The Development of the Egg and Fertilization in *Pinus Strobus*.

BY

MARGARET C. FERGUSON.

—♦—
With Plates XXIII, XXIV, and XXV.
—♦—

DURING the course of the present studies, the development of the archegonium and fertilization, together with the phenomena immediately preceding and following fecundation, were first carefully worked out in *Pinus Strobus*. Later, a far less extended study was made of *Pinus austriaca*, *P. rigida*, *P. resinosa*, and *P. montana*, var. *uncinata*. A complete series of stages in the development of these species was not obtained, and, therefore, the conclusions as set forth in this paper refer, unless otherwise stated, to *Pinus Strobus*, the other species being used only by way of comparison. The methods employed in the preparation of the material for this research were fully described in my earlier paper ('01) and need not be repeated here.

These investigations were begun, at the suggestion of Prof. George F. Atkinson, in the fall of 1897, and the principal results of the work, as herein described, were read before the Botanical Society of America at its Boston meeting in 1898. At this time Blackman's ('98) excellent article, 'Fertilization and Related Phenomena in *Pinus sylvestris*,' had

[Annals of Botany, Vol. XV. No. LIX. September, 1901.]

not appeared. In general, Blackman's observations have been confirmed for *Pinus Strobis*, and other interesting phenomena, not yet recorded as occurring in plants, have been noted.

EARLY DEVELOPMENT OF THE ARCHEGONIUM.

The archegonia in *Pinus*, as is well known, do not make their appearance until the ovules have entered upon the second year of their development. In material fixed during the latter part of May, the cells comprising the upper part of the prothallium, that is, its micropylar end, are seen to be much more regular in shape and arrangement than those which constitute the rest of the endosperm. Cell-division is now taking place in any or all parts of the prothallium, but the regular, more or less rectangular cells in its upper portion are especially active, and they are comparatively rich in protoplasm. Very soon some of the uppermost of these cells cease to divide, but continue to grow, so that they are distinguished from the adjacent cells by their greater size, larger nuclei, and more vacuolate cytoplasm. These are the initial cells of the archegonia (Pl. XXIII, Figs. 1 and 2). At this time the prothallium has, as a rule, become a solid mass of tissue, although it not infrequently happens that there still remains, at its centre, an open space into which the cells of the growing endosperm have not, as yet, extended.

While the primary cell of the archegonium is still quite inconspicuous it divides, giving rise to a small, upper cell, the mother-cell of the neck, and a large, lower cell which forms the venter of the archegonium (Figs. 3 and 4). The small cell immediately divides by an anticlinal wall, and the two cells, thus formed, divide by walls that are perpendicular to the first, the resulting four cells all lying in the same plane. These constitute what may be called the normal neck in *Pinus Strobis* (Figs. 5-9 and 12). Frequently, however, two of these cells divide again, as figured by Strasburger ('69), the six cells being arranged in a single layer (Figs. 10, 25, and 39). Occasionally all four cells divide by anticlinal walls, the neck then consisting of eight cells, all of which lie in the same

plane (Pl. XXIII, Figs. 3, 11, and Pl. XXIV, Fig. 40); in rare instances, the four cells divide by periclinal walls, when the eight cells which compose the neck of the archegonium are disposed in two tiers of four cells each (Fig. 20). This last seems to be the normal condition in *Pinus austriaca* and *P. rigida*, and is that figured by Blackman for *Pinus sylvestris*.

At first the growth of the central cell is not followed by a corresponding increase in the amount of protoplasm; and hence its cytoplasm early presents a very vacuolate appearance. There may be one large, irregular, central vacuole; or delicate strands of cytoplasm may extend out from the peripheral layer of the protoplasm, forming vacuoles of various sizes. But, as the central cell continues to enlarge, its cytoplasm begins to develop more rapidly, many strands extending out into and across the vacuoles; thus the size of the vacuoles is decreased while their number is greatly increased. The central vacuole, if present, may persist for a considerable time, or it may be replaced at once by smaller vacuoles (Figs. 4-7). Gradually the cytoplasm becomes more dense; and the vacuoles, receding from the periphery of the cell, especially from its base and sides, disappear last from its upper portion (Figs. 8 and 9). When the ventral canal-cell is cut off the vacuoles have nearly or quite been replaced by a finely granular cytoplasmic reticulum, in which a greater or less number of larger, more deeply staining granules are embedded. These granules are frequently surrounded by a clear court into which the protoplasmic network has not extended. The number of the so-called proteid vacuoles is usually small at this time (Fig. 10).

The nucleus of the central cell attains full size very soon after its formation. It has a delicate, more or less interrupted, reticulum, and is characterized by a large, vacuolate nucleolus which invariably occupies a central position; one or two smaller nucleoli may also be present. This nucleus always remains close beneath the neck cells, and is more or less concave on the side towards these cells (Figs. 4-9, 13, 14, and 16). Although, as Blackman has pointed out, the vacuolate

nature of the cytoplasm renders this nucleus very liable to displacement during the early stages in the development of the archegonium, yet, in well-fixed material, it is always found in its normal position. Hirase ('95) states that certain granules, which appear in the cytoplasm just beneath the nucleus of the central cell in *Ginkgo*, have been derived from the nucleus or from its nucleolus; and Ikeno ('98) describes the nucleus of this cell in *Cycas* as giving out a granular substance during its growth period. No comparable phenomenon has been observed in connexion with the nucleus of this cell in the species of pines which I have studied; but, as above stated, the nucleus quickly reaches its mature size, and remains apparently unchanged until the incept of its division.

Very early in the history of the archegonium, the cells immediately surrounding it become differentiated from the adjacent endosperm-cells by their more regular form, the greater density of their cytoplasm, and the increase in the size of their nuclei. Thus a distinct sheath, consisting as a rule of a single layer of cells, is formed about the venter of the archegonium. No special attempt has been made to count the number of chromosomes in the nuclei of the various parts of the sporophyte and gametophyte; but whenever a nucleus was observed in which the chromosomes were particularly clear and distinct their number was always noted. In such cases twelve chromosomes have invariably been found in the nuclei of the sheath-cells. Chamberlain ('99) has found the same number in the corresponding cells of *Pinus Laricio*.

The early development of the archegonium, as just described, agrees in the main with that given by Strasburger in 1878.

As the archegonium grows, the prothallium also continues to increase in size, several layers of cells being formed above the archegonium, except over its neck-cells. Here no prothallial tissue is laid down, and hence there arises an opening in the endosperm leading from the neck-cells to the nucellar cap (Figs. 9-12). In the last stages of development, the sides

of this tubular cavity often become very closely crowded together so that the passage is obscured.

The number of archegonia in a single ovule varies in *Pinus Strobilus*, *P. rigida*, and *P. resinosa* from one to five, the most usual number being three. In *Pinus austriaca* and *P. montana*, var. *uncinata*, the number is larger, averaging about five; as many as nine have been observed in a given prothallium in *Pinus montana*, var. *uncinata*. The form of the mature egg depends largely upon the number and arrangement of the archegonia. When there are not more than two or three, as is frequently the case in *Pinus Strobilus*, they may become almost spherical in outline.

DIVISION OF THE CENTRAL CELL.

As the central cell prepares for division, the cytoplasm between its nucleus and the neck-cells is apparently resolved into fine granules, and there is a more or less pronounced condensation of the protoplasm about the lower side of the nucleus. At the same time the nucleolus disappears wholly or in part, the nuclear reticulum becomes more open and broken, and the chromatin collects or condenses at various places on the network (Fig. 14). Soon a clear court, similar to that described by Hof ('98), Fulmer ('98), Nemec ('98 and '99), Strasburger (1900) and others, makes its appearance along the lower half of the nucleus; inasmuch as this nucleus is pressed close against the neck-cells such a court does not arise along its upper side (Figs. 15 and 16). Delicate, granular threads cross this court and press against the nuclear membrane; at the same time the upper and lower surfaces of the nucleus become irregularly indented (Fig. 17). As the chromatin condenses to form the spirem, an achromatic network, as already described for the corresponding stage in the division of the generative nucleus in *Pinus*, becomes apparent in the nuclear cavity (Figs. 14-17). When the spirem is fully established it presents a beautiful moniliform appearance and the longitudinal splitting of the band becomes apparent; at this time the threads which earlier arose in the cytoplasm

seem to have been again resolved into granules (Fig. 18). Whether any of them enter the nuclear cavity and contribute to the formation of the achromatic spindle has not been definitely ascertained. The spindle, when formed, lies wholly within the nucleus. During the early metaphase of the division the nuclear membrane can still be distinguished, and clearly consists of a weft of threads (Figs. 20, 21).

When the spindle arises it is 'multipolar in an axial plane,' and thus corresponds, with slight variation, to the mitotic figure described by Duggar ('00) in the microspore of *Symplocarpus foetidus*, and by Wiegand ('99) in the microspore of *Potamogeton foliosus*. In *Pinus*, however, the upper extremities of the threads do not at first unite into groups, but remain practically free, and are closely pressed against the neck-cells (Fig. 20). The several poles, formed at the inner or lower extremity of the karyokinetic figure, soon draw together, forming a single, very sharply defined pole; or the fully developed spindle may remain more or less truncate at its lower end. Blackman describes this spindle as bluntly truncate at both extremities. I have frequently observed such a spindle during a late anaphase of the division, but this is only one of the various aspects which may be presented during metakinesis and later stages in this mitosis. The upper extremities of the achromatic spindle-fibres may never draw together at all; they may unite to form two or more poles, or they may give rise to one pole which may be blunt or very slender (Figs. 22-26). But whatever form may be assumed by this spindle during the later stages in its development, there is always formed, at an early period, a diarch spindle (Strasburger, '00) which is multipolar at one extremity and monopolar, or nearly so at the other (Figs. 20 and 21). A like condition also exists at an early stage in the division of the generative nucleus in the pines; and it is suggested that such a figure may be characteristic, at least in the higher plants, of those indirect divisions which result in the formation of nuclei of different sizes.

The chromosomes, when oriented at the nuclear plate, are

invariably in the form of U's or V's. Blackman states that they are straight rods, but he does not so figure them. The cell-plate, during the early stages in its formation, lies midway between the developing nuclei, but when the daughter-nuclei are fully formed the nucleus of the oosphere is, as a rule, farther removed from the cell-plate than is the nucleus of the ventral canal-cell. It is very early evident that the cell-plate consists of two layers. As Chamberlain ('99) has shown, the lower portion of the spindle at this time is ordinarily convex, while the part within the ventral canal-cell is concave (Figs. 30 and 32).

I was able, in several preparations similar to that illustrated in Fig. 23, to count the number of chromosomes, and twelve or thirteen were found in both groups instead of eight as counted by Dixon ('94).

THE VENTRAL CANAL-CELL.

