The Gametophytes, Fertilization and Embryo of *Cephalotaxus drupacea*.

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With Plates I-IV.

INTRODUCTION.

TO the morphologist or to the cytologist there are few groups of plants which offer more attractive features than the Coniferales. This is not only true of the sporophyte structures, but is particularly true of the gametophyte, for here every phase in the history of the sexual generation reveals the development of structures which are of profound interest in regard to their morphological meaning. The facts concerning the behaviour and development of such gametophytic structures are, therefore, from the point of morphology alone, worthy of record.

The results of investigations on the comparative anatomy of the sporophyte, together with the evidence which is gradually accumulating from the study of fossil forms, is bringing us nearer to an understanding of the true phylogeny of the Coniferales, but at present such an understanding is far from perfect. That we are not yet warranted in establishing the true phylogenetic relationships of the Coniferales and their various sub-groups, is due mainly to the fact that the strongest kind of evidence, that of the fossils, is at present disconnected and in places contradictory. In view of the gaps in the chain of evidence from the paleobotanical side, a knowledge of the primitive structures associated with the gametophyte generation becomes imperative. That our knowledge of such structures has had much to do in modifying our ideas as to the phylogeny of the various groups of Gymnosperms, we need only refer to the effect of the discoveries of Hofmeister, Strasburger, Hirasé, Ikeno, and Webber, to say nothing of the great amount of literature which has more recently accumulated on the gametophytes of the Abietineae, Cupressineae, Taxodiaceae, and Taxaceae. Indeed, many of the discoveries recently made are so extraordinary that

a detailed history of the gametophytes of all living forms seems quite necessary if we hope to build up a phylogeny of the Coniferales and their various sub-groups.

The present investigation is intended as a contribution to our knowledge of the Taxaceae. It was undertaken with the idea of giving a complete account of the development of the gametophytes and embryonal sporophyte of *Cephalotaxus*. It thus fills in many of the gaps left by Sokolowa ('91), Strasburger ('79), and Arnoldi ('00), who have contributed fragmentary but valuable information concerning the gametophytes of this interesting genus. The work was commenced in the spring of 1903 but was delayed in its completion on account of the scarcity of material. The greater part of the work was carried on in the Botanical Laboratory of Stanford University, California, but it was finished in the Jodrell Laboratory at Kew Gardens.

I take this opportunity of expressing my gratitude to Lieut.-Col. Prain, C.I.E., F.R.S., Director of the Royal Gardens, Kew, and to Dr. D. H. Scott, F.R.S., Honorary Keeper of the Jodrell Laboratory, who kindly extended to me the privileges of the Jodrell Laboratory, and thus made the completion of the work possible. To Mr. L. A. Boodle, F.L.S., also I wish to express my sincere thanks. Upon my arrival at Kew Gardens I found that Mr. Boodle had commenced an investigation upon the gametophytes of *Cephalotaxus Fortunei* and *C. drupacea*, and had collected and prepared considerable material for this purpose. Upon learning that my investigation was so near completion, both as regards drawings and text, Mr. Boodle kindly invited me to compare some of his excellent preparations with my own. The result of this comparison was very gratifying, for it helped to confirm several important interpretations which might otherwise have remained in doubt.

METHODS.

The material for this investigation was obtained from three small shrubs growing on the campus of Stanford University, California. I began to make collections early in March, 1903, and continued to prepare material at short intervals, as long as time and the supply of material permitted, until the spring of the present year. There was not an abundance of fruit formed on the shrubs and I therefore thought it best to sacrifice the early stages of the female gametophyte in order to have enough specimens for investigating the later phases. I hope in the near future to trace out the formation of the megaspores and related structures.

For those who are investigating the cytology of the Gymnosperms I should like to emphasize the importance of fixing the material in the field or, when that is impossible, to otherwise provide against evaporation. With such critical stages as the division of the body-cell, the organization

of the ventral canal-cell, and fertilization, a rapid fixation is quite necessary. Better results were always obtained when the material was placed in the killing fluid as soon as possible after being detached from the tree. For many stages it is even desirable to carry on the dissections while the material is submerged in the fixing fluid.

The ordinary cytological methods were employed, details of which have already been published (Lawson, '04). We need only add that Flemming's weak solution and the chrom-acetic mixture gave the most satisfactory results as fixing reagents. The triple combination, safranin, gentian violet, and orange G. was used for staining.

THE MALE GAMETOPHYTE.

The first collection of the microsporangia was made on March 1, before the megasporangia were visible. From then on to the time of pollination collections were made almost daily. The microspores from the early collections contained a single large central nucleus and a large quantity of starch grains. The spore at this time also showed a well-developed exine. Fig. 1 represents a cross-section of a young microspore just before its first division. Sections of the sporangia taken a day or two before the pollen was shed showed two distinct nuclei in each microspore, although I was unable to find the spindle showing the actual division of the first nucleus. One of these nuclei was larger than the other and more centrally located. The former is undoubtedly the tube nucleus, while the history of the smaller one proved it to be the generative nucleus. From the observations of Strasburger ('92) and Coker ('04) it appears that in *Taxus*, *Juniperus*, and *Cupressus* there is no division of the microspores during their confinement within the sporangium, while in the Abietineae and in *Chamaecyparis*, *Cryptomeria*, *Thuja*, *Sequoia*, *Podocarpus*, and *Cephalotaxus* the microspores divide before they are shed.

In *Cephalotaxus drupacea* the first division of the microspore takes place at least a day or two before pollination. Fig. 2 represents a section of a pollen-grain at this time. It will be observed that the starch grains, which were formerly present in abundance, have entirely disappeared, and that there is an indication of a delicate membrane separating the two nuclei. In view of the importance of these early stages in the history of the male gametophyte, and especially in view of the fact that *Cephalotaxus* is regarded by many botanists as a primitive type of Conifer, a very careful search was made for any evidence of the survival of sterile prothallial cells as found in the Cycads, *Ginkgo*, and in the Abietineae, but no trace of such vestigial structures were found. There were but two nuclei present, and a careful study has convinced me that the division which gives rise to them (i. e. the generative and tube nuclei) is the only division in the male gametophyte which precedes the organization of the body and stalk

nuclei. A similar condition has been reported for *Taxus* (Coker, '04) and *Torreya* (Robertson, '04; Coulter and Land, '05). Indeed, from the number of types that have been investigated, it would seem that the survival of the sterile prothallial cells among the Coniferales, is almost entirely confined to the Abietineae. The single exception is that of *Podocarpus*, and even here there are strong suspicions that the Podocarpeae and Abietineae are closely related (Coker, '02).

