the spindles of the last simultaneous free nuclear division. Since the later divisions of the free nuclei here are far from simultaneous, this cannot be the case. Although the coming in of these first walls was not observed, the subsequent centripetal growth and division of the cells formed by their development are clearly seen (*fig. 25*). The inner cells of the centripetal rows are larger than the outer ones, the size of the latter being early diminished by repeated divisions, both tangential and radial (*fig. 25*). In most cases the nuclei of these cells, which are open to the interior of the sac (*fig. 27*), are much larger than those of the enclosed cells near the periphery. Most of the open cells have a single nucleus (*fig. 27*), but two nuclei are to be seen in some (*fig. 28*), and in a few, three nuclei. Binucleate cells (*figs. 29, 30*) are common in the tissue near the open central space, and even a few multinucleate cells are present (*fig. 31*), but since cells with a single nucleus are not found in the mature endosperm, these very cells must later become uninucleate. Whether this change results from a fusion of the several nuclei, a degeneration and absorption of the superfluous number, or their separation by the formation of intervening walls is not certainly known. In all cases of wall formation seen within cells, however, the wall is on a regular karyokinetic spindle. Usually the two nuclei in a cell are in contact, and it is not uncommon for one of them to be smaller and more dense than the other, suggesting its degeneration and absorption (*fig. 30*). From this it appears probable that cells which have at first two or more nuclei become uninucleate by a fusion of the several into one, or by the degeneration and absorption of the superfluous ones, and not by the formation of separating walls.

¹ One case of curious and irregular wall formation, abnormal and perhaps the result of pathological conditions, is seen in a sac of June 19. The plasmic layer containing free nuclei is as usual, but in addition there are eight little groups of nuclei, each group quite separate from the others and each with an inclosing membrane (*fig. 26*).

Wall formation in the cells open and growing toward the center, which is filled with sap, is carried on in the usual way at the peripheral end of each new cell, by the formation of a nuclear plate on the spindle of the division by which the new nucleus is formed (*fig. 32*).

The mode of formation of the side walls of these same cells, however, is not so easily determined. Curious double spindles (*fig. 33*) and extra fibers radiating from nuclei in the last stages of karyokinesis (*fig. 32*) indicate some such procedure as this: When a nucleus divides, lengthening the centripetal row, fibers radiate from it, not only to the sister nucleus, but radially as well, connecting with similar fibers radiating from other nuclei, and upon the resulting spindles are formed the plates which later develop into walls. This mode of wall formation has long been known in the endosperm of many angiosperms. In some cases are to be seen similar double spindles which lack nuclei at the two ends, the single nucleus concerned being in the middle. Further work is necessary to settle this point.

Centripetal growth continues through July and August, being most active at the base and filling the central cavity with tissue by the last week in August. In shape the prothallium is almost ellipsoidal, being slightly flattened on two opposite sides (*figs. 34, 35*). As a result of the shape and the equal growth from the sides, the closure of the tissue is in a plane parallel with the broader sides of the gametophyte. Only in sections cut at right angles to the flattened sides does the closure appear as a line (*fig. 34*). Upon approaching each other the open, centripetally growing cells of opposite sides do not unite and form a common end wall, as they are said to do in many other forms. Instead, each cell forms an independent end wall, separate from that of the other cells (*fig. 36*). The resulting body is not a solid mass, but a tissue which may be easily opened at the middle. Later these separate end walls, lying as they do against each other, may so unite as to appear and really form a common wall, but such is not the case in the oldest prothallia examined.

Growth.—Simultaneous with centripetal growth, the whole prothallium increases in bulk by growth and division of cells, the inner ones enlarging greatly and those at the periphery continuing meristematic (*figs. 25, 38*).

Archegonium initials appear very early; the two-celled neck has

been formed and the central cell has enlarged considerably before the tissue lining the sac equals in depth the width of the central cavity (*fig. 37*). HIRASÉ (4) has described the development of the archegonia.