As a rule the nucleus of the ventral canal-cell never presents a normal appearance, but shows signs of disintegration very early in its history. It is doubtful, in some cases, if a nuclear membrane is ever formed, and there are probably instances in which fusion of the chromosomes never takes place at all (Figs. 25 and 27); although Blackman, judging from such an appearance as that shown in Fig. 29, holds the latter condition to be impossible. The nuclear membrane, when present, very soon breaks down, and the chromatic substance becomes scattered throughout the cell (Figs. 30-32). This cell, immediately preceding and at the time of fertilization, ordinarily forms a deeply staining mass which lies just beneath the neck-cells and above, but in contact with, the egg (Figs. 12, 32, 40, and 42). The nucleus of the ventral canal-cell in *Pinus austriaca* has twice been observed to approximate in size that of the egg nucleus (Fig. 29), but in a study of several thousand archegonia of *Pinus Strobis* no instance has been found in which these two nuclei were similar in form; the nearest approach to a normal nucleus

that has been observed in the ventral canal-cell of this species is that shown in Fig. 28. Occasionally this cell is somewhat enlarged and is furnished with a rather scanty amount of cytoplasm in which distinct chromosomes, or chromatic figures of various forms, are embedded (Figs. 83 and 84, Pl. XXV). There seems to be a definite relation between the structure of the ventral canal-cell and the character of the upper part of the mitotic figure formed in the division of the central cell. This is clearly demonstrated on a comparison of Figs. 22-29.

The separation of the canal-cell from the cytoplasm of the oosphere, as Strasburger ('72) and Blackman ('98) have described in *Pinus*, is, I believe, due to a shrinkage of the egg-protoplasm caused by imperfect fixation; and it is possible that a similar appearance in *Cycas*, Ikeno ('98), has a like origin.

MATURATION OF THE EGG.

The egg-nucleus is no sooner formed than it begins to increase in size, becoming greatly enlarged even before the disappearance of the spindle-fibres (Figs. 27-31). As the nucleus moves toward the centre of the oosphere, threads of more or less delicacy extend, in a radial manner, from its wall into the surrounding cytoplasm. These fibres are not equally well defined in all preparations, but, whatever the degree of their prominence, they are invariably more strongly differentiated about the upper side of the nucleus, and may extend from the nucleus to the top of the egg (Figs. 32, 34, and 35).

As already stated few, if any, vacuoles persist within the venter of the archegonium at the time of the division of the central cell. Following their disappearance there arise numerous spherical bodies, the so-called proteid vacuoles. Co-ordinate with the downward movement of the egg-nucleus these bodies assume a position about the periphery of the oosphere, more especially at its base (the organic apex of Strasburger), and sides (Figs. 11, 12, and 41). Under a low

power, the cytoplasm of the mature egg appears dense and finely granular; the 'proteid vacuoles' do not seem to differ materially from the protoplasm in which they are embedded; and many deeply staining granules are scattered throughout the cell. With greater magnification, however, a very beautiful, granular reticulum becomes apparent; there is no suggestion of the alveolar structure described by Butschli ('94). At times this reticulum is everywhere crossed by short fibres, which have no definite arrangement, and are, apparently, not confined to any fixed period in the history of this cell (Fig. 30). The spheres in the outer and basal portions of the cytoplasm are resolved into very complex structures which, although they simulate the appearance of nuclei, could never be mistaken for such bodies by one familiar with cell-structures (Figs. 32 and 34).

Immediately preceding fertilization a cavity appears in the egg-cytoplasm, just beneath, or in the near vicinity of, the neck-cells (Figs. 12 and 38). In some cases this opening may not arise until the instant of fertilization. This cavity, which was thought by the earlier writers to represent the lower portion of the pollen-tube within the oosphere, has been explained by Blackman ('98) as due to the sudden inrush of the contents of the pollen-tube, and by Arnoldi ('00) in *Cephalotaxus*, as caused by the downward movement of the conjugation-nucleus. Shaw ('98) suggests that the concavity in the upper part of the egg of *Onoclea*, just prior to fertilization, may correspond to the receptive spot; and there is every evidence that in *Pinus Strobilus* this opening in the cytoplasm represents the last act of the egg in its preparation for the reception of the sperm-nucleus. If it were formed by the movement of nuclei or other bodies through the protoplasm, we should expect the cytoplasm to draw together again, as during the downward movement of the egg-nucleus; but, in reality, this opening persists throughout the entire later history of the archegonium (Pl. XXV, Figs. 77 and 78). The regular, clear outline of this cavity, together with the fact of its presence in the unfertilized as well as the fertilized

egg, warrants one in considering it a definite character of the mature oosphere.

No cytoplasmic radiations, similar to those described by Belajeff ('91) in *Taxus baccata* and by Dixon ('94) in *Pinus sylvestris*, have been observed in connexion with the fully developed egg-nucleus in any of the species of Pines which I have studied.

During the growth and downward movement of the egg-nucleus, it never presents a definite network, such as is observed in the nucleus of the ordinary resting-cell; but it is characterized at a very early stage by an open, interrupted reticulum, on which are arranged irregular granules of various sizes. This meshwork may be extremely delicate; it may assume a heavy appearance; or it may become very much interrupted and broken, many detached portions lying loose within the nuclear cavity (Figs. 27-36). Nucleoli have rarely been observed in this nucleus in *Pinus Strobus* during the first stages of its development (Figs. 27, 28, 30, and 31); but in *P. austriaca* they occasionally arise very early (Fig. 29). When the nucleus has attained considerable size, small nucleolus-like bodies, containing a single central vacuole, appear in connexion with the nuclear net; and at the same time a slightly larger nucleolus is observed in the lower part of the nucleus, usually in connexion with its membrane (Fig. 32). As the nucleus continues to grow, this nucleolus also increases in size, gradually becoming large and very vacuolate (Figs. 34-36).

When the egg-nucleus reaches maturity, it has attained huge dimensions, and its outline, depending on the form of the egg, is spherical or elliptical. The nucleolus, if demonstrable, is always found in the lower part of the nucleus; and there are usually several smaller bodies, designated in this paper as secondary nucleoli, scattered throughout the nucleus (Fig. 36). These secondary nucleoli are invariably found in connexion with the reticulum, but, as Montgomery ('98) believed, they are probably caught in, not vitally united to it. They may be present in great abundance, or they may be entirely

absent from the nucleus. The reticulum, on which the chromatic substance is disposed, presents numerous aspects, as already indicated in the description of this nucleus during its period of growth. Under very high magnification, it does not show, in normal conditions, a true granular structure; but it may present a most delicate, interrupted, granular network; or it may consist of large, irregular, diffusely staining masses which are united into an imperfect reticulum (Figs. 37*a* and 37*g*). In the latter instance the chromatic granules are either too minute to be distinguished, or they have been dissolved in the linin ground-work. The linin, always very abundant in this nucleus, may form heavy hyaline cords, on which the chromatin is collected at irregular intervals (Figs. 37*e* and 37*f*); but it more often consists of less conspicuous strands (Figs. 37*b*–37*d*). Great as the variations are in the structure of this nucleus, its chromatin has always been found, in the species of Pines studied by the writer, to exist either in the form of irregular granules of varying sizes or apparently dissolved in the linin. Such a resolving of the chromatin into nucleoli as that described by Chamberlain ('99) in *Pinus Laricio* and illustrated in his Figs. 14 and 15 has not been observed.

Whether the various appearances presented by the egg-nucleus represent normal phases in its life-history, or whether one is normal and the others artefacts resulting from the action of fixing agents, is, of course, a mere matter of conjecture. But inasmuch as these different aspects are characteristic of this nucleus during its period of growth, also after it has to all appearances reached maturity, and again at the time of its conjugation with the sperm-nucleus, it seems reasonable to conclude that all are normal and correspond to certain physiological activities which take place within the nucleus.

Strasburger ('84) described the nucleus of the oosphere in the Abietineae as being densely filled with a granular substance, which entirely obscured or masked the chromatin. This substance he called metaplasm, and virtually considered

the nucleus a vacuole filled with a nuclear sap capable of taking up or elaborating this material. Ikeno ('98) found a similar substance in the sexual nuclei in *Cycas*, and Arnoldi ('00) in *Cephalotaxus*. Blackman ('98) devoted several paragraphs to a discussion of metaplasma, as it manifested itself in the egg-nucleus of *Pinus sylvestris*. He found that it was present in the young nucleus in the form of granules, but that it later united with the chromatin to form the nuclear reticulum. Chamberlain ('99) does not recognize the presence of this substance in the egg-nucleus in *Pinus Laricio*; and there is no evidence of its existence in the sexual nuclei of the species of Pines studied by myself.

According to Wilson ('99), 'protoplasmic substances represent the active, metaplastic structures the passive elements' of the cell. During the development of the egg-nucleus in the species of Pines which have formed the basis of these studies, there is never any deposit within the normal nucleus of a granular substance; but the linin, as already stated, becomes very abundant. Just what proportion of it is active in cell-division we are unable to say. Without doubt a large part of the linin merges into the cytoplasmic network during the first segmentation of the oosphere-nucleus, but even so it cannot be classified with the passive elements of the cell.

Blackman ('98) wrote: 'The stage in which the nucleus is found in a position between the apex and the centre of the egg is rarely met with'; and Chamberlain ('99) stated 'that in over three hundred preparations, less than a dozen' show early stages in the development of the egg-nucleus. During the course of these investigations upon the Pines, more than twenty-five hundred preparations, representing several thousand archegonia, have been studied, and no developmental stage has been more frequently met with than that by which the nucleus assumes its central position in the egg. Such an appearance as that illustrated by Chamberlain in his Figs. 18 and 19 has often been observed in both the young and the mature egg-nucleus, in the conjugating nuclei, and also in the various nuclei of the proembryo. They have been wholly disregarded

in the present discussion of the maturation of the egg, for, in our material, these figures, and also Blackman's Fig. 11, would be interpreted as representing disintegration stages. Every step has been repeatedly traced from the ordinary nuclear reticulum to nuclei which can scarcely be distinguished from the surrounding cytoplasm, and then to archegonia which appear perfectly normal, except that no nuclei can be demonstrated within them. It is a well-known fact that the number of seeds derived from a Pine cone is very small in comparison with the number of ovules formed in a given cone. An examination of fresh material shows that development may cease at any point between the early stages in the formation of the ovule and the last steps in the ripening of the seed. This cessation of growth does not at once become apparent, and so cannot be avoided, in its earliest stages, when one is putting up material for cytological work. Under such conditions, it is inevitable that, with a limited amount of material, the abnormal will be interpreted for the normal.

The entire development of the archegonium in *Pinus Strobis* is passed through in about two weeks, probably not more than five days elapsing between the cutting off of the ventral canal-cell and fertilization. In *Pinus montana*, var. *uncinata*, these processes are apparently much more closely united in point of time, as the pollen-tube, in some cases, has reached the endosperm before the division of the central cell is completed (Fig. 33).

CONJUGATION OF THE SEXUAL NUCLEI.

When the time for fertilization arrives, the apex of the pollen-tube is ruptured, and almost all its contents pass into the cytoplasm of the egg. The sperm-nuclei, still surrounded by a common mass of protoplasm; the vegetative nucleus; the stalk-cell; a part of the cytoplasm from the pollen-tube; and some of the starch-grains from the male gametophyte can all be distinctly recognized in the upper part of the oosphere (Figs. 39-42). Dixon ('94) noted the passage into

the oosphere of the four nuclei of the pollen-tube, but he could not distinguish between these after their entrance into the egg. Blackman confirmed Dixon's observations as to the passage of these nuclei into the oosphere, and believed that the cytoplasm of the 'sperm-cells' passed into the egg along with the sperm-nuclei, but he was unable to demonstrate the fact. There can be no doubt that the cytoplasm of the sperm-cell enters the egg in *Pinus Strobis* (Fig. 39). This cytoplasm very soon fuses with that of the egg, and the larger sperm-nucleus moves towards the nucleus of the oosphere; the other elements from the pollen-tube remain for some time in the upper part of the ovum. There is no evidence that the sperm-nucleus increases in size after entering the oosphere; neither is there an increase in stainable substance, but, on the contrary, the nucleus loses its dense structure; and occasionally a nucleolus becomes apparent within it. (Compare the sperm-nuclei in Figs. 39 and 40 with those in Figs. 42-50.)