In this connexion it is interesting to note that Miss Robertson ('04)¹ correlates the survival of the vegetative prothallial cells with the winged character of the pollen-grain. No one doubts the primitive character of the Cycads and *Ginkgo*, and here the prothallial cells are evident. They are also constantly present in the Abietineae, but in the Taxeae, Taxodineae, and Cupressineae they are conspicuous by their absence. Miss Robertson ('04) infers that the superior buoyancy produced by the bladder-like swellings of the exine in *Podocarpus* and in the Abietineae 'would make any difference in weight of very little importance, and so natural selection would not come into play to eliminate the vestigial sterile cells in the pollen-grain.' In her explanation Miss Robertson assumes that the elimination of the one or two prothallial cells within the pollen-grain was accomplished in order to bring about a reduction in its specific gravity; and also that it was easier for natural selection to develop new structures in the form of winged bladders than to eliminate one or two very minute cells which have long since become functionless. Personally, I question whether the presence of one or two very minute prothallial cells within the pollen-grain would make any perceptible difference in its specific gravity. The early reduction in the number and size of the cells in the prothallium was no doubt accomplished to meet this end, thus affording an easy transportation; but I am more inclined to believe that the final elimination of the last two functionless vestigial cells in the Taxodineae, Cupressineae, and Taxineae was the result of long disuse. My belief in this regard is strengthened by a fact which Miss Robertson has evidently overlooked. It is well known that in the Pine pollen the vestigial prothallial cells become disorganized very soon after they are formed, and practically vanish *before* the pollen is discharged from the sporangium. This is also true for other members of the Abietineae. Miyake ('03) reports that in the pollen-grain of *Picea*, just before pollination, 'the disintegrating remains of the first two prothallial cells can be seen merely as two thin and darkly staining bodies between the stalk-cell and the pollen wall.' With the practical disappearance of the prothallial cells in the winged pollen, *before* the bladder-like appendages have an opportunity of producing any buoyancy, it is difficult to understand how these latter structures can be responsible for the survival of the former.

¹ See also Jeffrey, E. C., *The Comparative Anatomy and Phylogeny of the Coniferales*, Part II. Mem. Bost. Soc. Nat. Hist., 1904.

During the time that the pollen is free, there is practically no difference in the cellular contents of the grain of the winged pollen of the Abietineae and the wingless pollen of the majority of other Conifers. It thus seems to me that the correlation of the winged character of the pollen and the survival of the prothallial cells is not quite consistent. To my mind the survival of these latter structures is an indication of the primitive character of the Abietineae as a group.

The discharge of the pollen in *Cephalotaxus* begins late in March and continues for about three weeks. A considerable difference in the time of pollination was noted from year to year, which was probably due to the variable seasons in California. When the pollen is shed, the megasporangium is but a very small pointed protuberance surrounded by a well-developed integument. The micropyle is a little longer than the sporangium, as shown in Fig. 3, and remains open for some time after pollination. The microspores become lodged on the top of the megasporangium at the base of the micropyle, and here they remain; and although they enlarge considerably, there is not much further germination until the following spring. Early in May of the following year the pollen-tube becomes visible and begins its downward penetration into the tissue of the nucellus. In some cases there was observed a slight indication of the branching of the tube at this time, as shown in Fig. 4, but the main growth was always in a downward direction, directly toward the female prothallium. Previous to the penetration of the tube, the division of the generative nucleus takes place, for, as shown in Fig. 4, the body-cell is already organized, as well as the stalk-nucleus. The young tube now contains one large cell and two free nuclei, and in this respect conforms with the conditions met with at this time in most Conifers. The body-cell is distinctly oval in shape, and is surrounded by a distinct membrane. It contains a dense granular cytoplasm and a large, deeply-staining nucleus, which is quite four or five times the size of the stalk or tube-nucleus. One constant character of the body-cell is that its long axis is parallel to the long axis of the tube, and that its large nucleus is invariably found near the cell-membrane, at the side away from the stalk and tube-nuclei, as shown in Figs. 4 and 5. The stalk and tube-nuclei show no perceptible increase in size as the tube advances. They are almost identical. Indeed, it is quite impossible, at this time, to distinguish the one from the other. They lie close together, imbedded in a mass of cytoplasm close to and always in advance of the body-cell.

From this time on, the growth of the tube is comparatively rapid. The condition of the tube and its contents, shown in Fig. 4, was found on May 11, while that in Fig. 5 was found on May 21, taking just ten days for the tube to penetrate completely through the nucellar tissue and reach the archegonial chambers at the upper part of the female prothallium. The nucellar tissue, through which the tubes push their way, has a lacerated appearance,

and the cells near the tubes become thoroughly disorganized. The number of tubes varied from one to four, but more generally only one or two were found in a single nucellus. As the tube advances, the body-cell and the tube and stalk-nuclei were invariably situated near the tip, as shown in Fig. 5. When the tip of the tube has penetrated through the nucellar tissue and has reached the space between the nucellus and the archegonial chambers, it becomes quite distended, as if a great osmotic pressure were acting from within. It would seem that the sudden release from the confinement of the surrounding nucellar tissue permitted the wall of the tip of the tube to stretch, allowing the latter to take on an almost spherical or bulb-shaped form, as represented in Figs. 5 and 6. It will also be seen from these figures that the bulk of the cytoplasm is at the tip of the tube, with a thin layer lining the wall.

As soon as the tube reaches this condition the nucleus of the body-cell enlarges and prepares for division. This stage is shown in Fig. 6. I was unable to find the spindle of the dividing body-nucleus, but a careful study of the cytoplasm before and after the spindle stage revealed nothing that would indicate the presence of blepharoplasts, which occur during the division of the body-cell in the Cycads (Ikeno, '98; Webber, '97) and *Ginkgo* (Hirase, '95). The cytoplasm immediately before division was finely granular at the periphery, but distinctly fibrillar in its structure in the vicinity of the nucleus, which indicated the preliminary stages in the formation of the spindle.

The result of the division of the body-nucleus proved very interesting because it differed in a marked degree from that reported for *Taxus* (Belajeff, '93) and *Torreya taxifolia* (Coulter and Land, '05). It will be remembered that in *Taxus* this division results in the organization of two male cells of unequal size. In *Torreya taxifolia* a similar condition prevails, although Miss Robertson ('04) reports an equal division of the body nucleus in *Torreya californica*, and thus similar to the conditions found in *Cephalotaxus Fortunei* by Arnoldi ('00). Miss Robertson, however, believes that only one of the male nuclei enters the archegonium. The discovery of Juel ('04) that the body-cell in *Cupressus Gorweniana* gives rise to a complex of cells is an interesting and important one, especially as Thomson ('05) and Lopriore ('05) recently report that in *Agathis australis* and in *Araucaria* the pollen-tube may contain six or seven nuclei. In these latter cases it will be interesting to learn whether these nuclei are vegetative or generative.

