# A morphological Study of Juniperus communis var. depressa<sup>1</sup>)

by

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#### With 4 figures and plates VIII-XVII.

During recent years considerable attention has been given by a large number of investigators to the morphology of different gymnosperms, so that at the present time the number of living genera which have not been worked on to some extent is comparatively small Exhaustive studies, however, have as yet been made in only a few cases. At the time when the present work was projected (1906) practically the only accounts dealing with the sporangia, gametophytes, fertilization, and embryo in any one gymnosperm were those of Coker (1903b) on Taxodium, and of Miss Ferguson (1904) on *Pinus*. Norén (1904) and Sludsky (1905) had already published short preliminary papers dealing with the morphology of Juniperus communis, but no investigations of this species based on American material had ever been made. Since there seemed to be considerable doubt among systematic botanists as to the identity of the European J. communis with the American var. depressa, it was thought worth while to make a detailed morphological investigation of the American form.

#### Material and methods.

The writer is indebted to Dr. P. A. Rydberg for confirming his determination of the plants from which material for study was collected. Dr. Rydberg writes: "It is *Juniperus communis depressa* of Gray's Manual. This has been referred both to *J. communis* and *J. sibirica* (= *J. nana*). I think, though, that it is distinct from the typical forms of both species, but I am in doubt whether it should be referred as a variety to either of them or be regarded as a distinct species." It will be seen by a comparison of the

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writer's results with those of Norén that the cytological phenomena in the American variety resemble in most important respects those in the European form.

Collections of material were made near New Haven, Connecticut during the years 1906, 1907, and 1908. The frequency of collections and the manner of treating the material varied somewhat. Staminate cones and first year ovules were collected at intervals of from two days to a week and placed directly in the killing fluid. The younger second year ovules were collected every one or two days and split lengthwise with a sharp knife before treating with reagents. After the development of a firm integument it was found necessary to carefully dissect out the nucellus before immersing it in the killing fluid. Collections were made daily after the appearance of archegonia. In the later stages material was killed and fixed in the field, but in the earlier stages this was usually done in the laboratory. Material brought indoors and kept in water for not more than a week appears to develop normally. Various killing and fixing solutions were tried, and excellent results were obtained with the modification of Flemming's chrom-acet-osmic fluid re-commended by Mottier (1897). The writer expresses his thanks to Prof. Alexander W. Evans, at whose suggestion this work was undertaken, for his helpful criticism and kind advice.

# The microsporangium.

Development of the microspore mother cells. - Hofmeister (1848) describes the microsporangium of Pinus maritima as being in the spore mother cell condition in November, and this seems to be the first published reference to the development of this structure among the Gymnosperms. Goebel (1881) makes several observations in regard to the development of the microsporangia in this group, and in Pinus traces back the archesporium to a single hypodermal cell. In Thuja (Biota), which he cites as typical of the *Cupresseae*, he finds that the archesporium is likewise of hypodermal origin, but whether it arises from one or several cells he does not make out. He also makes the state-ment, later upheld by Coulter and Chamberlain (1901) and other recent writers, that in all important respects the development of the microsporangium in the Gymnosperms follows the same course as in the eusporangiate pteridophytes. In the cycad Stangeria Lang (1897) finds that the sporogenous cells "are derived by periclinal division from cells of the sub-epidermal layer". A similar origin is reported by Coker (1903b) in Taxodium, and Coulter and Land (1905) are of the opinion that in Torreya the archesporium arises from a hypodermal cell.

The staminate cones of *Juniperus communis* var. *depressa* become recognizable late in the summer and pass the winter in a more or less rudimentary condition. In material collected November 28<sup>th</sup> it is in most cases impossible to distinguish vegetative leaves from sporophylls, and many of the latter do not begin to

develop until the following season. The earliest stages to be described are from material collected February 11<sup>th</sup> and kept indoors for two days. The differentiation of the tapetum and inner layer of the sporangium wall from the sporogenous complex takes place during the first week in April. A prolonged period of growth then follows, accompanied by cell division, culminating about May 1 <sup>st</sup> in the formation of the microspore mother cells.