There is every indication that the movement within the egg of the sperm-nucleus which becomes active in fertilization is both rapid and direct. It almost invariably traverses the shortest distance between its point of entrance into the egg and the egg-nucleus. The relative position which the conjugating nuclei may occupy with reference to the major axis of the oosphere varies considerably, but always bears a definite relation to the position of the neck-cells. When these cells are directly above the centre of the oosphere, the sperm-nucleus comes into contact with the upper part of the egg-nucleus (Figs. 41, 44, 45, 48, and 50); but if the neck be excentrically placed, the sperm-nucleus will be found against one side of the oosphere-nucleus (Figs. 43, 46, and 47). It has not been observed beneath the egg-nucleus as figured by Coulter ('97) in *Pinus Laricio*. Neither is there a bulging of the egg-nucleus towards the sperm-nucleus, nor do the sexual nuclei ever approximate in size as shown in this same figure of Coulter's; but a somewhat similar figure has been observed in *Pinus Strobis* after the first division of the

'segmentation-nucleus.' The sperm-nucleus is usually described as being more dense than the egg-nucleus at the time of their conjugation, and this is sometimes found to be the case in *Pinus Strobilus*; as a rule, however, the conjugation-nuclei differ in size only, as observed by Arnoldi ('00) in *Cephalotaxus*.

Just before the sexual nuclei come into contact, the side of the egg-nucleus adjacent to the sperm-nucleus becomes slightly concave (Fig. 43). This concavity is doubtless formed under the influence of the approaching sperm-nucleus, and suggests the crater-like depression developed at an earlier period in the egg-nucleus of *Cycas* (Ikeno, '98). As noted by Blackman, the sperm-nucleus does not penetrate the membrane of the egg-nucleus; it lies in a pocket-like indentation formed in the side of the oosphere-nucleus, both nuclei then occupying the space originally filled by the egg-nucleus. The sperm-nucleus, when in contact with the nucleus of the egg, ordinarily assumes the form of a biconvex lens, but it may vary much in outline, presenting in some cases the figure of a crescent, and in others that of an ellipse. Occasionally it forms a deep, tongue-like depression in the nucleus of the oosphere (Figs. 41-50).

THE FIRST DIVISION FOLLOWING FECUNDATION.

While the sexual nuclei lie in intimate contact, but still, to all appearances, perfectly distinct, certain changes in their structure indicate that each is in the early prophase of division. The chromatin condenses or collects in irregular granules about the periphery of the sperm-nucleus, while that of the egg-nucleus is deposited just beneath the sperm-nucleus. The remainder of each nucleus is filled with a granular, achromatic reticulum of great beauty, reminding one of delicate frost-work (Fig. 51). This condition suggests an early stage of fertilization in the sea-urchin as described by Wilson ('95). Wilson thinks that the sudden increase in linin may be only apparent, resulting from the

'rapid condensation and localization of the chromatic substance'; but he is inclined to believe that 'a considerable portion of the chromatin breaks down at this time into linin.' It would appear that the prominence of the achromatic reticulum in the conjugating nuclei of *Pinus* results from both these processes. For, while there is always a large quantity of linin in the egg-nucleus and a comparatively small amount of chromatin, the size of the chromatic spirem, when formed, seems disproportionate to the entire bulk of the chromatin earlier existing in the nucleus.

The chromatin continues to separate out from these nuclei until a spirem, studded with irregular granules, lies just within the wall of the sperm-nucleus, and a similar one arises directly below in the egg-nucleus. Frequently the cytoplasm caught between the two nuclei collects into spherical masses; between these spheres of cytoplasm the membranes of the nuclei come into close contact (Fig. 52). Very soon the spirem of each nucleus becomes coiled and regularly moniliform; and the chromatic band of the sperm-nucleus takes up a position along that side of its nucleus which is nearest to the spirem formed in the egg-nucleus. At this time delicate, minutely granular threads, some of which pass from nucleus to nucleus, appear in the regions of the two chromatic spirems. The rest of the achromatic contents of these nuclei is largely transformed into long, comparatively heavy threads, which are furnished with innumerable granules. The two nuclei are still perfectly distinct; the nucleolus of the egg-nucleus may persist at this stage; and the nuclear membranes are yet present, although they are very irregular in outline, and have given way at several points (Fig. 54). The nucleolus is not always present at this time, but nucleolus-like masses, which from their position are evidently derived from the egg-nucleus, may be present as late as the telophase of the division. Delicate, granular fibres continue to arise in the regions of the two spirems; the coarser, achromatic threads of the nuclei become finer in structure, and extend in all directions towards the forming spindle; and the nuclear membranes fade entirely

out, not only along the line of contact of the two nuclei, but from their entire outer surfaces as well (Fig. 55). Blackman found that, while the chromatic portions of these nuclei remained distinct in *Pinus sylvestris*, the nuclei fused at an early stage in the prophase of the division. There is, apparently, no such fusion of the sexual nuclei in *Pinus Strobus*; but the entire membrane of each nucleus disappears during an early prophase of the mitosis, and the contents of the nuclei lie free in the cytoplasm of the egg.

The spindle-fibres continue to increase in number, becoming even more delicate in structure, and losing their granular appearance. The long, now quite delicate, but still granular, achromatic threads of the nuclei are very numerous, and many extend into the areas occupied by the chromatic spirems. They probably feed the growing spindle, some of them, doubtless, being directly transformed into spindle-fibres. The chromatic bands have now become perfectly homogeneous. Before their segmentation, the very irregular, multipolar polyarch spindle has become a multipolar diarch (Strasburger, '00) spindle; and the achromatic substance not used in spindle-formation has been gradually resolved, from the periphery of the nucleus inwards, into a granular, or finely reticulated structure, which later merges into the general cytoplasm of the egg (Figs. 56-58). When the spindle has become a true multipolar diarch, it frequently consists of two nearly equal parts, which seem to belong respectively to the male and the female nucleus (Plate XXV, Figs. 58 and 59). This appearance, however, may be only accidental, as the great irregularity which characterizes this spindle in the first stages of its formation renders such an origin of the two halves of the nearly completed spindle very problematic.

Two chromatic groups are distinctly recognized at the time of the segmentation of the spirems, and can still be clearly made out during the early development of the chromosomes (Figs. 59 and 60). When the chromosomes are oriented at the nuclear plate, the maternal and paternal elements can no longer be distinguished (Fig. 61). One

beautiful preparation was obtained at this stage in which a single section through the nuclear plate showed twenty-four entire chromosomes, and no chromosomes were found in the other sections of the series (Fig. 62). As twelve chromosomes had previously been counted in the egg-nucleus, there can be little doubt that the same number is brought into the egg by the sperm-nucleus.

The smallness of the mitotic figure in the first division following fecundation compared with the size of the egg-nucleus has been commented upon by all students of the Abietineae. This spindle may occupy various positions in the space originally filled by the egg-nucleus; but, as is clearly demonstrated by a study of its development, it invariably lies partly within the sperm- and partly within the egg-nucleus, its major axis being always parallel with the outer free surface of the sperm-nucleus. While, then, the division-figure bears a certain definite, fixed relation to the conjugating nuclei, it will be readily seen that its position may vary, depending upon the shape of the sperm-nucleus and its line of contact with the egg-nucleus, as, also, upon the plane at which the section is cut with regard to the sexual nuclei. For instance, when the sperm-nucleus is elliptical in outline, and lies in a deep depression in the egg-nucleus, as illustrated in Fig. 48, the spindle will appear to occupy the centre of the egg-nucleus. Cases like the above and many others were first satisfactorily interpreted after a careful study of something like two hundred preparations showing fertilization-stages.

During matakinesis the mitotic figure may present every variation between the extremely broad, multipolar diarch, shown in Fig. 63, and the narrow, almost bipolar spindle, illustrated in Fig. 64. It is at this time that the longitudinal splitting of the chromosomes first becomes apparent. Each chromatic element divides at the point where the spindle-fibres are attached, forming a small diamond-shaped opening. While this opening is still inconspicuous, the two halves of each chromosome become distinct throughout the entire length of the segment. Such a condition was several times

observed in the division of the 'segmentation nucleus,' but was not sketched because of lack of space. A similar stage in the division of one of the four nuclei of the proembryo is shown in Fig. 80 *b*.

In general the chromosomes at the nuclear plate are in the form of U's and V's; in rare instances they are long and somewhat coiled, and the spindle-fibres are not attached to their centres (Figs. 61-64). They pass to the poles as narrow U's (Fig. 66). Sometimes the arms of the U are pressed so closely together that the chromosomes look like longitudinally split rods. In a late anaphase of the division, the chromatic elements present a crinkled appearance, and the poles of the spindle terminate in granular areas from which threads extend into the surrounding cytoplasm. These fibres may be quite inconspicuous or they may be very prominent, frequently forming fantastic figures (Figs. 67 and 68).

A portion of the achromatic constituents of the sexual nuclei may persist in the region of the mitotic figure until the formation of the daughter-nuclei, but, as a rule, all traces of the original nuclei have disappeared at this time. Blackman finds no suggestion of a cell-wall in connexion with the first division which takes place within the oosphere. But here, again, great variation has been found in *Pinus Strobus*. The spindle either becomes constricted at the centre with little or no sign of thickening along its median line, or it may be very broad, in which case prominent thickenings occur, only to disappear at a later stage, in the line of the cell-plate (Figs. 66 and 69). As the half-chromosomes unite to form the daughter-nuclei, the poles of the spindle often become very slender, and seem to press against the forming nuclei, rendering them concave along their inner surfaces; and delicate fibres now extend from all sides of the division-figure into the cytoplasm (Fig. 69). As already indicated, there is no evidence that any portion of this spindle is derived from the cytoplasm, and it is probable that a large part, if not all, of its fibres are formed by a rearrangement of a portion of the achromatic, nuclear reticula. During the dissolution of the

mitotic figure, some of the substance of the spindle-threads probably passes into the daughter-nuclei, but the greater part of the fibres merge into the cytoplasmic reticulum and become indistinguishable from it. We have here another evidence that cytoplasmic and nuclear elements are but different expressions of the fundamental or ground substance of the cell. When the daughter-nuclei are formed, they present very beautiful, moniliform reticula, which later undergo changes very similar to those described for the growing egg-nucleus.