In *Cephalotaxus drupacea* I was able to follow a very complete series of stages after the division of the body-cell nucleus, and my observations are these:—There is no cell-plate formed between the two male nuclei, and consequently there are not two male cells formed, but simply two large nuclei, which lie within the old membrane of the body-cell. These nuclei

are at first perfectly spherical and of equal size. As shown in Figs. 7 and 8 they eventually lie close together and soon lose their spherical form. They become slightly elongated and seem to cling or fold about each other, as shown in Fig. 8. This condition was very frequently observed just prior to their entrance into the archegonium. When the sperm-nuclei are completely organized the chromatin is in the form of uniformly small granules with two or three deeply-staining nucleoli.

Surrounding the nuclei there is very little starch present, which is in contrast to the conditions generally found at this stage in the Cupressineae, and to which Coker ('03) seems to attach some importance.

As we shall point out later, both sperm-nuclei, still folding over one another, may enter a single archegonium together, or they may separate a short distance apart just before entering. It invariably happens, however, that they both enter the same archegonium.

The history of the male gametophyte in *Cephalotaxus drupacea* covers a period of about fifteen months. In none of its phases does it show any noticeably primitive characters as compared with other Coniferales.

THE FEMALE GAMETOPHYTE.

There were two difficulties in the way which prevented the securing of a satisfactory series of preparations to show the development of the megaspores. First, because the three trees, from which all the material was obtained, did not produce flowers in abundance, I hesitated to sacrifice the later history of the gametophyte for the sake of the early megaspore stages; and second, because the hard thick tissue enveloping the young ovule prevented the rapid penetration of the killing fluid, it was almost impossible to obtain a satisfactory fixation of the cells concerned, the ovules themselves being too small to remove without injury. In one preparation I was able to make out a single large megaspore mother-cell and in another a large megaspore with two smaller cells, one above the other, which I interpret as disorganizing megaspores. As to the reduction division and the events immediately following I was unable to trace further.

It seems almost certain, however, that only one megaspore germinates, and that its early germination proceeds very slowly. As the megaspore enlarges, the usual free nuclear division takes place. In several cases six or eight free nuclei were found in the cytoplasm. A central vacuole very soon makes its appearance, which increases in size and gradually forces the cytoplasm and free nuclei to the periphery. Fig. 9 shows a longitudinal section through the young female prothallium, with the very large central vacuole and the parietal layer of cytoplasm, in which the free nuclei are distributed at more or less regular intervals. Up to this time the parietal layer of cytoplasm is but a very delicate film. As shown in Fig. 12 it is not even as wide as the diameter of the nucleus. The character of the

parietal layer was very similar to that which I have studied in *Sequoia* and *Cryptomeria*. As indicated in Fig. 10, the central vacuole increases enormously without much perceptible difference in the thickness of the parietal layer of cytoplasm, although there is a very noticeable increase in the number of nuclei, which shows that free nuclear division proceeds during the enlargement of the vacuole.

During these early stages in the formation of the prothallium considerable attention was given to the nucellar tissue which immediately surrounds it. In *Taxodium* Coker ('03) describes a distinct layer of large cells which surrounds, and persists throughout the growth of the young prothallium. It is thought that this layer takes an active part in the nourishment of the growing gametophyte, and is, therefore, considered to be a tapetum. A similar more or less differentiated tapetum has been found during the early stages in the development of the prothallium in the Abietineae and the Cupressineae, but seems to be entirely wanting in *Cephalotaxus*. It is also absent in *Taxus* and *Torreya*.

For *Cephalotaxus Fortunei* Mlle Sokolowa ('91) figures a distinct but very thin megaspore-membrane. In *C. drupacea* I was unable to detect the presence of a megaspore-membrane about the young prothallium, but during the fertilization period and early embryo stages an extremely thin membrane was observed. In two other species of *Cephalotaxus* Thomson ('05) was unable to find a trace of this membrane. He also reports that in *Taxus* the megaspore-membrane is very poorly developed. The absence of the tapetum and the extreme evanescent condition of the megaspore membrane is significant. Indeed, from this point of view alone Thomson ('05) regards the Taxeae as the most recent group of the Coniferales.

After the stage shown in Fig. 10 the parietal layer of cytoplasm increases in thickness, but the nuclei remain on the inner surface near the vacuole. The formation of the first cell-walls is not unlike that found in other Conifers. The nuclei, instead of dividing freely as heretofore, have walls formed between them, thus forming the primary prothallial cells or 'alveoli' of Mlle Sokolowa ('91). These primary cells are open on the inner side and exposed to the sap of the central vacuole. The primary cells elongate rapidly and encroach upon the vacuole. Before the complete closure of the central vacuole by the inward growth of the prothallial cells numerous oblique and cross-walls are formed. These early cross-walls are also present in *C. Fortunei* (Sokolowa, '91) and *Torreya* (Robertson, '04). Fig. 11 represents a section of the upper half of the prothallium before the ingrowing cells have met in the central region. It will be noticed that the nuclei are not only distributed on the inner exposed surface but they are quite numerous at the periphery of the prothallium. The cytoplasm also seems to be concentrated at these two definite regions. Nothing unusual was observed in the final formation of permanent prothallial tissue. From

Fig. 13 it will be seen that the cells are arranged in rows which converge towards the centre of the prothallium. By comparing this with Fig. 11 one may safely infer that these slanting rows or lines of cells were formed from the ingrowing primary cells.

THE ARCHEGONIA.

The first indications of archegonial initials were observed in material collected early in April, somewhat over a year from the time the megasporangium first made its appearance. They are generally but four in number and are clearly of superficial origin. In the earliest stages observed the initial cells were not much larger than the surrounding sterile cells, and only their granular cytoplasm and deeply-staining nuclei differentiated them from the latter. At a very early stage the initial divides, giving rise to the first neck-cell and the central cell. The primary neck-cell almost immediately divides by an anticlinal wall, and thus forms the two neck-cells. It frequently happens that one of these divides again anticlinally, so that the mature archegonium may present two or three cells in the neck in cross-section, as shown in Fig. 15. The neck-cells are easily distinguished from the other superficial cells of the prothallium by their densely granular contents. Among the Coniferales the number of neck-cells to the archegonium seems to vary considerably; even in the same species the number is not at all constant. There are never less than two, however, and that seemed to be the prevalent number in *Cephalotaxus*. Fig. 14 shows a longitudinal section of a young archegonium and the position of the two neck-cells from this view.