During the winter the lower part of the sporophyll is composed of meristematic cells of uniform size and structure, while the upper portion is already occupied by large vacuolate cells containing numerous compound starch grains. The latter are conspicuous in many of the vegetative cells of the sporophyll until shortly before pollination. The archesporium first becomes recognizable as a plate of radially elongated hypodermal cells, four to six in number when viewed in longitudinal section (fig. 5). Structurally there is little to distinguish them from the adjacent sterile cells save their somewhat denser cytoplasm. This layer soon divides by periclinal walls (fig. 6), and the archesporium continues to increase in size by the growth and division of its cells without any differentiation becoming apparent. By the time the stage shown in fig. 7 is reached the archesporium is without difficulty distinguishable from the vegetative tissue of the sporophyll through the denser contents of its cells, while the base of the sporophyll has begun to bulge slightly in the region where the microsporangium is being developed.

After a considerable mass of cells has been formed those of the outer layer divide, and two layers of tabular-shaped cells are cut off, which completely enclose a central mass of cells (fig. 8). The latter may be termed the primary sporogenous cells, since after a period of further growth and division they give rise to the spore mother cells. The inner of the two enveloping layers becomes the tapetum, while the outer layer develops into what is usually termed the inner layer of the sporangium wall. This latter layer, if viewed in a not quite mature sporangium, does appear to be a part of the wall, since, after the breaking down of their contents, the crushed cells form a thin layer which is closely appressed to the outer layer of the wall (figs. 11, 12). Morphologically, however, it is more closely correlated to the tapetum, both tissues being derived in the manner described above from the archesporium, while the outer layer of the wall has quite a different origin, as will be seen presently. Moreover the subsequent history of this layer shows that physiologically also it is a sort of accessory tapetum, contributing nourishment to the developing spores and eventually disappearing entirely. An analogous case has been noted in *Stangeria* (Lang 1897) where the inner layers of the wall are disorganized during the growth of the microsporangium, and similar conditions are found in other Gymnosperms.

Soon after the differentiation of the tapetum and the inner wall layer from the sporogenous cells the majority of the sterile cells in the lower part of the sporophyll become vacuolate. Certain

epidermal cells, however, which are in direct contact with the wall layer remain in an embryonic condition, dividing by anticlinal walls to keep pace with the now rapidly enlarging sporogenous mass, and these eventually give rise to the one-layered wall of the mature sporangium. At first these cells are scarcely distinguishable from those of the archesporium, but they may be recognized by their position and prismatic shape. This derivation of all or a part of the sporangium wall directly from the epidermal layer is of universal occurence among gymnosperms and is in marked contrast to the conditions found among the Angiosperms where the epidermis plays no important part in the formation of the wall of the mature pollen sac.

The microsporangium now increases enormously in size, rounds out, and becomes easily visible to the naked eye. The sporogenous cells continue to grow and divide actively until shortly before synapsis, but cell division in the two wall layers soon stops. The cells of the outer wall layer for a time enlarge and develop large vacuoles which become filled with some amorphous substance, presumably of a resinous character, while the cells of the inner wall layer cease to grow and, in consequence of the pressure from within the sporangium, become stretched and flattened, and their nuclei and cytoplasm disorganize, so that by the conclusion of the reduction division the cells are usually crushed and structureless.

The cells of the tapetum continue to divide by anticlinal walls until shortly before the maturity of the mother cells. Growth still goes on in them after the cessation of division, vacuoles appear, and they may be distinguished from the sporogenous cells by their paler color and smaller nuclei. The tapetum attains its maximum development during the cell divisions which precede spore formation and disorganizes rapidly after the formation of the pollen, disappearing entirely before pollination (figs. 9-12).

During the brief period of rest which precedes synapsis (fig. 9), the mother cell complex appears as a compact mass of thin-walled, polyhedral cells with large nuclei and dense protoplasmic contents. As noted by Norén (1907), the mother cells are comparatively small — about 22  $\mu$  in diameter — and their nuclei rarely exceed 12  $\mu$ . It is therefore difficult and sometimes impossible to follow with certainty many of the complex nuclear phenomena which characterize the heterotypic division. For research along these lines *Larix* and *Pinus* have been the favorite objects among the conifers, for in these genera the nuclei of the pollen mother cells are from 25 to 35  $\mu$  in diameter.