As recorded by Wilson ('96 and '00), Van Beneden ('83 and '87) made the very interesting discovery, confirmed by Herla ('93), that the chromosomes are formed separately in the sexual nuclei of *Ascaris megalocephala*. The differentiation of the chromatic segments takes place after the entrance of the sperm-nucleus into the egg, but before the two nuclei have come into contact. Thus the exact equivalence of the chromatic substance in the paternal and maternal nuclei was demonstrated. In the following year, Strasburger ('88) suggested that in the coming together of the nuclear threads lay the important point in fertilization. A separating-out of the chromatic elements similar to that described by Van Beneden, has since been found to occur during fertilization in many animals, but has not yet been demonstrated as of frequent occurrence in plants. In 1891, Guignard described the formation of two distinct chromatic spirems in the conjugation nucleus of *Lilium Martagon*, but he did not figure them, and his statement seems to have been overlooked by most later writers. Strasburger was able, in 1897, to distinguish the maternal and paternal portions of the fertilized nucleus in *Fucus* up to the time when the spindle was fully formed. But the results of more recent writers¹ seem to indicate that

¹ Arnoldi ('00) in *Cephalotaxus*, Caldwell ('99) in *Lemna*, Campbell ('99) in *Sparganium*, Farmer and Williams ('98) in *Fucus*, Guignard ('99) in *Lilium*, Harper ('00) in *Pyronema*, Ikeno ('98) in *Cycas*, Jäger ('99) in *Taxus*, Land ('00) in *Erigeron* and *Silphium*, Lotsy ('99) in *Gnetum*, Merrell ('00) in *Silphium*, Mottier ('98) in *Lilium*, Mottier ('00) in *Dictyota*, Nawaschin ('99) in *Lilium*, Nawaschin ('00) in *Helianthus*, *Delphinium*, and *Rudbeckia*, Osterhout ('00) in *Batrachospermum*, Shaw ('98) in *Onoclea*, Thom ('99) in *Adiantum* and *Aspidium*,

fertilization in plants consists in the fusion of two nuclei to form a resting nucleus.

Students of *Pinus*, however, have attained quite different results, and find, in this genus, phenomena very similar to those occurring during fertilization in some animals. Blackman concludes that in *Pinus sylvestris* 'no resting fertilized nucleus is ever formed,' and that 'the half-chromosomes, derived from the male and female nuclei respectively, fuse together at the poles of the first segmentation-spindle'; and Chamberlain found that two chromatic spirems were formed in *Pinus Laricio*, but, as so many stages were lacking in his material, he hesitated to draw definite conclusions. As a result of the present studies, it is found that, in *Pinus Strobis*, the chromatic portions of the sexual nuclei remain distinct until the daughter-nuclei are formed; and there is never any true fusion of the conjugating nuclei; i. e. the two nuclei do not form one individual enclosed by a definite membrane.

No centrosome or centrosome-like body has been observed in connexion with the sexual nuclei, either before or during this division. Although the centrosome as an organ has failed to be demonstrated, yet a detailed study of this mitosis makes the conclusion inevitable that the *force* initiating and controlling the division is supplied by the sperm- and not by the egg-nucleus—this force manifesting itself only in the presence of the egg-cytoplasm.

THE DIVISION OF THE TWO SEGMENTATION-NUCLEI.

The two daughter-nuclei remain in the upper part of the egg and pass through the same stages in their development as those described in the maturation of the egg-nucleus, except that, as a rule, no nucleolus becomes apparent within them. These nuclei have been observed to approximate in

Thomas ('00) in *Caltha*, Wager ('00) in *Peronospora*, and Wuiczki ('99) (according to Arnaldi and to reviews in the Bot. Zeit. and in the Journ. Roy. Micr. Soc.) in *Larix*.

size to the mature egg-nucleus; but they usually cease to grow and begin to divide while they are still much smaller than the fully developed nucleus of the oosphere. The steps in the division of these two nuclei are almost exactly like those of the first division. The nuclear reticulum is resolved into a beautiful, open and interrupted, granular, achromatic network, which is crossed by several coarsely granular, deeply staining threads. These threads, which represent the chromatic portion of the nucleus, have at first no definite arrangement; but they soon unite to form two distinct coiled or angled spirems which draw together at one side of the nucleus (Figs. 71 and 72). It is an interesting fact that these spirems are always found on the adjacent sides of the two nuclei. This position suggests that there is a certain attraction, comparable to that existing between the sexual nuclei, active between these nuclei; or the relation of the inner sides of these nuclei with the poles of the spindle, in the early stages of their formation, may have some influence upon the position which these spirems assume in the dividing nuclei.

When the two spirems, which are still roughly beaded with the chromatic substance, come to lie side by side along the inner wall of the nucleus, the nuclear wall resolves itself into a web of fibres. These threads pass into the surrounding cytoplasm and soon wholly disappear; at the same time, achromatic fibres arise in the regions of the spirems (Figs. 72 and 73). These threads quickly draw together, forming a sharply bipolar spindle on which the two now perfectly homogeneous, chromatic bands lie. The spindle does not become bipolar in some instances until after the segmentation of the spirems (Figs. 72-74). We have preparations representing a very complete series in this division, but, as it is exactly similar, especially in its later stages, to the first division, it is thought best not to multiply sketches by repeating like figures.

There can be little doubt that the two spirems formed in each of these nuclei represent the separated-out paternal and maternal chromatic substance, although, to all appearances,

the chromosomes were completely fused in the reticula of the daughter-nuclei. One is reminded by these phenomena, of Strasburger's ('92) remark, when he states that he accepts the view of a complete fusion of the segments into a network in the daughter-nuclei, and then asks if he must, therefore, conclude that the chromosomes in the following divisions do not correspond in material.

Rückert ('95) found that the chromatic portions of the conjugating nuclei in *Cyclops* not only remained distinct during the first division, but that two groups of chromosomes, representing respectively the maternal and paternal chromatic elements, could still be recognized after several divisions had taken place. In this case, however, the two groups do not fuse in the daughter-nuclei but a double nucleus is formed in the resting stage. In the same year Zoja ('95) observed that in *Ascaris* the maternal and paternal chromosomes remain entirely distinct during several successive divisions of the segmentation-nucleus. We have, then, in this second division, a further point in which fertilization-phenomena in *Pinus* correspond to those which occur within the ova of some animals. I have, as yet, made no attempt to obtain a complete series of stages in the development subsequent to the formation of the first four nuclei of the proembryo. But, from a comparison of Fig. 79 *b* with 71, and 80 *b* with 64, one is led to expect that the third division following fertilization will correspond in all points with the second. It would be interesting to determine if two chromatic groups are characteristic of all the divisions which normally occur within the oosphere of *Pinus*, and I hope to be able, shortly, to investigate this question more thoroughly.

THE FOUR SEGMENTATION-NUCLEI.

As a rule these nuclei retain their position in the upper half of the egg until their growth is completed (Fig. 76). Here, again, as in the development of the two segmentation-nuclei, the steps described for the maturation of the egg-

nucleus are repeated, except that a nucleolus does not generally become apparent within these nuclei. When they have attained full size they pass to the base of the oosphere. During their descent many fibres arise in the cytoplasm surrounding the nuclei. Some of these threads run parallel with the walls of the nuclei, while others extend out from the nuclei in a radial manner. These fibres become more prominent as the nuclei approach the base of the oosphere, and, as in the case of the egg-nucleus, they are most strongly developed along the upper sides of the nuclei (Figs. 77, 77 *b*, 78, and 78 *b*). When these nuclei have nearly reached the bottom of the egg, the nutritive spheres have almost disappeared from the cytoplasm, those which still persist being much reduced in contents (Fig. 78). After the four nuclei have arranged themselves at the 'organic apex' of the oosphere, in a plane perpendicular to the major axis of the archegonium, a marked change occurs in the cytoplasm of their immediate vicinity. It becomes dense, coarse, more or less granular, and has a great affinity for stains (Figs. 79 and 79 *b*). Blackman describes the formation of cell-walls between these nuclei, but, in the five species of pines which I have studied, cell-walls do not arise until after eight nuclei have been formed.

The early prophases, as also the meta- and ana-phases in the mitosis of the four segmentation-nuclei, correspond in every respect with the same stages in the second division following fertilization; and it is probable that the chromosomes are derived from two distinct spirems as in the first and second divisions occurring within the egg; but, as already indicated, the steps in the origin and development of the chromosomes have not been carefully traced in this division. These nuclei divide simultaneously. Chamberlain states that 'in the division of the four nuclei the spindle is extremely broad and multipolar.' I have occasionally observed such a figure during this mitosis, but here, again, great variation exists. Every transitional form may be presented during metakinesis between a multipolar diarch spindle, which fills

the entire breadth of the nucleus, and a slender bipolar spindle, such as is shown in Fig. 80 *b*. As the halves of each chromosome separate at the point where the spindle fibres are attached, the longitudinal splitting of the segments becomes evident throughout the entire length of the chromosomes (Fig. 80 *b*).

During mitosis, the deeply staining substance surrounding these nuclei condenses into large irregular masses. When the eight nuclei are formed, this deeply staining material collects about them and extends in irregular strands into the cytoplasm. Each nucleus is now surrounded by its own cytoplasm, though no cell-walls have yet been laid down (Figs. 79 *b*-80 *b*). The deeply staining, cytoplasmic substance appears to be repelled from all sides of these nuclei and is deposited in lines which indicate the position of the future cell-walls; the cell-membranes appear to arise by a direct transformation of this substance. The process seems to be very similar to that described by Farmer and Williams ('98) in *Fucus*. Mottier ('00) inclines to the view that the cell-plate is deposited in the form of a homogeneous fluid, the kinoplasm, even though its presence cannot be demonstrated, being the active agent in its deposition. The substance which is cast out, or passes out, from the region of the eight nuclei in the formation of cell-walls at the base of the oosphere in *Pinus*, has the appearance at times of a homogeneous, deeply staining fluid, in which numerous irregular granules are embedded; but there is never any evidence of its being purely fluid in nature.

The origin and disappearance of the achromatic spindle in the several divisions, which have been followed within the oosphere of *Pinus*, indicate that, as in the division of the generative cell of the pines, so here, the spindle-fibres are not the expression of a special kinoplasmic substance.

THE SO-CALLED PROTEID VACUOLES.

The true nature of the proteid vacuoles is a subject which attracted my attention very early in the course of these investi-

gations. There can be no doubt that there is an intimate relation between the sheath-cells of the pines and the substance of the egg, such as is believed to exist between the follicle-cells and the egg in animals. But the exact nature of this connexion, in *Pinus*, is not easily determined. I have rarely examined a preparation showing archegonia without studying the relation of the sheath-cells to the oosphere; and yet no entirely satisfactory evidence, because not demonstrable beyond question, of the origin and nature of the so-called proteid vacuoles has been found.

Hirase ('95) observed that the granules in the egg of *Ginkgo* were of nucleolar origin, being derived both from the nucleus of the central cell and from the nuclei of the sheath-cells. Arnoldi ('00) found that substantially the same thing was true in *Cephalotaxus*. He was not able to detect the passage of the nucleoli from the sheath-cells into the egg, but, since these granules were present on both sides of the membrane of the egg-cell, he accepted the fact of their transference. I have frequently seen a nucleolus partly without and partly within the nucleus of a sheath-cell; but in no instance could one be sure that such a condition was not the result of mechanical displacement.