As soon as the neck-cells became organized, the central cell grows very rapidly, and its nucleus, which at this early stage is centrally located, increases to fully five or six times its original size. Its cytoplasm also becomes much more densely granular and stains more deeply. The four archegonia, as may be seen from Figs. 15 and 16, lie close together, with but a few layers of small prothallial cells between them, and with their necks lying freely exposed, during these early stages, at the more or less flat or convex surface of the top of the prothallium. As the archegonia continue their development, the upper sterile portion of the prothallium grows forward, leaving the archegonia behind. This growth progresses until a considerable cavity or archegonial chamber is formed immediately over each archegonium, as shown in Figs. 24 and 25. A similar depression of the archegonia occurs in *Torreya californica* (Robertson, '04), also in *Taxus* (Jäger, '99).

At a very early stage in the development of the archegonium (Fig. 14), the sterile cells immediately surrounding it become very densely granular and very soon acquire the characteristics of the sheath or jacket-cells so commonly met with throughout the Conifers. These cells increase

in number, as development progresses, and eventually each mature archegonium is completely clothed by a single layer of them. In her description of the jacket-cells for *Torreya californica*, Miss Robertson ('04) merely states that they are more rich in protoplasm than their neighbours and have conspicuous nuclei. For *Torreya taxifolia* Coulter and Land ('05) report that the jacket-cells do not make their appearance until after fertilization, and even then they are not well organized. In *Cephalotaxus* the jacket is a constant and striking feature of the archegonia from the very early stages until they become disorganized by the developing embryo. In view of the fact that Arnoldi ('00) has described and elaborately figured the passage of jacket-cell nuclei through the wall and into the egg-cytoplasm, the behaviour and character of these cells demanded considerable attention. After a careful and detailed study I was unable to detect any evidence of nuclear transference or any indications of perforations in the cell-walls through which the nuclei might pass. The nuclei of the jacket-cells, to all appearances, were perfectly normal and active until the disorganization of the archegonium by the developing embryo. The so-called 'proteid vacuoles' were observed in the egg-cytoplasm some considerable time before fertilization, and during the period of the actual fusion of the male and female nuclei they were particularly abundant. As to their origin I am unable to speak, but it seems highly improbable that they have any direct relationship to the nuclei of the jacket-cells. There is not a common jacket for the group of archegonia as occurs in *Cryptomeria* (Arnoldi, '01; Lawson, '04) and other Cupressineae, but each archegonium is surrounded by its own single layer of nourishing cells, as shown in Figs. 16 and 24. In this regard they more nearly resemble the conditions occurring in the Abietineae.

Previous to the division of the central nucleus the cytoplasm of the archegonium is finely granular and contains numerous vacuoles of various sizes. These latter become so numerous as to give a frothy appearance to the whole mass of cytoplasm. Mr. Boodle tells me he finds identically the same condition in *C. Fortunei*. A very similar appearance has also been figured for the young archegonium of *Picea* by Miyake ('03). The nucleus during this period takes up its position directly under the neck-cells as indicated in Fig. 17. The chromatin is in the spireme condition and there is generally present one and frequently two nucleoli. The nucleus remains in this position during the period of its mitosis, which gives rise to the ventral canal nucleus and egg-nucleus. In this connexion it is worthy of note that Coulter and Land ('05) were unable to detect a ventral canal-cell or nucleus in *Torreya taxifolia*, and express it as their opinion that a division of the central nucleus in this species does not occur. Miss Robertson ('04), on the other hand, reports that in *Torreya californica* she was able to detect two archegonia with

the central nucleus in process of division. In *Cephalotaxus drupacea* there can be no doubt of the division of the central nucleus and the organization of the ventral canal nucleus. I was not only able to find numerous cases showing the ventral canal nucleus (Fig. 23) but also a series of stages clearly showing the formation of the spindle.

The first indication of the division of the central nucleus is the usual enlargement and the condensation of the chromatin thread for the organization of definite chromosomes. During this period of changes within the nucleus, the cytoplasm surrounding it becomes quite dense, and kinoplasmic fibrils soon make their appearance. Fig. 18 represents a stage when the nuclear membrane is partly broken down and the fibrils of the young spindle are quite visible, although the general contour of the nucleus is still retained. Fig. 19 shows a little older stage more highly magnified. Here the elongated chromosomes are clearly defined and one of the poles of the spindle is already indicated by the drawing out and converging of the fibrils towards a definite point. I was unable to detect any differentiated areas of cytoplasm which would even suggest the presence of centrospheres, and feel confident that the spindle is formed in the manner which prevails among the Angiosperms.

Fig. 20 shows this spindle with the chromosomes at the poles, and Fig. 21 shows the same stage more highly magnified. Being the last mitosis in the history of the female gametophyte it will be interesting to compare the chromatin with that of the first mitosis of the sporophyte which immediately follows fertilization, and which is represented in Fig. 35. This, however, will be touched upon again when we describe the details of fertilization. Suffice it to say that the chromosomes are very long bodies and that these are, as nearly as could be estimated, ten in number.

As shown in Fig. 21, the continuous fibrils of the spindle persist between the two masses of chromatin for some little time. It is these fibrils which, in some Conifers, take part in the organization of the cell-plate which separates the ventral canal-cell from the egg. In *Cephalotaxus* these fibrils completely disappear without forming a membrane. A ventral canal membrane is developed in *Pinus* (Blackman, '98; Coulter and Chamberlain, '01; Ferguson, '01), *Tsuga* (Murrill, '00), *Picea* and *Abies* (Miyake, '03). As in many other Conifers, the ventral canal-cell in *Cephalotaxus* is represented only by the nucleus. As indicated in Fig. 22, the egg-nucleus and the ventral nucleus are practically identical as to size and shape. For a short time they occupy a position, one above the other, in the neck region of the archegonium. There seemed to be no special differentiation of the cytoplasm about either of them. There was not a trace of a membrane formed at any time. In fact, I feel tolerably certain none is formed. In this respect *Cephalotaxus* resembles *Juniperus* (Strasburger, '79), *Thuja* (Land, '02), *Taxodium* (Coker, '03), *Cryptomeria* (Lawson,

'04), *Sequoia* (Lawson, '04), *Podocarpus* (Coker, '03), and probably *Torreya* (Robertson, '04). It is an interesting fact that in all those forms so far investigated in regard to the ventral canal-cell, the membrane which separates the egg from the ventral nucleus has only been found in representatives of the Abietineae. This fact itself may not be of great importance, especially as there are many forms yet to be investigated. It seems to be significant, however, when we take it in connexion with the survival of the vestigial prothallial cells in the pollen-grain, for here too these vestigial structures are only found, so far as we know at present, in representatives of the Abietineae (the single exception being that of *Podocarpus*). If the retention of such evanescent vestigial structures as the prothallial cells in the pollen or the membrane of the ventral canal-cell has any phylogenetic bearing, then we have an argument in support of the primitive character of the Abietineae as a group.