The nucleus of the mother cell (fig. 15) possesses a well defined membrane and a reticulum consisting of deeply staining, knot-like masses connected by inconspicuous, lightly staining threads, the whole forming an irregularly anastomosing network. Concerning the nucleus at this time Norén (1907, p. 8) writes: "Wir sehen die Chromatinkörner zu sehr kleinen, scharf begrenzten Körpern gruppiert, die meistens paarweise auf kurzen Lininfäden sitzend hauptsächlich in der Peripherie des Kerns gelegen sind." If such

a pairing of the chromatic material is present in the nucleus of the resting mother cell of the form under consideration the writer is unable to make it out. Nor is it possible to recognize in the nucleus, either at this time or during the subsequent stages leading up to the formation of microspores, a sharp distinction between chromatin and linin, such as is present in the nucleus of the body cell just before its division. In view, however, of the small size of these nuclei this fact by no means precludes the probability that such a differentiation may be present.

One or more large nucleoli are conspicuous in the nucleus. The cytoplasm is uniformly distributed through the cell, and in most cases presents merely a granular appearance; but in well prepared sections a distinct alveolar structure is evident, the contour of the non-staining "alveolar spheres" being outlined by the thickly scattered microsomes (see Wilson 1900, p. 46). Minute, glistening granules of starch are present in the cytoplasm, and these are more or less prominent during the succeeding stages of development.

Tetrad division. — The behavior of the chromatic material in the nuclei of the microspore mother cells during the early prophase of the heterotypic division is very similar to that described by Allen (1905) in the pollen mother cells of Lilium canadense. As the nucleus approaches synapsis the finer threads of the chromatic network are drawn in, the material of the knots shows a tendency to become distributed along the coarser connecting strands, and the reticular structure gradually gives way to a series of more or less united, lumpy bands. At the same time it becomes evident, as shown in figs. 16 and 17, that in many places separate strands or masses of chromatin have come to lie alongside one another. Sometimes they approach so close that they appear to have united, but again it can be made out that there are two distinct strands lying side by side. Coincident with these changes the chromatin begins to aggregate toward the central region of the nucleus, and a heaping up of the nuclear materials begins. This continues until a mass of tightly interwoven strands is formed which moves to one side of the nuclear cavity and assumes a position close to the nuclear membrane (fig. 18). Just what conditions prevail during synapsis it is impossible to make out with certainty. Occasionally, however, threads are found which project out from the almost homogeneous mass, and from the appearance of these, and in view of the preceeding and subsequent phenomena, it may be inferred that there is present at first a series of double threads, and that toward the close of synapsis these coalesce and unite end to end to form a single bivalent spirem. There appears to be no rule as to which side of the nuclear cavity the synaptic mass occupies (fig. 10). The nucleoli in most cases protrude from the chromatic ball and occasionally are entirely extruded, but they never exhibit the flattened appearance described by Allen (1905).

Considerable theoretical interest is attached to the view held by several eminent cytologists, among them Berghs (1904, 1905), Allen (1905), and Overton (1905), that there is a presynaptic

pairing of the maternal and paternal elements of the chromatin, followed during synapsis by their apparent fusion. Miss Ferguson (1904) and Lewis (1908) are unable to find such procedure in the microspore mother cells of *Pinus* and *Thuja* but maintain that there the reticulum of the nucleus contracts directly into the synaptic condition. Lewis finds occasional paired threads but attaches no special significance to them. The writer's observations, however, have led to the conclusion that in the species under discussion a presynaptic pairing does take place.

The nuclei remain in synapsis for five or six days. Emerging from it they undergo the heterotypic and homotypic divisions, and within a week the tetrads have formed and have given rise to the microspores. The ripening of the pollen takes about two weeks, and pollination occurs about May 25<sup>th</sup>.