Ikeno ('98) found direct evidence that the nutritive spheres in *Cycas* are of nuclear origin. But no such phenomenon as he observed in *Cycas* occurs in *Pinus*. Platner ('86) described the passage of the follicle-cells into the ovum in *Helix*, and a few other such instances have been recorded in animals. Arnoldi ('00) has recently noted a most remarkable migration of whole nuclei from the sheath-cells into the egg in several species of pines. He has observed, in a single series, as many as one hundred and fifty nuclei passing into the ovum. From the fact that Arnoldi writes *Strobilus* in a parenthesis after *Pinus Peuce*, I infer that he employs the terms as synonyms; but I find no authority for such a usage, and cannot accept his conclusions as holding good for *Pinus Strobilus*. It does not seem possible that, in a careful examination of several thousand archegonia, so obvious

a phenomenon as that described by Arnoldi could have escaped detection; and I must, therefore, conclude that it does not take place in the species of pines which I have studied.

Some interesting observations have been made, however, regarding the nature of the nucleolus of the egg-nucleus. As already indicated, this nucleolus does not arise in *Pinus Strobos* until the egg-nucleus has attained considerable size. It appears in the lower part of the nucleus as a minute, solid, spherical body; during growth a small, central vacuole appears, then other vacuoles, until, at maturity, it is completely filled with vacuoles of various sizes (Figs. 32-36). A limiting membrane is not always apparent in this nucleolus (Fig. 80, Pl. XXV); but, in some instances, there seems to be very strong evidence of such a membrane (Figs. 36, Pl. XXIV, and 90, Pl. XXIII). In Fig. 36 the nucleolar wall has been broken at one place, and a vacuole, lying near the point of rupture, has been indented along its outer surface, thus becoming crescent-shaped. Montgomery ('98) sounded a word of warning against interpreting the peripheral stratum of the ground-substance of the nucleolus as a wall-layer; and there is a possibility that, in the figures above referred to, what appears like a limiting membrane is only the outer, unmodified portion of the nucleolus.

The attitude of this nucleolus towards dyes varies much at different periods in its history. It may or may not take the safranin stain characteristic of Flemming's triple combination; it may stain intensely with gentian-violet or iron-hematoxylin (Figs. 36, Pl. XXIV, and 88, Pl. XXIII); it may show a weak reaction to these stains (Fig. 90, Pl. XXIII), or it may be absolutely unaffected by them, remaining as a hyaline or greenish-yellow structure (Fig. 89, Pl. XXIII). When the nucleolus resists the action of dyes, its nucleus is usually totally free of the secondary nucleoli, which have been described in connexion with the maturation of the egg-nucleus, and the cytoplasm of the egg is studded, to an unusual degree, with large, deeply staining granules. But

the nucleus containing a nucleolus which stains with avidity, generally contains, also, innumerable secondary nucleoli; at the same time there are comparatively few deeply staining granules in the cytoplasm of the egg.

The position of the secondary nucleoli with reference to the primary nucleolus is frequently such as to indicate that the former originate in the latter (Figs. 54, Pl. XXIV, and 88, Pl. XXIII). The only observations which would militate against such an origin are the few cases found in which the secondary nuclei seem to appear earlier than the primary nucleolus (Fig. 29). It may be that, in these cases, the primary nucleolus has not yet become differentiated in structure from the secondary nucleoli, as would evidently be true in a stage slightly younger than that shown in Fig. 32; or it may be true that the primary nucleolus is present, but fails, at this time, to stain.

The nuclei of the cells surrounding the young archegonia contain from three to five nucleoli, and one or more nucleolus-like structures may be present in the cytoplasm of these cells. Each nucleolus is surrounded by a clear court which, as Zimmermann ('96) has pointed out, is evidently not an artefact. These nucleoli may be spherical, elliptical, irregular, or long and almost dumbbell-like in outline. The ordinary cells of the prothallium do not now show nucleoli. If such bodies be present, they are small and obscured by the nuclear reticulum. At about the time of the cutting off of the ventral canal-cell, many small, nucleolus-like masses appear in the nuclei of the sheath-cells—twenty or more occurring in a single nucleus. When the egg has reached maturity, and during the later stages of its history, no nucleolus, or but one or two nucleoli, can be demonstrated in the nuclei of the sheath-cells. These nucleoli are no longer surrounded by a hyaline court, but are embedded in the chromatic network.

The nucleoli of the sheath-cells present the same attitude towards stains as does the nucleolus of the egg-nucleus. But while the nucleoli of the sheath-cells frequently stain but feebly, they rarely fail entirely to stain.

During an earlier study of the sporogeny of *Pinus Strobilus*, similar colour-reactions were observed in connexion with the nucleoli. The occurrence of unstained nucleoli in the same nucleus in which others were deeply coloured was common, especially at about the time of synapsis.

Various views are held regarding the nature of the nucleolus. We cannot here enter into a discussion of the voluminous literature dealing with the origin, function, and destiny of these structures; but a few of the many views which have been advanced may be noted.

Strasburger ('95, '97, and '00) expresses his conviction that nucleolar substance contributes to the formation of spindle-fibres. A similar view is held by Fairchild ('97), Harper ('97), Debski ('97), and other students of the Bonn Laboratory. Strasburger further holds that the nucleoli make active the spindle-forming substance in the cytoplasm, or that they enhance the activity of the kinoplasm.

Flemming ('82), Humphrey ('95), Zimmermann ('93), Sargant ('96 and '97), Duggar ('99), Mottier ('00), and many others believe that the nucleoli represent reserve supplies of chromatin. Dixon ('99) finds in them a vehicle of inheritance. Hirase ('98) thinks that they give rise to the attractive spheres; and according to Karsten ('93), Lavdowsky ('94), and Wilcox ('95) they are centrosomes.

Jordan ('93) states that 'their function is almost certainly one of nutrition either concerned in the storage or elaboration of nutritive material'; Lukjanow ('88) and Macallum ('91) consider the nucleoli to be excretory organs which are intimately related to the nutritive spheres of the egg, these spheres arising through a process of deposition from the nucleolus; and Häcker ('93) observes that the nucleolus is a contractile vacuole which absorbs proteid substances. The absorbed materials undergo a chemical change within the nucleolus, and are then periodically discharged.

Fleming ('82), Zacharias ('85), and Zimmermann ('93) ascribe to the nucleolus the dignity of a nuclear organ; and Montgomery ('98) makes the following suggestion: 'That though

the nucleolus consists of substances which stand in some relation to the nutritive processes of the nucleus, and so, at the time of its formation, may be a functionless, inert mass of matter, yet it may at later periods in the history of the resting nucleus, acquire some active function, and thus gradually come to acquire the value of a nuclear organ.'

The nucleolus of the egg-nucleus, as also the nucleoli of the sheath-cells in *Pinus Strobis*, appear to represent active portions of the cell rather than inert masses of matter. Certain aspects presented by these nucleoli are surely suggestive of plastids. The uncoloured framework of the egg-nucleus reminds one very strongly of a chlorophyll-body from which the pigment has been extracted. Yet we would not, in the present state of our knowledge, denominate them plastids. I believe, however, although the phenomena are not of such a nature as to admit of definite demonstration, that the nucleolus of the egg-nucleus, as also the nucleoli of the sheath-cells, is actively engaged in the formation of a substance which in the egg-nucleus assumes the shape of secondary nucleoli. These nucleoli become diffused throughout the nucleus, from which they pass, probably in solution, into the egg-cytoplasm. Here they are again differentiated, and by a gradual development, give rise to the 'proteid vacuoles' or nutritive spheres of the oosphere. It may be that the greater size of the egg-nucleus, in comparison with that of the sperm-nucleus, is correlated with the physiological rôle, as above suggested, which it plays in the cell.

THE FATE WITHIN THE EGG OF THE SMALLER SPERM-NUCLEUS, THE VEGETATIVE NUCLEUS, AND THE STALK-CELL.

When these nuclei first enter the oosphere there is no question as to their identity, to one who has become familiar with them before their exit from the pollen-tube (Figs. 40-42). Remnants of these cells have been found in the upper part of the egg as late as the formation of the eight-celled stage of the proembryo. The stalk-cell remains for some

time unchanged, and finally disintegrates. In so far as I have been able to determine, it assumes a more or less granular appearance, and at last blends with the cytoplasm of the egg. The vegetative nucleus undergoes various changes. Occasionally it seems to contract, becoming gradually smaller until it is no longer demonstrable; it may change little, if at all, in size, but its reticulum often becomes more prominent than when within the pollen-tube; rarely it enlarges rapidly after its entrance into the egg and develops a beautiful reticulum (Fig. 39). The sperm-nucleus not active in fertilization increases but little in size, and its network becomes less dense, resembling that of the conjugating nuclei; it may pass through the ordinary processes of disintegration; and in a few cases, not sketched for lack of space, it has been observed to divide amitotically as described by Arnoldi ('00) in *Cephalotaxus*.

But frequently the sperm-nucleus and occasionally the vegetative nucleus attempt to divide mitotically. One or two small, abortive, karyokinetic figures are not uncommon in the upper part of the egg at the time of the division of the two segmentation-nuclei. I have said, 'attempt to divide,' for no instance has been observed in which the division of these nuclei has extended beyond a late prophase. A bipolar spindle, with the chromatic segments scattered irregularly upon it, represents the most advanced stage which has been seen in the division of the smaller sperm-nucleus (Fig. 87 *b*). (A rupture was made during sectioning in the cytoplasm at one end of this spindle so that the upper pole has been separated into two.) The stalk-cell still persists at this late date (Fig. 87 *b*), and in another section of the series a second mitotic figure appears (Fig. 87). This evidently represents the vegetative nucleus. The achromatic part of the figure presents the appearance of a normal bipolar spindle; but the chromatic spirem has not become homogeneous, and probably would not have developed further. In some cases a well-developed spirem is formed in the upper part of the egg, but no achromatic threads become apparent (Fig. 85); again,

a nucleus seems to have been entirely resolved, during its disintegration, into achromatic fibres. As above stated, in no case observed did the division of these nuclei reach telokinesis; but at some point in the development prior to such a late stage, activity ceased, and disintegration of the nuclear elements took place.

It might be suggested that these division-figures result from the conjugation of the nucleus of the ventral canal-cell with the smaller sperm-nucleus. There is no evidence that such is the case, and I am convinced that they could not have had such an origin. In an examination of many hundred archegonia just before fertilization, no ventral canal-cell containing a normal nucleus has been observed. Shall we, then, conclude that, in a far less number of preparations representing stages immediately following fecundation, fifty or more instances occur in which the nucleus of the ventral canal-cell has conjugated with another nucleus and subsequently divided?

It is generally recognized, especially by cytologists on the animal side, that the stimulus to division is given, not by the egg-nucleus, but by the cytoplasm of the egg. If this be true, it is not strange that these nuclei, lying in a position where everything is most favourable for growth and development—in a medium not only rich in nutritive substances but especially adapted to incite activity in nuclei—should divide. It is a well-known fact that when several spermatozoa enter the ovum of certain animals, only one unites with the egg-nucleus, the others degenerate, or, as is frequently the case, they divide mitotically. And herein we find a further similarity between the processes attending fertilization in some animals and those taking place within the oosphere of *Pinus*.

SUMMARY.