The outer wall of the prothallium, the first wall built by the plasmodic sac, becomes much thickened, and the megaspore membrane or embryo sac becomes exceedingly thick and dense, making the entrance of the killing and the fixing fluids difficult. The structure and development of the megaspore wall is set forth in a paper by THOMSON (11), but as my results differ slightly from his, I shall give them. THOMSON states the thickness of the megaspore coat from a mature seed to be $4.5\text{--}5\text{ }\mu$. The wall shown in *fig. 22* measures $2.1\text{ }\mu$ in thickness and that of an endosperm which has just closed in the center measures $6.16\text{ }\mu$. At first the membrane, which as shown above is in reality the mother cell wall, is thin and delicately granular (*fig. 20*). It rapidly thickens and becomes coarsely granular. A cross-section shows a very thin inner layer acting as a base for the transversely placed rods of the thick outer layer (*fig. 22*). THOMSON reports these rods as being quite irregularly placed. Subsequent thickening takes place in the outer layer only, the rods increasing in length and thickness, and the thin inner layer finally disappearing (*fig. 25*). Not infrequently, in material of this stage, little bunches of the rods forming the wall are found torn out and scattered over the slide. The result of the ease with which the outer layer is torn across between the rods and the resistance offered by the thin inner layer is shown at the upper end of the wall in *fig. 22*. The ends of the rods, presented at right angles at the surface of the spore coat, are the cause of the slight roughness there. After the disappearance of the smooth inner layer, the inner surface becomes similarly rough.

The complete independence of the heavy embryo sac and the outer wall of the prothallium explains the position of the former with reference to the archegonial chamber of the mature endosperm. Being entirely free from the tissue of the endosperm, it is lifted up by the growth of the tissue around the archegonial chamber until it is some distance from the floor of the latter and forms a roof over it.

From early in May the ovules are green throughout, but as they increase in size the inner tissue becomes hyaline, the chlorophyll being

abundant in one place only—a shallow region at the surface of the single heavy integument. Soon after the appearance of walls and long before the filling of the sac with tissue, the gametophyte becomes green. In a few weeks it is by far the greenest thing in the ovule. An alcoholic solution of this green pigment gives the spectrum of chlorophyll. The presence of chlorophyll, evidently functional, within a gametophyte wholly enclosed within an ovule has not been reported before. The thin walls of the cells of the integument, the paucity of chlorophyll there, and the presence of numerous large cavities full of a clear viscid liquid favor the transmission of light to the gametophyte, and, as a meristematic tissue, it responds by the formation of the pigment. WARMING (12) reports that the endosperm of *Cycas circinalis*, if fertilization fails to take place, sometimes grows out through the micropyle and in the light becomes green. No mention, however, is made of its becoming green before protruding.

The cells of the endosperm early fill with starch, the large grains characteristic of the storage forms being abundant in the inner cells (figs. 26–38), while smaller grains in all stages of formation are to be seen in the periphery.

Some of this starch is undoubtedly manufactured by photosynthesis within the prothallium, but some may be the result of absorption from surrounding nucellar and integumentary tissues. Starch is plentiful, however, only in the outer layers of integument, never close to the endosperm.

Spongy tissue

The spongy tissue, in the midst of which the mother cell appears, performs an important function in the nutrition of the prothallium. As the megaspore mother cell forms the tetrad, the whole spongy mass increases in bulk, the individual cells multiplying by spindles at right angles to the periphery of the mass (fig. 39). The nucellar tissue immediately surrounding at once shows signs of being absorbed, the protoplasmic contents of the cells becoming dark and granular even before the walls suffer collapse (fig. 39). This mode of increase in bulk of the spongy tissue and the adaptation of the surrounding nucellar tissue is described at length by Miss FERGUSON (4) in her work on *Pinus*. The result of this activity is a growing mass, at first

ovoidal, then ellipsoidal, of glandular cells encroaching upon and absorbing the surrounding nucellar tissue, the whole being enclosed in an enlarging cavity whose wall is made of elongating dividing cells. Although at first densely granular and non-vacuolate (*figs. 5, 6*), the absorbing cells soon become vacuolate (*fig. 18*), then multinucleate (*fig. 22*). Whether these nuclei are the result of direct or indirect division is not known. That the former is probably the case is suggested by the occurrence of such division in the absorbing cells of other sporangia. Moreover, spindles are seen in early stages only, and in those cases a plate is always present.

Until the fourth week in June the spongy tissue cells show great activity (*fig. 22*) in encroaching upon and absorbing the adjacent tissues, but soon afterward they themselves show signs of being absorbed by the enlarging prothallium (*fig. 24*), the latter being still in the free nuclear stage, but having formed the enclosing wall.

By the time the radial walls are formed and centripetal growth has brought the tissues one-third of the distance across the sac, the spongy tissue cells have been absorbed (*fig. 25*), their remains being only a thin mass of collapsed and heavily staining walls. The embryo sac now lies against the undifferentiated nucellar tissue, separated from the living cells of the latter only by this mass of dead cells which have given up their substance. When the prothallium has become a mass of tissue, it is quite near the surface of the nucellus, instead of far below it as at first. Most of the upper part of the nucellus has been absorbed, and the nucellar beak, at first so conspicuous, has collapsed (*fig. 34*).