Toward the close of synapsis the tightly interwoven threads begin to loosen up, and the mass of chromatic material gradually resolves itself into a slender, somewhat roughened spirem of uniform thickness, which twists and coils throughout the nuclear cavity (figs. 19, 20). The nucleus at this period bears a striking resemblance to the homologous stage as figured in Drosera by Berghs (1905, fig. 8), and no trace of the bivalent nature of the spirem can be detected. As soon as the spirem has become completely disentangled it begins to shorten and thicken, and it can be seen in places that different portions of the thread are in contact (fig. 21). Miss Ferguson (1904) finds that in Pinus neighboring portions of the thread meet and fuse, and Norén (1907) states that in J. communis the conditions are similar. That parts of the spirem should touch one another is unavoidable, but a careful examination has led to the conclusion that there is no actual coalescence at the points of contact and that the chromosomes arise, not from an "incompletely reticulated structure", but from a continuous spirem.

A longitudinal splitting of the thick spirem soon becomes apparent, and there seems to be no reason for not assuming that the adjacent threads thus formed represent merely a reseparation of the chromatic elements which have been actually or seemingly fused during synapsis. Almost simultaneously transverse breaks appear, so that the spirem becomes divided up into a number of double segments which are arranged end to end (fig. 22). Whether the number of segments formed at this time corresponds with the reduced number of chromosomes found during the later stages of the heterotypic division cannot be stated with certainty, but subsequent steps make this conclusion seem extremely probable. The end to end arrangement of the segments is of short duration, and the nucleus rapidly passes into the condition known as 'diakinesis', the segments becoming variously oriented but for the most part lying in the peripheral region of the nucleus (fig. 23). These segments now represent the bivalent chromosomes of the heterotypic division, and their double nature can readily be made out in well stained preparations. The two halves usually lie side by side

and in close contact, either parallel or twisted around each other corkscrew-fashion, but frequently they are in contact at only one or two points.

Coincident with these nuclear metamorphoses, changes have been taking place in the cells themselves. They have lost their angular shape and compact arrangement and have become more or less separated from one another (fig. 11). This separation is apparently brought about by the dissolution of the middle lamella of the mother cell in the manner described by Strasburger (1882). Allen (1905) reports an interesting process in connection with the separation of the pollen mother cells in *Lilium canadense*. There the wall of the mother cell dissolves, leaving the cells protected only by a plasma membrane, and subsequently an entirely new wall is developed about the cell. In Juniperus, immediately after the separation of the mother cells, the wall appears very thin, but at all times places may be found where the cytoplasm has shrunk away from the surrounding membrane, which would hardly be true if the cells were surrounded merely by a differentiated layer of cytoplasm.

Up to this time the cytoplasm has exhibited a fairly uniform alveolar structure. As the nucleus emerges from synapsis, however, a fiber-like, radial arrangement of the cytoplasmic materials is faintly discernible (fig. 20), similar to that figured in the pollen mother cells of *Lilium* by Mottier (1897, fig. 3), and in *Larix* by Allen (1903, fig. 6). Shortly before diakinesis there is evident a concentration of the cytoplasm toward the nucleus, and by the time the segmentation of the spirem has taken place the nucleus is enclosed by a dense granular layer, while toward the periphery of the cell the cytoplasm has become thin and stains very lightly (fig. 21). This dense layer of cytoplasm around the nucleus doubtless represents the 'felted' structure described in Larix by Belajeff (1894), Strasburger (1895), and Allen (1903), but on account of the small size of the cells it is impossible to ascertain definitely its nature here. Occasional indications are seen, however, of a fibrous structure like that described by the above mentioned authors.

The nuclear membrane, which until now has been sharply outlined, disappears soon after the formation of the 'felt', and the dense cytoplasmic layer appears to press into the nuclear cavity. The origin of the spindle fibers, however, cannot be clearly demonstrated. Osterhout (1897) and Mottier (1897) find that during diakinesis, in the species which they studied, fibers of unmistakably nuclear origin become attached to the chromosomes. But a careful study of the chromosomes of *Juniperus* before the dissolution of the nuclear membrane has failed to reveal any such structure, and it is impossible to state whether the spindle fibers are derived entirely from the cytoplasm or whether they originate partly within the nucleus.