The time at which the archegonia appear, in the species of *Pinus* which I have studied, varies somewhat, but in general they can first be detected about two weeks before fertilization. They are normally found at the micropylar end of the prothallium, and arise by the differentiation of certain of the peripheral cells. By the later growth of the female gametophyte, the mature egg is sunk to a considerable depth in the prothallial tissue, but there always remains an open channel leading from the neck-cells to the nucellar cap. The number of archegonia varies in the different species from one to nine. When the number of oospheres formed is small, they are almost spherical in outline; but this shape may be greatly modified according to the number and arrangement of the archegonia.

In *Pinus Strobus*, the typical neck of the archegonium consists of four cells, all lying in the same plane, while in *Pinus austriaca* and *P. rigida* it is made up of eight, disposed in two layers of four cells each; but there is a lack of uniformity both in the number and in the arrangement of these cells, not only in different, but in the same species.

The central cell is very vacuolate at first, its nucleus always remains close beneath the neck-cells, and is more or less concave on the side toward those cells. When the ventral canal-cell is cut off, about a week before fertilization, the vacuoles have nearly disappeared from the venter of the archegonium.

The spindle in the division of the central cell arises as a multipolar diarch one, and lies wholly within the nucleus. That portion of the mitotic figure which gives rise to the ventral canal-cell varies much in the later stages of its development; but, whatever irregularity characterizes the upper part of this spindle, it always becomes monopolar, or nearly so, at its lower, inner extremity.

The form and structure of the nucleus of the ventral canal-cell are very variable, and are correlated with the irregularities

occurring in the upper, outer portion of the achromatic spindle during the division of the central cell. There are probably instances in which no membrane is developed about this nucleus; in such cases the chromosomes never fuse to form a network. The ventral canal-cell rarely presents the appearance of a normal cell; at the time of fertilization it usually persists as a small, somewhat crescent-shaped, deeply staining body, which lies just beneath the neck-cells of the archegonium and above, but in contact with, the cytoplasm of the egg.

During the maturation of the egg, many nutritive spheres arise in its cytoplasm. At first these are irregularly scattered throughout the cell, though more prominent at its periphery; in the mature egg, they are largely confined to the peripheral portions of the lower half of the cytoplasm. It is suggested, though not definitely demonstrated, that these nutritive spheres are the products of nucleolar activity, having originated within the nucleolus of the egg and the nucleoli of the sheath-cells.

As the egg-nucleus assumes its central position in the oosphere, it increases much in size, and many fibres arise in the cytoplasm surrounding it. These threads have, in general, a radial arrangement and are most prominent along the upper side of the nucleus. The structure presented by the growing, and also by the mature, egg-nucleus may vary from a most delicate network bearing minute granules, to an interrupted, imperfect reticulum composed of large, irregular, diffusely-staining elements. These various aspects are doubtless the expressions of the different physiological activities with which this nucleus is concerned. The normal egg-nucleus has one large, vacuolate nucleolus and a variable number of small, secondary nucleoli. There is no evidence of the presence in this nucleus of a special metaplasmic substance.

The egg-cytoplasm presents a delicate reticulum, in which, at times, many fibres occur. Immediately preceding fertilization, an opening arises in this cytoplasm, just below, or in the near vicinity of, the neck-cells. This cavity is apparently formed for the reception of the sperm-cell.

At the time of fertilization, an opening is formed in the apex of the pollen-tube, and the cells of the male gametophyte which still persist, together with a portion of the cytoplasm and some of the starch of the pollen-tube, pass into the cytoplasm of the egg.

The larger sperm-nucleus escapes from the protoplasm of the sperm-cell and moves directly toward the egg-nucleus; the other nuclei from the pollen-tube may persist, in a modified form, in the upper part of the archegonium until the eight-celled stage of the proembryo; but the cytoplasm of the sperm-cell fuses at once with that of the oosphere. The stalk-cell gradually disintegrates and blends with the egg-cytoplasm. The vegetative nucleus and the smaller sperm-nucleus may share the fate of the stalk-cell, but, during the second division following fertilization, they not infrequently give rise to mitotic figures. The smaller sperm-nucleus, then, may pass through a slow process of disintegration, it may divide amitotically, or it may give rise to a karyokinetic figure of more or less definiteness.

There is no apparent change in the diameter of the sperm-nucleus after its entrance into the oosphere. At the time of conjugation, the egg-nucleus is several times larger than the sperm-nucleus, and the sperm-nucleus does not increase in size after its contact with the egg-nucleus. The inequality in the size of the sexual nuclei may be due to the difference in the size of their cells. But if, as has been suggested, the egg-nucleus functions as a manufacturer of nutritive material, may we not find in this activity a feasible explanation of its greater size? The conjugating nuclei always dissimilar in size may, or may not, be similar in structure.

The egg-nucleus becomes slightly concave on the side nearest to the approaching sperm-nucleus. This nucleus embeds itself in the side of the egg-nucleus but does not penetrate its membrane. There is never any fusion, as ordinarily understood, of these two nuclei. A chromatic spirem arises, and a prominent achromatic reticulum becomes apparent in each nucleus. Soon afterwards the nuclear mem-

branes entirely disappear. The two chromatic groups remain distinct until the nuclear plate stage.

The spindle of the first division following fecundation always lies between the conjugating nuclei and parallel with the outer, free surface of the sperm-nucleus. It is multipolar in origin and is probably derived equally from the paternal and the maternal nucleus. The spindle-fibres appear to arise by a rearrangement of the achromatic nuclear reticula and are evidently not the expression of a special kinoplasmic substance. After the formation of the daughter-nuclei, the greater portion, if not all, of these threads pass into the cytoplasmic network. During metakinesis and later stages this spindle may vary from a broad, multipolar diarch to a slender bipolar spindle. The chromosomes pass to the poles in the form of narrow U's.

No individualized centrosomes or centrospheres have been found to occur in connexion with the first division following fertilization. But the entire activity connected with this mitosis indicates that the sperm-nucleus, under the influence of the egg-cytoplasm, is the agent which initiates and controls the division.

The two segmentation-nuclei present a reticulated structure in which the paternal and the maternal chromatin appear to be completely fused. They divide in the upper part of the egg, passing through practically the same steps as those noted for the first division. The two chromatic spirems of each nucleus take up a position along the adjacent sides of the nuclei. A longitudinal splitting of the chromosomes first becomes apparent during an early stage in metakinesis.

The four segmentation-nuclei attain full size while still in the upper part of the egg. As they pass to the base of the oosphere, fibres occur in the cytoplasm similar to the threads observed around the descending egg-nucleus. The steps in the division of these nuclei have not been carefully traced, but, from the stages observed, it is probable that this mitosis does not differ from the division of the two segmentation-nuclei.

No cell-wall is laid down at the base of the oosphere, in the species of pines which we have studied, until after the eight-

celled stage of the proembryo has been reached. These eight nuclei are surrounded by a deeply staining substance which extends out from each nucleus in irregular strands. This substance finally comes to lie in the lines of the future cell-walls, and is evidently transformed into cell-wall.

The number of chromosomes in the nucleus of the ventral canal-cell, in the nuclei of the sheath-cells, and in the egg-nucleus has been found to be 12, while the mitotic figure, in the first division following fertilization, shows 24 chromatic segments.

It is interesting to note the many points of similarity between fertilization as it has been observed in *Pinus*, and the processes known to take place during fertilization in some animals. (1) The egg in *Pinus* is very large and is abundantly supplied with nutritive spheres. (2) The sexual nuclei do not fuse, and no structure which could properly be called a segmentation-nucleus is ever formed. (3) An achromatic nuclear reticulum becomes very prominent in the sexual nuclei during the prophase of division. (4) The chromatin of the sexual nuclei forms two definite groups which remain distinct until metaphase. (5) Two chromatic groups, representing respectively the paternal and the maternal chromatin, appear in the second division following fecundation; and the indications are that they will again occur in the third division and perhaps are characteristic of all the divisions which take place within the oosphere. (6) The nuclei, which enter the egg from the pollen-tube but play no part in fertilization, show a tendency to divide mitotically.

I am pleased to express my gratitude to Professor George F. Atkinson for his most helpful advice and encouragement, and his never-failing kindness, throughout the entire progress of these studies.

LIST OF PAPERS CITED.

Papers starred have not been consulted by the writer because not available to her. The references have been made from other literature in which these articles are commented upon.

- ARNOLDI, W., 1900: Beiträge zur Morphologie der Gymnospermen, iii. Embryogenie von *Cephalotaxus Fortunei*. Flora, Bd. lxxxvii, Heft 1, pp. 46-63, Taf. 1-3.
- 1900: Beiträge zur Morphologie der Gymnospermen, iv. Was sind die 'Keimbläschen' oder Hofmeisters-Körperchen in der Eizelle der Abietineen? Flora, Bd. lxxxvii, Heft 2, pp. 194-204, Taf. 6.
- BELAJEFF, W., '91: Zur Lehre von dem Pollenschlauche der Gymnospermen. Ber. d. deutsch. bot. Gesell., Bd. ix, pp. 280-287, Taf. 18.
- '93: Zur Lehre von dem Pollenschlauche der Gymnospermen. Ber. d. deutsch. bot. Gesell., Bd. xi, pp. 196-201, Taf. 12.
- *VAN BENEDEN, E., '83: Recherches sur la maturation de l'œuf, la fécondation et la division cellulaire. Archives de Biologie, Bd. iv.
- *——— et NEYT, A., '87: Nouvelles recherches sur la fécondation et la division mitotique chez l'Ascaride mégalocephale. Bull. d. l'Acad. roy. d. Belgique, 3^e sér., 7.
- BLACKMAN, VERNON H., '98: On the Cytological Features of Fertilization and Related Phenomena in *Pinus sylvestris*, L. Phil. Trans. Roy. Soc., Series B, vol. cxc, pp. 395-427, Pl. 13-14.
- BRAUER, A., '91: Ueber die Entwicklung von *Hydra*. Zeit. f. wiss. Zool., Bd. lii, pp. 169-216, Taf. 9-11.
- BUTSCHLI, O., '94: Investigations on Microscopic Foams and on Protoplasm. Eng. Transl., pp. 1-366, 12 plates.
- CALDWELL, OTIS W., '99: On the Life-History of Lemna Minor. Bot. Gaz., vol. xxvii, pp. 36-66.
- CAMPBELL, DOUGLAS H., '99: Notes on the Structure of the Embryo-sac in Sparganium and Lysichiton. Bot. Gaz., vol. xxvii, pp. 153-166, Pl. 1.
- CHAMBERLAIN, J. CHARLES, '99: Oogenesis in *Pinus Laricio*, with Remarks on Fertilization and Embryology. Bot. Gaz., vol. xxvii, pp. 268-281, Pl. 4-6.
- COULTER, J. M., '97: Notes on the Fertilization and Embryogeny of Conifers. Bot. Gaz., vol. xxiii, pp. 40-43, Pl. 6.
- DEBSKI, B., '97: Beobachtungen über Kerntheilung bei *Chara fragilis*. Jahrb. f. wiss. Bot., Bd. xxx, pp. 227-249, Taf. 9-10.
- DIXON, H. H., '94: Fertilization of *Pinus sylvestris*. Ann. Bot., vol. viii, pp. 21-34, Pl. 3-5.
- '99: The Possible Function of the Nucleolus in Heredity. Ann. Bot., vol. xiii, pp. 269-278.
- DUGGAR, B. M., '99: On the Development of the Pollen Grain and the Embryo-sac in *Bignonia venusta*. Bull. Torrey Bot. Club, vol. xxvi, pp. 89-105, Pl. 352-354.
- 1900: Studies in the Development of the Pollen Grain in *Symplocarpus foetidus* and *Peltandra undulata*. Bot. Gaz., vol. xxix, pp. 81-98, Pl. 1-2.