Integument

The tissue of the single, thick integument is homogeneous at first, but by the last of May it is differentiated into three distinct tissues (*fig. 40*): an outer layer of large thin-walled cells with many mucilage-filled cavities; a middle layer of small, isodiametric cells; and an inner region of large, very thin-walled cells loosely held together. This inner layer is further differentiated into an outer layer having cells transversely elongated, and an inner layer having cells vertically elongated. This innermost layer of vertically elongated, delicate-walled cells appears only in the free portion of the integument, but the

remaining layers extend through its entire length, being the same both where it is separate from the nucellus and where it is continuous with it. The outer of these three layers becomes the fleshy, juicy part of the ripened fruit (*figs. 34, 35*); the middle layer, by a great thickening of walls and compacting of cells, becomes the stony coat; the inner fleshy layer, which is delicate and watery in early stages, becomes crushed and dry, forming the papery layer which lines the stony coat and adheres to it in the mature fruit. The nucellar tissue is quite distinct from the watery tissue of the integument, and in the upper part the two are not even in contact (*fig. 40*), so the former cannot form the papery coat as it is said to do by SEWARD and GOWAN (9). Below the line of junction of the nucellus and integument the former may contribute some small part to the papery coat, but it is almost entirely absorbed by the prothallium.

At the base of the ovule two bundles enter the watery tissue, coming up through a little gap in the tissue of a stony coat (*fig. 40*). Their course lies along the inner surface of the watery tissue, one curving up on each side and ending just below the point at which the integument becomes free from the nucellus. The abundant liquid in this watery tissue doubtless comes up through these bundles. Upon removal of the integument, which splits readily into two lips, the nucellus is seen to be flattened on two opposite sides, the intervening sides being angled (*figs. 34, 35*). It is in these angled sides that the bundles end (*fig. 35*). In some cases there is a three-lipped integument and a three-angled nucellus, there being also three bundles, one ending in each angled side.

SUMMARY

1. The ovule has a conspicuous nucellar beak and pollen chamber.
2. The sporogenous tissue is deep within the nucellus.
3. Differentiation of the mother cell has taken place by the first of May.
4. One mother cell is usual, but more may occur.
5. A peculiar kinoplasmic mass is present in the mother cell.
6. The gametophyte number of chromosomes is eight.
7. The first spindle is obliquely placed in the mother cell.
8. The tetrad may be complete or incomplete.

9. The tetrad is usually linear, but sometimes bilateral, or a combination of bilateral and linear.

10. The lowest spore is the functional cell.

11. The spore is vacuolate from the first and the free nuclei of following stages are probably always parietally placed.

12. Free nuclear division is at first simultaneous but gradually becomes irregular.

13. Free nuclear division extends, approximately, from the second week in May until the first week in July.

14. The cytoplasm, at first delicate, becomes granular and forms upon its outer surface a delicate wall.

15. Centripetally growing walls are formed between the nuclei and with their outer edges fastened to the membrane developed at the outer surface of the plasmic sac.

16. The sac fills with tissue by centripetal growth and division of cells, the inner ends being open.

17. These open cells are usually uninucleate but sometimes multinucleate.

18. Binucleate and multinucleate cells are frequent in young prothallial tissue, but later they become uninucleate, probably by fusion of the several nuclei or by degeneration of the superfluous ones.

19. When the centripetally growing cells meet at the center each forms an independent end wall.

20. The megaspore wall and the outer wall of the prothallium become much thickened.

21. The megaspore wall is composed of an inner, thin, firm layer, and a very thick, outer layer made up of rods formed at right angles to the surface.

22. The archegonia are quite far developed while there is still a large central cavity.

23. The gametophyte develops abundant chlorophyll, becoming the greenest tissue of the ovule.

24. The spongy tissue surrounding the mother cell is tapetal in function, absorbing the surrounding tissue, and finally being itself absorbed by the growing prothallium.

25. The spongy tissue cells become vacuolate and multinucleate.

26. The integument is early differentiated into three distinct

tissues, which become respectively the fleshy coat, the stony coat, and the papery coat.

27. Abundant liquid is brought to the watery layer by two bundles which enter through a gap in the stony coat and terminate just below the point of separation of the integument and nucellus.

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EXPLANATION OF PLATES V AND VI

With the exception of *figs. 3, 4, 34, 35, 37, and 40*, the figures were drawn with the aid of an Abbé camera lucida and all were reduced one-half in reproduction.

PLATE V

- FIG. 1. Vertical section of a single ovule; April 15, 1906. $\times 15$.
 FIG. 2. Habit sketch of spur shoot, showing opened fertile bud; April 25, 1906. $\times 2$.
 FIG. 3. Vertical section of a pair of ovules; May 1, 1905. $\times 12$.