Great care was taken to follow up the further history of the ventral nucleus. It remains in the neck region until the egg-nucleus moves down and takes a position in the centre of the archegonium and it then shows signs of disorganization. It completely disintegrates, and deeply-staining nuclear fragments of it were frequently observed just below the neck-cells. At the time of fertilization very little trace of it was left. By the time the ventral nucleus has become disorganized the egg-nucleus is found in the widest part of the archegonium (Fig. 27), and here, much enlarged, it remains until its fusion with the male nucleus.

For a short time before and during fertilization the cytoplasm takes on an extraordinary modification. Peculiar dense centres of cytoplasm make their appearance and, as shown in Figs. 26, 27, and 28, they are arranged in a single row which extends from the egg-nucleus to the base of the archegonium. In a general way they have the appearance of asters, for from each of them the cytoplasm is arranged in a series of radiations. These radiations do not seem to be more fibrous than the rest of the cytoplasm but are peculiar in that they extend out from these definite centres. I am unable to understand the meaning of these structures, but they are a constant and striking feature of the mature archegonia.

FERTILIZATION.

From our description above we have seen how the pollen-tube pushes its way through the nucellar tissue until it reaches the cavity in the female prothallium immediately above one of the archegonia. At this period of its development the tube contains the stalk- and tube-nuclei, lying freely in the cytoplasm, and the two sperm-nuclei, both enveloped in the old wall of the body-cell. All of these structures are at the tip of the tube, which has become greatly distended. In some cases it was found that the contents of

the tube had been discharged into one of the archegonial chambers before the neck-cells had been reached. Whether this early discharge of the tube contents is normal it is difficult to say. It may be due to the bursting of the tube by the fixing fluids, as the tip at this time has all the appearance of being under considerable osmotic pressure. In many cases examined the tip of the tube was carried between the neck-cells and into the archegonium before its contents were liberated. Fig. 25 shows the swollen end of the pollen-tube within the archegonium, and its contents are apparently still intact. As the tip of the tube approaches the neck-cells, the two sperm-nuclei may be separated from each other for a very short distance, but quite as frequently they cling close together until the interior of the archegonium has been reached. In Fig. 27 the tip of the pollen-tube is represented just outside the neck-cell; there is a space between the two sperm-nuclei, and these latter appear to be spherical in form. Arnoldi's ('00) description of fertilization in *Cephalotaxus Fortunei* is very meagre, and from his figures it is difficult to make out just what happens during these stages. In several cases I was able to observe the two male nuclei, after entering the archegonium, to be still enveloped by the membrane of the body-cell, and frequently accompanied by the tube- and stalk-nuclei. From the disturbed condition of the cytoplasm above the egg-nucleus, the discharge of the tube contents and the migration of the sperm-nucleus towards that of the egg must take place with considerable force. This is clearly illustrated in Figs. 28, 29, 30, and 31.

It seems to be a common occurrence among the Coniferales for the entire contents of the pollen-tube to enter a single archegonium. According to Blackman ('98), Coulter and Chamberlain ('01), and Ferguson ('01), the pollen-tube itself does not enter the archegonium in *Pinus*, but its entire contents are forced between the neck-cells, and are later found in the cytoplasm of the egg. Practically the same thing occurs in *Taxodium*, *Picea*, and *Abies* (Coker, '03; Miyake, '03), and a similar condition has recently been reported for *Torreya taxifolia* by Coulter and Land ('05). Exceptions to this are found in *Torreya californica* (Robertson, '04) and in *Sequoia* (Lawson, '04), where but a single sperm-nucleus enters the egg. From Belajeff's ('91) figures it seems that this may also be true for *Taxus*. In *Cryptomeria* (Lawson, '04) a single male cell enters the archegonium.

Among the Gymnosperms there seems to be a decided tendency to modify and reduce the structure of the male gametes. In the Cycads and *Ginkgo* we have the two free-swimming ciliated sperms. In the Cupressineae and Taxodineae there are two distinct male cells of equal size organized, and each is surrounded by an independent cell-wall. In *Taxus*, *Podocarpus*, and *Torreya taxifolia* there are two distinct male cells organized, but one of them is dwarfed and functionless and much smaller than the other. In the Abietineae, *Cephalotaxus* and *Torreya californica*, the two male gametes are

represented only by nuclei, since there is no cell-wall separating the one from the other. It is obvious that this reduction and modification of the male gametes of the Coniferales is working along two distinct lines. One of these is to reduce and practically eliminate one of the male cells, and the other is to reduce the structure of the gametes to nuclei. Inasmuch as the nucleus is the essential part of the gamete, the degeneration of one of the male cells must be regarded as a recent and highly specialized condition.

Returning to the conditions found in *Cephalotaxus drupacea*, it seems that very little time elapses between the entrance of the tube-contents and the dissolving of the membrane which surrounds the two sperm-nuclei within the archegonium. As soon as they are released, one of them immediately advances towards the egg-nucleus. When separated from each other they become perfectly spherical, and there is no perceptible difference in their size, shape, or in the structure of the chromatin; indeed, it is quite impossible to determine which one is destined to unite with the egg-nucleus until one of them approaches the latter. In one case I observed the two sperm-nuclei in touch with the female nucleus, but whether there was ever an actual triple fusion I was unable to determine. In a large number of preparations I was able to see but one of the sperm-nuclei functioning; the second one invariably remained behind, near the neck-cells. Arnoldi ('00) reports that the functionless sperm-nucleus in *Cephalotaxus Fortunei* may give rise to several smaller nuclei by dividing amitotically. I was unable to confirm this for *C. drupacea*. In one case, during a pro-embryo stage, I found the second sperm-nucleus in the upper part of the archegonium, apparently having undergone no division whatever.

As shown in Fig. 29, the path taken by the advancing sperm-nucleus may be followed by the vacuole-like track in its rear. From the fact that the cytoplasm has not closed in around it as it advances towards the female nucleus, its movement is evidently a rapid one. It enlarges slightly as it approaches the egg-nucleus, and at the time of contact it is about one-third the size of the latter. Fig. 30 shows the sperm-nucleus in contact with the egg-nucleus. As shown in this figure and those following, the conditions are similar to those which occur in *Pinus* and other Abietineae. The sperm-nucleus does not break through the membrane of the egg-nucleus, but forces its way into the latter without losing any of its almost spherical form. Fig. 31 shows the male nucleus almost inside of the female, while the male cytoplasm partially envelops them both. The second male nucleus may be seen in the neck region of the egg-cytoplasm. Fig. 32 shows a little later condition; here the functioning sperm-nucleus has enlarged slightly, and the chromatin in the egg-nucleus has undergone a change. Fig. 33 shows the fusing nuclei more highly magnified. It will be seen from these figures that there is a very obvious difference in the structure of the

chromatin of the sex-nuclei. The male chromatin is very finely and uniformly granular, while that of the female consists of much larger granules arranged on a network of linin.