- FAIRCHILD, D. G., '97: Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum*. Jahrb. f. wiss. Bot., Bd. xxx, pp. 285-297, Taf. 13-14.
- FARMER, J. B., and WILLIAMS, J. LL., '98: Contributions to our Knowledge of the Fucaceae: their Life-History and Cytology. Phil. Trans. Roy. Soc., Series B, vol. cxc, pp. 623-647, Pl. 19-24.
- FERGUSON, MARGARET C., '01: The Development of the Pollen Tube and the Division of the Generative Nucleus in Certain Species of Pines. Ann. Bot., vol. xv, pp. 193-223, Pl. 12-14.
- *FLEMMING, W.: Zellsubstanz, Kern- und Zelltheilung. Leipzig, 1882.
- FULMER, EDWARD L., '98: Cell-Division in Pine Seedlings. Bot. Gaz., vol. xxvi, pp. 239-246, Pl. 23-24.
- GUIGNARD, L., '91: Nouvelles Études sur la Fécondation. Ann. des Sci. Nat., Bot., 7^e série, tom. xiv, pp. 163-296, Pl. 9-18.
- GUIGNARD, L., '99: Sur les Anthérozoïdes et la double copulation sexuelle chez les végétaux angiospermes. Académie des Sciences, Comptes Rendus, tom. cxxviii. An abstract, Flora, 1899, p. 35.
- HÄCKER, V., '93: Das Keimbläschen, seine Elemente und Lage. Arch. f. mikr. Anat., Bd. xlii, pp. 279-318, Taf. 19-21.
- HARPER, R. A., '97: Kerntheilung und freie Zellbildung im Ascus. Jahrb. f. wiss. Bot., Bd. xxx, Heft 3, pp. 249-285, Taf. 11-12.
- 1900: Sexual Reproduction in *Pyronema confluens* and the morphology of the Ascocarp. Ann. Bot., vol. xiv, pp. 321-401, Pl. 19-21.
- HERLA, VICTOR, '93: Étude des variations de la mitose chez l'*Ascaride mégalocéphale*. Arch. de Biologie, tom. xiii, pp. 423-515, Pl. 15-19.
- HIRASE, S., '95: Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Journ. Coll. Sci., Univ. Imp. Tokio, vol. viii, pp. 307-322, Pl. 31-32.
- '98: Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Journ. Coll. Sci., Univ. Imp. Tokio, vol. xii, pp. 103-150, Pl. 7-9.
- HOF, A. C., '98: Histologische Studien an Vegetationspunkten. Bot. Centr., Bd. lxxvi, pp. 65-69, 113-118, 166-171, 221-226, Taf. 3-4.
- HUMPHREY, J. E., '95: On some Constituents of the Cell. Ann. Bot., vol. ix, pp. 361-381, Pl. 20.
- IKENO, S., '97: Vorläufige Mittheilung über die Spermatozoiden bei *Cycas revoluta*. Bot. Centr., Bd. lxix, pp. 1-3.
- '98, 1: Zur Kenntniss des sogenannten centrosomähnlichen Körpers im Pollenschlauche der Cycadeen. Flora, Bd. lxxxv, pp. 15-18.
- '98, 2: Untersuchungen über die Entwicklung der Geschlechtsorgane und den Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. f. wiss. Bot., Bd. xxxii, pp. 557-602, Taf. 8-10.
- JÄGER, L., '99: Beiträge zur Kenntniss der Endospermibildung und zur Embryologie von *Taxus baccata*, L. Flora, Bd. lxxxvi, pp. 240-288, Taf. 15-19.
- JORDAN, E. O., '93: The Habits and Development of the Newt (*Diemyctylus viridescens*). Journ. of Morph., vol. viii, pp. 269-367, Pl. 14-18.
- KARSTEN, G., '93: Ueber Beziehungen der Nucleolen zu den Centrosomen bei *Psilotum triquetrum*. Ber. d. deutsch. bot. Gesell., Bd. xi, pp. 555-563, Taf. 29.
- LAND, W. J. G., 1900: Double Fertilization in Compositae. Bot. Gaz., vol. xxx, pp. 252-260, Pl. 15-16.
- LAVDOWSKY, M., '94: Von der Entstehung der chromatischen und achromatischen

- Substanzen in den tierischen und pflanzlichen Zellen. Merkel und Bonnet's Anat. Hefte, Bd. iv, Heft 13, pp. 353-447, Taf. 26-31.
- *LOTSY, J., '99: Contributions to the Life-History of the Genus *Gnetum*. Ann. du Jardin Bot. de Buitenzorg, Series 2, vol. i, pp. 46-114, Pl. 2-11.
- LUKJANOW, S. M., '88: Ueber eine eigenthümliche Kolbenform des Kernkörperchens. Arch. f. mikr. Anat., Bd. xxxii, pp. 474-478, Taf. 19.
- MACALLUM, A. B., '91: Contributions to the Morphology and Physiology of the Cell. Trans. Canadian Inst., vol. i, pp. 247-278, Pl. 2.
- MERRELL, W. D., 1900: A Contribution to the Life-History of *Silphium*. Bot. Gaz., vol. xxix, pp. 99-133, Pl. 3-8.
- MONTGOMERY, TH. H., '98: Comparative Cytological Studies, with special reference to the Morphology of the Nucleolus. Journ. of Morph., vol. xv, pp. 265-582, Pl. 21-30.
- MOTTIER, D. M., '98: Ueber das Verhalten der Kerne bei der Entwicklung des Embryosacks und der Vorgänge bei der Befruchtung. Jahrb. für wiss. Bot., Bd. xxxi, pp. 125-158, Taf. 2-3.
- 1900: Nuclear and Cell-Division in *Dictyota dichotoma*. Ann. Bot., vol. xiv, pp. 163-192, Pl. 11.
- NAWASCHIN, S., '99: Neue Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*. Bot. Centr., Bd. lxxvii.
- 1900: Ueber die Befruchtungsvorgänge bei einigen Dicotyledonen. Ber. d. deutsch. Bot. Gesell., Bd. xviii, pp. 224-230, Taf. 9.
- NEMEC, B., '98: Ueber die Ausbildung der achromatischen Kerntheilungsfigur im vegetativen und Fortpflanzungsgewebe. Bot. Centr., Bd. lxxiv, pp. 1-5. Eight text figures.
- '99, 1: Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*. Jahrb. f. wiss. Bot., Bd. xxxiii, pp. 313-336, Taf. 3.
- '99, 2: Ueber Kern- und Zelltheilung bei *Solanum tuberosum*. Flora, Bd. lxxxvi, pp. 214-227. Nine text figures.
- '99, 3: Zur Physiologie der Kern- und Zelltheilung. Bot. Centr., Bd. lxxvii, pp. 241-252. Seven text figures.
- OSTERHOUT, W. J. V., 1900: Befruchtung bei *Batrachospermum*. Flora, Bd. lxxxvii, pp. 109-115, Taf. 5.
- PLATNER, G., '86: Zur Bildung der Geschlechtsprodukte bei den Pulmonaten. Arch. für mikr. Anat., Bd. xxvi, pp. 599-622, Taf. 29-30.
- RÜCKERT, J., '95: Ueber das Selbständigbleiben der väterlichen und mütterlichen Kernsubstanz während der ersten Entwicklung des befruchteten Cyclops-Eies. Arch. f. mikr. Anat., Bd. xlv, pp. 339-370, Taf. 21-22.
- SARGANT, ETHEL, '96: The Formation of the Sexual Nuclei in *Lilium Martagon*. I. Oögenesis. Ann. Bot., vol. x, pp. 445-478, Pl. 22-23.
- '97: The Formation of the Sexual Nuclei in *Lilium Martagon*. II. Spermatogenesis. Ann. Bot., vol. xi, pp. 187-224, Pl. 10-11.
- SHAW, WALTER H., '98: The Fertilization of Onoclea. Ann. Bot., vol. xii, pp. 261-285, Pl. 19.
- STRASBURGER, E., '69: Die Befruchtung bei den Coniferen. Jena. Taf. 3.
- '72: Die Coniferen und die Gnetaceen. Jena. pp. 274-298, Taf. 5-17.
- '78: Ueber Befruchtung und Zelltheilung. Jena.
- '84: Neue Untersuchungen über den Befruchtungsvorgang bei

- den Phanerogamen als Grundlage für eine Theorie der Zeugung. Jena, pp. 1-176, Taf. 1 and 2.
- STRASBURGER, E., '88: Ueber Kern- und Zelltheilung im Pflanzenreich. Jena.
- '92: Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. pp. 1-156, Charts 1-3.
- '95: Karyokinetische Probleme. Jahrb. f. wiss. Bot., Bd. xxviii, pp. 151-204, Taf. 2-3.
- '97, 1: Ueber Cytoplasmastrukturen, Kern- und Zelltheilung. Jahrb. f. wiss. Bot., Bd. xxx, Heft 3, pp. 375-405.
- '97, 2: Ueber Befruchtung. Jahrb. f. wiss. Bot., Bd. xxx, Heft 3, pp. 406-422.
- '97, 3: Kerntheilung und Befruchtung bei *Fucus*. Jahrb. f. wiss. Bot., Bd. xxx, Heft 3, pp. 351-374, Taf. 17-18.
- '00: Ueber Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Jena, pp. 1-224, Taf. 1-4.
- THOM, CHARLES, '99: The Process of Fertilization in *Aspidium* and *Adiantum*. Trans. Acad. Sci. St. Louis, vol. ix, no. 8, pp. 285-314, Pl. 36-38.
- THOMAS, ETHEL N., 1900: Double Fertilization in a Dicotyledon, *Caltha palustris*. Ann. Bot., vol. xiv, pp. 527-535, Pl. 30.
- WAGER, HAROLD, '00: On the Fertilization of *Peronospora parasitica*. Ann. Bot., vol. xiv, pp. 163-180.
- WEBBER, H. J., '97: Notes on the Fecundation of *Zamia* and the Pollen-tube Apparatus of *Ginkgo*. Bot. Gaz., vol. xxiv, pp. 225-236, Pl. 10.
- WIEGAND, KARL M., '99: Development of the Microsporangium and Microspores in *Convallaria* and *Potamogeton*. Bot. Gaz., vol. xxviii, pp. 328-359, Pl. 24-25.
- WILCOX, E. V., '95: Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. Bull. Mus. Comp. Zool. Harvard Univ., vol. xxvii, pp. 3-34, Pl. 1-5.
- WILSON, E. B., '95: Archoplasm, Centrosome, and Chromatin in the Sea-Urchin's Egg. Journ. of Morph., vol. xi, pp. 443-479. Twelve prototypes and text figures.
- '96: The Cell in Development and Inheritance. New York.
- '99: The Structure of Protoplasm. Science, vol. x, No. 237, pp. 33-44. Nine figures.
- '00: The Cell in Development and Inheritance. New York. pp. 1-481, Figs. 1-194.
- *WUICIZKI, '99: Ueber die Befruchtung bei den Coniferen. Warschau (Russisch). An abstract, Bot. Zeit., Bd. lviii, pt. 2, p. 39, 1900, also Journ. Roy. Micr. Soc., August, 1900.
- ZACHARIAS, '85: Ueber den Nucleolus. Bot. Zeit., Bd. xliii, pp. 257-265, 273-283, 289-295.
- *ZIMMERMANN, A., '93: Ueber das Verhalten der Nucleolen während der Karyokinese. Beitr. zur Morph. und Phys. der Pflanzenzelle, Bd. ii.
- '96: Die Morphologie und Physiologie des pflanzlichen Zellkernes. Eine kritische Literaturstudie. Jena.
- ZOJA, R., '95: Sulla indipendenza della cromatina paterna e materna nel nucleo delle cellule embrionali. Anat. Anz., Bd. xi, pp. 289-293. Three text figures.