- FIG. 4. Vertical section of a single ovule; May 1, 1905. $\times 12$.
- FIG. 5. Vertical section through sporogenous mass with single mother cell; May 1, 1905. $\times 325$.
- FIG. 6. Vertical section through sporogenous mass with two mother cells; May 1, 1905. $\times 325$.
- FIG. 7. Young mother cell; May 1, 1905. $\times 650$.
- FIG. 8. Mature mother cell, showing vacuoles and kinoplasmic mass; May 1, 1905. $\times 650$.
- FIG. 9. Mother cell in synapsis; May 1, 1905. $\times 650$.
- FIG. 10. Mother cell just after synapsis, showing the eight chromosomes already divided for the heterotypic division, and the spindle fibers in the cytoplasm; May 1, 1905. $\times 650$.
- FIG. 11. Mother cell showing oblique spindle of heterotypic division; May 1, 1905. $\times 650$.
- FIG. 12. Mother cell showing oblique division; May 1, 1905. $\times 650$.
- FIG. 13. First division of mother cell completed; May 1, 1905. $\times 650$.
- FIG. 14. Lower cell showing spindle of homotypic division; upper cell preparing for division; May 1, 1905. $\times 650$.
- FIG. 15. Division of lower cell completed; May 1, 1905. $\times 650$.
- FIG. 16. Incomplete tetrad; upper cell failing to divide; May 1, 1905. $\times 650$.
- FIG. 17. Linear tetrad; lowest cell enlarging; May 1, 1905. $\times 650$.
- FIG. 18. Bilateral tetrad; May 1, 1905. $\times 650$.
- FIG. 19. Combination linear and bilateral tetrad; wall failing to appear in upper cell after vertical division of nucleus; May 1, 1905. $\times 650$.
- FIG. 20. Vertical section of a sixteen-nucleate sac and tapetum; May 18, 1905. $\times 225$.

PLATE VI

- FIG. 21. Portion of sac showing spindles of free nuclear division in slightly different phases; May 18, 1905. $\times 650$.
- FIG. 22. Portion of embryo sac showing granular cytoplasm, free nuclei, and multinucleate tapetal cells encroaching upon nucellus; June 5, 1905. $\times 650$.
- FIG. 23. Face view of plasmic sac with free nuclei; June 5, 1905. $\times 650$.
- FIG. 24. Portion of embryo sac showing absorption of tapetum; free nuclei in different conditions; and a cell wall developed upon the outer surface of the plasmic sac; June 20, 1905. $\times 650$.
- FIG. 25. Section of prothallium, showing centripetal walls fastened to outer membrane which is separate and distinct from embryo sac; July 19, 1905. $\times 325$.
- FIG. 26. Peculiar case of wall formation; June 5, 1905. $\times 650$.
- FIG. 27. Open cell from inner end of centripetally growing row; July 19, 1905. $\times 325$.
- FIG. 28. Open inner cell with two nuclei; July 28, 1905. $\times 325$.
- FIG. 29. Binucleate cell from prothallial tissue; July 28, 1905. $\times 325$.

FIG. 30. Binucleate cell from gametophyte; one nucleus apparently undergoing absorption; July 19, 1905. $\times 325$.

FIG. 31. Multinucleate cell from prothallial tissue; July 28, 1905. $\times 325$.

FIG. 32. Open inner cell showing centripetal growth by means of spindles; extra fibers being attached to outer nucleus; July 19, 1905 $\times 325$.

FIG. 33. Curious incomplete double spindle from open inner cell; July 19, 1905. $\times 325$.

FIG. 34. Vertical section of ovule, showing embryo sac filled with tissue; the closure appearing as a line; August 21, 1905. $\times 1$.

FIG. 35. Transverse section of ovule, showing embryo sac filled with tissue; showing radiation of cell rows from line of closure; August 21, 1905. $\times 1$.

FIG. 36. Vertical section of tissue at line of closure, showing independent end walls of opposite cells; August 21, 1905. $\times 325$.

FIG. 37. Diagram of vertical section of prothallium, showing amount of tissue present at time when archegonia are becoming conspicuous; July 19, 1905. $\times 2$.

FIG. 38. Typical mature cell from gametophyte, showing single nucleus and abundance of starch; August 21, 1905. $\times 325$.

FIG. 39. Vertical section through spongy mass, showing mode of increase in size, and effect upon surrounding cells; May 1, 1905 $\times 325$.

FIG. 40. Diagram of vertical section of ovule, showing differentiation of tissues in the integument; June 10, 1905. $\times 4$.

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