In the difference in size of the sex-nuclei at the time of fusion, *Cephalotaxus* resembles the conditions described by Blackman ('98) and others for *Pinus* and the Abietineae in general, but differs from those found in *Sequoia* and *Cryptomeria* (Lawson, '04), where the male nucleus enlarges almost to the size of the female before actual fusion takes place.

The early stages in the formation of the first segmentation-spindle were not found. It seemed perfectly clear, however, that very little time elapses between the fusion of the chromatin masses and the organization of the first spindle. According to Blackman ('98), Chamberlain ('99), and Ferguson ('01), no resting fertilized nucleus is formed in *Pinus*. If such a nucleus is formed in *Cephalotaxus*, its resting period is a very short one. I was unable to identify the male and female chromatin after the stage shown in Fig. 33, but the first segmentation-spindle was frequently met with.

According to Coker ('03), the fusion of the male and female nuclei takes place at the base of the archegonium in *Taxodium*. Jäger ('99) reports a similar condition for *Taxus*. As shown in Fig. 34, this cannot be true in *Cephalotaxus*, for the first cleavage-spindle is always formed in the identical place where the fusion of the nuclei occurred—that is, in the middle region of the archegonium. The first cleavage-spindle, more highly magnified, is shown in Fig. 35. Here the chromosomes are at the equator, and are very long bodies, extending almost to the poles of the spindle. A repeated counting of the chromosomes convinces me that these are twenty in number in the sporophyte, which is twice the number found in the gametophyte.

THE EMBRYO.

Upon the fusion of the sperm-nucleus with that of the egg, the embryonal sporophyte begins its existence. The first cleavage-spindle is immediately organized and its formation takes place within the area occupied by the fusion-nucleus, and, as stated above, this fusion occurs in the middle region of the archegonium. The position of the first spindle is shown in Fig. 34. Its axis is not parallel to the long axis of the archegonium and may even lie at right angles to the latter. The dense granular cytoplasm brought in by the sperm-nuclei may be distinguished until after the first division, and forms a complete zone about the spindle.

The result of the first division of the fusion-nucleus is shown in Fig. 36. There are two free nuclei formed. When first organized they are some distance apart, but very soon approach each other and become enveloped in a common dense sheath of starch and other granular substances. As they lie very close together they might easily be mistaken for the sex-

nuclei in process of fusion, had these latter stages not been carefully studied beforehand (Fig. 36). As soon as they are formed these two free nuclei move towards the base of the archegonium. They travel but a very short distance in this direction, however, before the second division occurs. Each of them divides and we thus have four free nuclei in the pro-embryo. Fig. 37 represents a longitudinal section of the archegonium, at this time showing three of the four nuclei and their relative position to each other. The four of them are grouped very near to each other and are apparently not very far removed from the original position of the fusion-nucleus.

In *Taxus*, Jäger ('99) reports that the free nuclei are organized at the base of the archegonium, and a similar condition apparently occurs in *Podocarpus* (Coker, '02). In *Torreya* the free nuclei are also found at the base of the archegonium, but the formation of walls between the nuclei takes place after the second division (Robertson, '04), (Coulter and Land, '05). It will thus be seen that even in the early stages of the pro-embryo *Cephalotaxus* is unlike either of these types of the Taxaceae, and, as we shall point out, in the later stages the dissimilarity is much more striking.

Very soon after the second division, all of the starch, 'proteid vacuoles,' and other cytoplasmic granules sink to the lower or basal region of the egg, thus dividing the archegonium into two distinct and sharply differentiated parts. The less dense or upper part of the archegonium apparently becomes disorganized and functions no further, while the lower part or basal region becomes occupied with the free nuclei of the pro-embryo. Fig. 38 shows the differentiation of the pro-embryonal region at this time and that the free nuclei do not sink to the extreme base of the archegonium as they do at this stage in so many other Conifers.

A third division of the free nuclei now follows, resulting in the formation of eight of them, and still there is no indication of cell-walls separating them. Fig. 39 shows a pro-embryo at this time; six of the free nuclei are shown as well as their relative position to each other. It will also be observed that the free nuclei are arranged in tiers, two for each tier. The next division is followed by the formation of membranes between the nuclei and the organization of the first cells of the embryo. These cells are arranged in tiers, but this arrangement is not as clearly defined as that which prevails in the Abietineae and Cupressineae, where three sharply defined tiers are present. In certain stages three tiers were formed, and in others four could be distinguished. On account of irregular arrangement of the first cells that are formed and the rapid division that follows, it was difficult to say whether the fourth tier originated from a subdivision of the first tier at the tip or from the uppermost tier of nuclei. As Strasburger ('79) has pointed out, there is a group of terminal cells organized which serves as an organ of penetration. After a careful

study I am inclined to believe that these cap-cells originate from a subdivision of the first tier at the tip of the pro-embryo.

My observations on the development of the embryo proper confirm Strasburger's ('79) account of the embryo of *Cephalotaxus Fortunei*. As shown in Fig. 40 there are four distinct regions to be distinguished. The first is the penetrating cap which soon becomes thrown off, the second is the embryo itself, the third suspensors, and the fourth the rosette. Unlike most Conifers, the cells of the second tier, which constitute the embryo proper, divide very rapidly and become very numerous. There may be as many as sixteen or thirty-two cells formed before there is any perceptible elongation of the suspensors. This early merismatic activity of the embryo may explain the necessity for the development of the terminal cells into a penetrating cap. This explanation finds support in the fact that the cap-cells are thrown off very soon after the suspensors are developed. The cap-cells are in two layers. The first consists of five or six cells not much larger than the embryo-cells, while the second consists of a very large terminal cell, very much elongated and tapering to a point. Very soon after the suspensors have reached the length shown in Fig. 40 the cap-cells are discarded. The stages shown in Figs. 42 and 43 are soon after the cap-cells have been thrown off.

The suspensors now elongate very rapidly and carry the embryo down into the endosperm. The elongation of the suspensors is at first in a straight line, but as growth proceeds they become more or less twisted or curved. This curvature gradually becomes more marked until a distinct winding and twisting form is assumed. As shown in Fig. 43 the suspensors become many times the length of the archegonium, and on account of their winding growth it is quite impossible to trace them back to their point of origin from a single section.