EXPLANATION OF FIGURES IN PLATES XXIII,
XXIV, AND XXV.

Illustrating Miss Ferguson's paper on Fertilization in *Pinus Strobus*.

All figures were drawn with the aid of the Abbé camera lucida, projection 14.3 cm. A Zeiss microscope was used, the lenses being combined as follows: Figs. 8-12, 41, 75-82 with ocular 4 and 16 mm. objective; Figs. 1-7, 38-40, 42-50 *b*, with comp. ocular 12 and objective as before; Fig. 33 with ocular 4 and 4 mm. objective; Figs. 13-32, 34-36, 51-74, 77 *b*, 78 *b*, 79 *b*, 80 *b*, 81 *b*, and 82 *b* with ocular 4 and 2 mm. homo. immer. objective; Figs. 37 *a*-37 *g*, and 88-90 with comp. ocular 12 and 2 mm. homo. immer. objective. The amount of magnification accompanies the description of each figure. The drawings were reproduced without reduction. The lettering of the figures is to be interpreted thus: prothallium (*pr.*), ventral canal-cell (*v. c.*), neck-cells (*n. c.*), egg-nucleus (*e. n.*), sperm-nucleus (*s. n.*), sperm-cytoplasm (*s. c.*), vegetative nucleus (*v. n.*), stalk-cell (*st. c.*), starch (*sh.*), cytoplasm from pollen-tube (*c. p. t.*), nutritive spheres (*n. s.*), primary nucleolus (*py. ns.*), secondary nucleolus (*sy. ns.*).

All the figures, with the exception of Figs. 33 and 80 *b*, have been given their normal position, as nearly as it was possible to do so, on the plates, i. e. they are so placed that the primary axis of the ovule would be parallel with longer axis of the half-plates; and the portion of a figure nearest to the micropylar end of the ovule is always toward the top of the plate. Unless otherwise stated the figures represent stages in the development of *Pinus Strobus*.

PLATE XXIII.

Figs. 1-7. Stages in the early development of the archegonium. $\times 160$.

Figs. 8-11. Later stages in the growth of the archegonium. $\times 70$.

Fig. 12. Mature archegonium. $\times 70$.

Fig. 13. Nucleus of the central cell shortly before its division. $\times 540$.

Figs. 14-18. Prophases in the division of the central cell. $\times 540$. Nos. 16 and 18, *Pinus austriaca*.

Fig. 19. Cross-section of the ventral canal-cell soon after segmentation of the chromatic spirem. $\times 540$.

Figs. 20-21. Disappearance of the nuclear membrane and establishment of the achromatic spindle. $\times 540$.

Figs. 22-29. Separation of the half-chromosomes and formation of the daughter-nuclei. $\times 540$. Nos. 24 and 29, *Pinus austriaca*. These figures show some of the variations occurring in the mitotic figure for this division, and the corresponding variations in the structure of the nucleus of the ventral canal-cell.

Figs. 30-32. Later history of the ventral canal-cell and early stages in the development of the egg-nucleus. $\times 540$.

Fig. 33. Division of the central cell, showing also the lower portion of a pollen-tube which has already reached the endosperm. $\times 230$. *Pinus montana*, var. *uncinata*.

Fig. 88. The primary nucleolus from a mature egg-nucleus with secondary nucleoli clustered about it, and evidently being formed by it. The primary nucleolus has a great affinity for stains at this time. $\times 1200$.

Fig. 89. The framework of the primary nucleolus from a mature egg-nucleus. $\times 1200$. This nucleolus has remained of a light greenish-yellow colour after treatment with Flemming's triple stain.

Fig. 90. The primary nucleolus of a mature egg-nucleus. This nucleolus shows a weak reaction towards dyes, and apparently has an outer limiting membrane. $\times 1200$.

PLATE XXIV.

Figs. 34-35. Later stages in the downward movement and growth of the egg-nucleus. $\times 540$.

Fig. 36. Mature egg-nucleus. $\times 540$.

Fig. 37, a-g. Portions of the reticulum from different mature egg-nuclei, showing the variations which may exist in the structure of this nucleus. $\times 1200$.

Fig. 38. The upper part of an archegonium, showing cavity formed in the cytoplasm just prior to fertilization. $\times 160$.

Fig. 39. The upper part of an archegonium just after the entrance into the egg of the elements from the pollen-tube. $\times 160$.

Fig. 40. A slightly later stage. The cytoplasm of the sperm-cell has already fused with the cytoplasm of the egg. $\times 160$.

Fig. 41. An entire archegonium showing the sexual nuclei in contact, and, above them, the various elements which have come into the egg from the pollen-tube. $\times 70$.

Fig. 42. The upper part of an archegonium in the same stage as the above. $\times 160$.

Fig. 43. The sexual nuclei just before coming into contact. Note depression in egg-nucleus. $\times 160$.

Figs. 44-50. Various appearances presented by the conjugating nuclei. It will be borne in mind that these figures are so placed that the major axis of the archegonia in which they occur would lie parallel with the longer axis of the half plate. $\times 160$.

Fig. 50 b. Another section through the egg-nucleus shown in number 50. There is a greater difference in the size of the conjugating nuclei than would appear in figure 50. $\times 160$.

Fig. 51. An early prophase in the first division following fecundation. $\times 540$.

Fig. 52. A slightly later stage. The cytoplasm caught between the two nuclei has collected into spherical masses. $\times 540$.

Fig. 53. A still later stage in the formation of the two chromatic spirems. $\times 540$.

Fig. 54. A still later stage in which the paternal chromatic spirem has taken up a position near the maternal spirem, and a few delicate achromatic threads have made their appearance in the neighbourhood of these spirems. The nuclear membranes are still present, but have broken down at several points. $\times 540$.

Fig. 55. A later stage. The nuclear membrane has entirely disappeared; the spindle fibres have increased in number; and the rearrangement of the achromatic, nuclear reticula into granular threads is very apparent. $\times 540$.

Figs. 56-57. More advanced stages in the formation of the spindle. $\times 540$.

PLATE XXV.

Fig. 58. The spindle fully established; the two chromatic spirems still perfectly distinct. $\times 540$.

Fig. 59. The two spirems after segmentation; the two halves of the spindle seem to indicate the maternal and the paternal portions of the mitotic figure. $\times 540$.

Fig. 60. Early stage in the formation of the chromosomes. The chromatic elements still occur in two distinct groups. $\times 540$.

Fig. 61. The chromosomes being oriented at the nuclear plate. The distinction between paternal and maternal elements no longer evident. $\times 540$.

Fig. 62. A cross-section through the nuclear plate just before the splitting of the chromosomes; twenty-four segments are distinctly shown. $\times 540$.

Figs. 63-65. Some of the aspects presented by this mitotic figure during metaphase. $\times 540$.

Fig. 66. An anaphase of the mitosis.

Fig. 67. A late anaphase of the division; the poles terminate in granular areas from which delicate threads extend into the cytoplasm; some of the nucleolar substance from the egg-nucleus still persists. $\times 540$.

Fig. 68. One end of a spindle in the same stage as the above; the fibres which radiate from the polar region of the spindle are very abundant and stain deeply. $\times 540$.

Fig. 69. One aspect presented by the karyokinetic figure in the telophase of this division. $\times 540$.

Fig. 70. The two segmentation-nuclei fully formed. $\times 540$.

Fig. 71. One of the two segmentation-nuclei in an early prophase of division. $\times 540$.

Figs. 72-73 *b*. Later stages in the second division, showing two chromatic spirems. $\times 540$.

Fig. 74. A still later stage. $\times 540$.

Fig. 75. An entire archegonium showing the position of the two segmentation-nuclei during division. $\times 70$.

Fig. 76. An archegonium showing the original position of the four segmentation-nuclei. $\times 70$.

Fig. 77. The same after the nuclei have begun their downward movement. $\times 70$.

Fig. 78. The same after the nuclei have almost reached the base of the oosphere. $\times 70$.

Fig. 78 *b*. A portion of number 78, showing details in nuclear structure, and fibres in the surrounding cytoplasm. $\times 540$.

Fig. 79. The lower part of an archegonium after the four nuclei have arranged themselves at the 'organic apex' of the oosphere. $\times 70$.

Fig. 79 *b*. A portion of the above; the nucleus is in the early prophase of division; the cytoplasm surrounding the nucleus has become dense and deeply staining. $\times 540$.

Fig. 80. The basal portion of an egg; the four segmentation-nuclei are in a metaphase of the mitosis. $\times 70$.

Fig. 80 *b*. A part of the same showing details. $\times 540$.

Fig. 81. A portion of the lower part of an oosphere after the formation of the eight nuclei of the proembryo. $\times 70$.

Fig. 81 *b*. A part of the above giving details. No cell-walls have as yet been formed. $\times 540$.

Fig. 82. A somewhat later stage than number 81. $\times 70$.

Fig. 82 *b*. A portion of the above, showing cell-walls in the process of formation. $\times 540$.

Figs. 83-84. Some of the aspects presented by the ventral canal-cell. $\times 540$.

Figs. 85-86. Figures occurring in the upper part of archegonia during the division of the segmentation-nuclei. $\times 540$.

Figs. 87-87 *b*. Figures occurring in the upper part of an archegonium at the time of the second division following fertilization: Fig. 87 represents the vegetative nucleus, and the karyokinetic structure in Fig. 87 *b* the smaller sperm-nucleus; just above this figure is the stalk-cell. $\times 540$.

CORNELL UNIVERSITY.

Oct. 1, 1900.

M.C. Ferguson, del.

M. C. Ferguson, del.

University Press, Oxford.

M.C. Ferguson, del.

58.

59.

60.

61.

62.

63.

65.

64.

66.

68.

67.

70.

71.

69.

M. C. Ferguson, del.

FERGUSON — FERTILIZATION IN *PINUS STROBUS*.

University Press, Oxford.

Ferguson, Margaret Clay. 1901. "The development of the egg and fertilization in *Pinus strobus*." *Annals of botany* 15, 435–479.

<https://doi.org/10.1093/oxfordjournals.aob.a088827>.

View This Item Online: <https://www.biodiversitylibrary.org/item/236929>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a088827>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318631>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.