At an early stage during the elongation of the suspensors there appeared to be an occasional budding from the main group of embryo-cells and the formation of small secondary embryos. These however were not frequently found. As a rule there is but one embryo formed from a single archegonium. The small secondary embryos when they appear do not develop very far. In one case one of these smaller embryos was observed growing towards the neck end of the archegonium, quite in the opposite direction taken by the main embryo. Even when two or three main embryos from separate archegonia develop, one of them takes the lead and grows much more rapidly than the others. Such conditions are shown in Figs. 43 and 44.

The next period in the development of the embryo is marked by the appearance of the embryonal tubes or secondary suspensors and the disorganization of the primary suspensors. The tubes or secondary suspensors were first observed soon after the stage shown in Fig. 43. The

layer of embryo-cells next to the suspensors first elongate and this is followed by the second, third, fourth, and so on in regular series. Several layers of the embryo-cells thus become very much elongated, and continue the function of the primary suspensors in carrying the tip of the embryo deeper down into the tissue of the endosperm. Fig. 44 shows an advanced stage in the development of the embryo. It will be observed from this figure, that the elongation of these embryonal cells may exceed the length of the primary suspensors and that the first of them are the longest and appear gradually shorter as they approach the growing tip of the embryo.

SUMMARY AND CONCLUSIONS.

At the time of pollination the microspore consists of two cells—the tube-cell and the generative cell. Vestigial cells or nuclei representing the vegetative prothallial cells of the male gametophyte are entirely lacking.

Pollination takes place late in March. Three or four pollen-grains become lodged on the top of the nucellus at the base of the micropyle, and while they enlarge considerably they show no further nuclear activity until the following spring.

There are usually three or four pollen-tubes produced. At the time of the penetration of the tube into the nucellar tissue, the generative nucleus divides, giving rise to the body-cell and the stalk-nucleus. The tube thus contains one large cell and two free nuclei.

It takes about ten days for the tip of the tube to reach the archegonial chamber. When this has been reached, the body-cell divides, giving rise to two sperm-nuclei. The two sperm-nuclei are of equal size, and no cell-wall is formed between them. There are therefore not two male *cells* formed, but simply two large nuclei which lie close together within the membrane of the body-cell.

Only one megaspore germinates, which gives rise to the female prothallium. The megaspore first enlarges, and this is followed by free nuclear division. A central vacuole soon makes its appearance, and by increasing in size, forces the cytoplasm and free nuclei to the wall. During the growth of the vacuole, free nuclear division proceeds at a rapid rate. The presence of a megaspore-membrane could not be detected in the young prothallium, but during the later fertilization-stages an extremely thin membrane was observed. The prothallium very soon consists of a large central vacuole and a very thin parietal layer of cytoplasm in which the free nuclei are distributed at intervals.

When the parietal layer has reached a certain thickness a series of walls are formed between the free nuclei, thus constituting the primary cells of the prothallium which are open and exposed to the sap of the vacuole on the inner side. By their inward growth the primary cells eventually close

up the space occupied by the sap of the vacuole. Before the vacuole is completely closed however, numerous cross-walls are formed.

As soon as the prothallial tissue is organized the archegonial initials make their appearance in the form of superficial cells at the apex. There are generally two, but frequently three, cells formed in the neck of the archegonium. The sterile tissue around the necks of the archegonia grows forward leaving the archegonia behind, thus forming four distinct cavities or archegonial chambers. There are four archegonia organized, and each is surrounded by a single layer of jacket-cells. There was no evidence to show any migration of the jacket-cell nuclei into the archegonium.

A distinct ventral canal-nucleus is organized, which in size, shape, and chromatin contents resembles that of the egg. It degenerates before fertilization takes place.

The entire contents of the pollen-tube enter the egg. The two sperm-nuclei are not released from the membrane of the body-cell until the interior of the archegonium has been reached. The sperm-nuclei are perfectly similar, and it is impossible to say which one will function until one of them moves towards the egg-nucleus. The second male nucleus remains behind in the neck region of the archegonium.

The fusion of the sex nuclei takes place in the middle of the archegonium, and at this time the male is about one-third the size of the female. If a resting fertilized nucleus is formed, its resting period is very brief, for the first cleavage spindle is organized immediately after fusion.

The first division of the fusion nucleus takes place in the middle of the archegonium. The first division is immediately followed by a second which results in the formation of four free nuclei in the pro-embryo.

After the second division, all of the starch 'proteid vacuoles' and other cytoplasmic granules sink to the lower part of the egg, and the archegonium thus becomes sharply differentiated into two distinct regions. The lower dense region becomes occupied by the free nuclei of the pro-embryo.

By repeated free nuclear division there are sixteen nuclei organized before the formation of cell-walls. The cells eventually become arranged in four tiers. The end tier develops into a penetrating cap, the second tier forms the embryo proper, the third the suspensors, and the uppermost the rosette.

There may be as many as sixteen or thirty-two cells in the embryo before there is any perceptible elongation of the suspensors. This early merismatic activity of the embryo-cells may account for the necessity of organizing a penetrating cap from the terminal cells. Soon after the suspensors have developed the penetrating cap is thrown off.

When the primary suspensors have reached their full length their function is continued by a series of long embryonal tubes or secondary suspensors which are developed from the proximal cells of the embryo.

Several cases of budding from the main group of embryo-cells were found, resulting in the formation of small secondary embryos, but as a rule one archegonium produces but a single embryo.

From this account of the gametophytes it becomes obvious that *Cephalotaxus* cannot be regarded as a primitive type of Conifer, although this is contrary to results obtained from certain studies on the sporophyte. From his investigations on the vascular structure of the ovule Worsdell ('00) regards *Cephalotaxus* as the most ancient of the Coniferous genera, and concludes that 'this genus forms in some measure a connecting link between Cycadaceae and Coniferae, and helps us to trace, however faintly, a fragment of the line of descent of the latter group.' By comparing the gametophytes of *Cephalotaxus* with the Cycadales and with other Coniferales I cannot accept Worsdell's view. In fact I am forced to the conclusion that this genus represents a very recent type of Conifer.

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EXPLANATION OF FIGURES IN PLATES I—IV.

Illustrating Dr. Lawson's Paper on *Cephalotaxus*.

All figures were drawn with the aid of the camera Lucida. The following oculars and objectives were used.

Figs. 1, 2, 6, 7, 8, 12, 19, 21, Zeiss oc. 1, obj. oil imm. $\frac{1}{12}$.

Figs. 3, 44, Zeiss oc. 2, obj. 3.

Figs. 4, 5, 9, 10, Zeiss oc. 3, obj. 3.

Figs. 11, 13, 14, 15, 16, 24, Zeiss oc. 1, obj. 3.

Figs. 17, 18, 20, 22, 26, 27, 28, 29, 30, 31, 32, 34, 36, 37, 38, 39, 40, 41, 42, 43, Zeiss oc. 6, obj. 3.

Fig. 25. Zeiss oc. 4, obj. 3.

Fig. 23. Zeiss oc. 4, obj. 7.

Fig. 33. Zeiss oc. 2, obj. oil imm. $\frac{1}{12}$.

Fig. 35. Zeiss oc. 6, obj. oil imm. $\frac{1}{12}$.

Fig. 1. A cross-section of a microspore some time before pollination, showing the single large nucleus and the presence of starch granules. March 7, 1904.

Fig. 2. A cross-section of a microspore just before pollination showing the tube and the generative nucleus.

Fig. 3. A longitudinal section of the ovule at the time of pollination showing the megasporangium, integument, and micropyle. April 19, 1904.

Fig. 4. A longitudinal section through the upper part of the nucellus showing a young pollen-tube containing the body-cell B., and the stalk and tube-nuclei *s. t.* May 11, 1904.

Fig. 5. The same ten days later showing a pollen-tube that has completely penetrated the nucellar tissue with the body-cell, and the stalk and tube-nuclei in the distended tip of the tube. May 21, 1904.

Fig. 6. A tip of a pollen tube more highly magnified showing the character of the cytoplasm in the tube and in the body-cell, and nucleus of the latter preparing for division. May 21, 1904.

Fig. 7. The two sperm-nuclei resulting from the division of the body-cell nucleus. They occupy the greater part of the cavity of the body-cell, the old membrane of which still envelops them. The stalk and tube-nuclei are also present. May 23, 1904.

Fig. 8. Two sperm-nuclei showing the characteristic way in which they fold over each other. May 26, 1904.

Fig. 9. A longitudinal section of a very young prothallium. The single embryo-sac consists of a thin parietal layer of cytoplasm and a large central vacuole.

Fig. 10. The same as above at a later stage.

Fig. 11. A later stage in the development of the prothallium showing the primary prothallial cells growing in and closing the vacuole.

Fig. 12. A highly magnified section of a part of the parietal layer of cytoplasm taken from the stage represented in Fig. 9.

Fig. 13. A longitudinal section through the upper region of a young prothallium showing a young archegonium with one neck-cell. April 27, 1904.

Fig. 14. A longitudinal section as above with two neck-cells in the archegonium and a single layer of jacket-cells already organized.

Fig. 15. A cross-section through the neck region of the archegonia. Two of the four show three cells in the neck, the remainder only two. May 21, 1904.

Fig. 16. A cross-section through the middle region of the archegonia showing their characteristic perfectly circular form in section and each enveloped in a single layer of jacket-cells. May 21, 1904.

Fig. 17. A longitudinal section of an archegonium with the central nucleus preparing for division. May 23, 1904.

Fig. 18. A longitudinal section of an archegonium with the central nucleus in the early stage of division. Spindle formation has begun. May 26, 1904.

Fig. 19. A more highly magnified section of the central nucleus during spindle formation. The long chromosomes are sharply defined and one pole of the spindle is already indicated by the conveying of the fibrils toward a definite point. The nuclear wall is partly broken down. May 26, 1904.

Fig. 20. A longitudinal section of an archegonium with the central nucleus in an advanced stage of mitosis, the chromosomes being at the poles of the spindle. May 21, 1904.

Fig. 21. The same spindle more highly magnified showing the character of the chromosomes and the absence of any cell-plate formation between the daughter-nuclei. May 21, 1904.

Fig. 22. A longitudinal section of an archegonium showing the ventral canal nucleus and egg-nucleus fully organized and the absence of a membrane separating them. May 30, 1904.

Fig. 23. A longitudinal section of the upper portion of an archegonium more highly magnified, illustrating the similarity of the ventral canal and the egg-nuclei and their relative position just after their organization. May 30, 1904.

Fig. 24. A longitudinal section of two mature archegonia showing their relative position to the archegonial chamber above, and the character and extent of the jacket-cells. May 23, 1904.

Fig. 25. A longitudinal section showing two pollen-tubes one of which has extended into the archegonium. May 30, 1904.

Fig. 26. A longitudinal section of a mature archegonium just before fertilization showing the peculiar cytoplasmic centres in the region below the nucleus. May 26, 1904.

Fig. 27. A longitudinal section showing the two sperm-nuclei still surrounded by the membrane of the body-cell and about to enter the archegonium. The egg-nucleus has enlarged considerably. May 26, 1904.

Fig. 28. A longitudinal section showing one sperm-nucleus within the archegonium, the second sperm-nucleus was observed in the section immediately following. They were both within the wall of the body-cell. May 28, 1904.

Fig. 29. A longitudinal section showing one of the sperm-nuclei approaching the egg-nucleus. Its path and rapid approach is indicated by the disturbed condition of the cytoplasm in its rear. May 28, 1904.

Fig. 30. A longitudinal section showing the actual union of one of the sperm-nuclei with the egg-nucleus while the second sperm-nucleus remains in the cytoplasm above. May 26, 1904.

Fig. 31. A longitudinal section showing a similar condition with the enveloping of the fusing nuclei by the male cytoplasm. The sperm-nucleus enters the egg-nucleus without losing its spherical form. May 26, 1904.

Fig. 32. A longitudinal section showing a little later stage in the fusing of the sex-nuclei; the male chromatin being in a less compact condition. May 28, 1904.

Fig. 33. A section of the fusing-nuclei more highly magnified showing the difference in the structure of the male and female chromatin. May 26, 1904.

Fig. 34. A longitudinal section showing the spindle of the division of the fusion-nucleus. The male cytoplasm forms a complete zone around the spindle, but some distance from it. May 23, 1904.

Fig. 35. A more highly magnified section of the first spindle of the sporophyte showing the increased number of chromosomes as compared with those found in the gametophyte. May 23, 1904.

Fig. 36. A longitudinal section showing the first two nuclei of the pro-embryo. They both lie embedded in a dense area of starch granules. May 23, 1904.

Fig. 37. A little later than above showing a four-nuclei stage of the pro-embryo. May 23, 1904.

Fig. 38. The same somewhat later showing all the granular substances of the cytoplasm accumulated in the lower part of the archegonium. May 23, 1904.

Fig. 39. A pro-embryo of eight free nuclei and still no trace of cell wall forming between them.

Fig. 40. An older embryo showing the young suspensors and the embryo proper at the ends with the terminal penetrating cap.

Fig. 41. The same as above but slightly older.

Fig. 42. The same still older. The cells forming the penetrating cap have been thrown off.

Fig. 43. An embryo showing the very much elongated and twining suspensors with three younger embryos above.

Fig. 44. A much older embryo. The primary suspensors can no longer be seen, but the secondary suspensors developed from the rear cells of the embryo proper are conspicuous. These secondary suspensors elongate enormously and carry the embryo at the end deep into the endosperm.

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