The spindle when first formed is multipolar (fig. 24), but rapidly resolves itself into a multipolar diarch (fig. 25), and

before the initiation of metakinesis becomes distinctly bipolar (fig. 26). The chromosomes, which after the disappearance of the nuclear membrane have become crowded together, are rapidly oriented toward the nuclear plane and assume a position approximately perpendicular to the axis of the spindle. In this position it is possible, in sections cut perpendicular to the spindle axis, to count twelve chromosomes, which represent the reduced number characteristic of the gametophyte (fig. 27). In the megaspore mother cell of the European J. communis Norén (1907) finds only eleven chromosomes. It is not surprising, however, that there should be a divergence of opinion on this point, since not only are the objects under consideration very minute, but even in sections which are favorably cut and stained the chromosomes are crowded and usually appear to overlie and merge into one another, so that their accurate enumeration is extremely difficult, nor is it impossible that the number in var. depressa differs from that in the European form. In view of Norén's results a careful recount was made, but although cases are frequent where only eleven can be seen, in some instances at any rate it was determined with considerable certainty that there are twelve chromosomes.

The chromosomes are short and thick, but as they lie in the equatorial plane the various L, V, X, Y, and O forms characteristic of the heterotypic division are often recognizable. The spindle fibers are attached at or near the inner ends of the daughter chromosomes, and their separation begins at this point. During the anaphase the daughter chromosomes are drawn apart and as they approach the poles it can be seen that they have undergone a second longitudinal fission (fig. 28). Just what significance should be attached to this fact is doubtful, but in the light of subsequent events it seems hardly possible that the segments formed at this time are homologous with those of the homotypic division, as is maintained by Strasburger (1900). At the poles the chromosomes come together and form a seemingly homogeneous, lumpy mass in which no structure can be made out (fig. 29).

The reconstruction of the daughter nuclei is apparently brought about without the formation of a definite spirem, in a manner similar to that described by Lawson (1903) and elaborated by Gregoire and Wygaerts (1904). Lacunae appear within the mass of chromosomes, increase in size, flow together, and force the surrounding chromatin outward (fig. 30). Alveoli then divide the chromatin into smaller masses, until there is formed eventually a well developed resting nucleus in which the identity of the individual chromosomes is completely lost (fig. 31). Miss Ferguson (1904) finds that in *Pinus* the chromosomes unite end to end and form a definite spirem which in turn gives rise to the reticulum of the daughter nuclei. There is no stage, however, in the organization of the daughter nuclei in *J. communis* where a continuous spirem can be recognized. The nuclei are spheroidal, somewhat broader than long and slightly flattened on the equatorial surface.

They possess a rather coarse reticulum and have a distinct nucleolus. The development of resting nuclei at the close of the heterotypic division has been described in *Larix* (Strasburger 1900), *Taxodium* (Coker 1903b), *Pinus* (Ferguson 1904), *Juniperus* (Norén 1907), and *Thuja* (Lewis 1908).

During the telophase (fig. 30) the spindle fibers are conspicuous as a barrel-shaped mass extending between the two daughter nuclei, and in many cases thickenings of the fibers in the equatorial plane give indications of an ephemeral cell plate, such as occurs in *Larix* (Strasburger 1895), and occasionally in *Pinus* (Ferguson 1904). Coker (1903b) reports that in *Taxodium* a cell plate is produced which extends entirely across the cell and persists throughout the second division. In *Juniperus*, however, the cell plate entirely disappears before the initiation of the homotypic division.

In the brief period of rest which succeeds the heterotypic division the daughter nuclei grow considerably in size, but within a short time the finer meshes of the reticulum are again drawn in, and a coarse, more or less anastomosing structure is produced which gives rise to the chromosomes of the homotypic division. If a spirem is formed, it is poorly defined and irregular. The spindles of the first division now disappear, new spindles are formed, and the nuclear membrane is lost sight of. As in the previous division, the chromosomes become oriented at the equator (fig. 32), longitudinal splitting takes place, and the granddaughter chromosomes are drawn toward their respective poles (figs. 33, 34) where the organization of the granddaughter nuclei is brought about. The nuclei thus formed are at first very small — about 7  $\mu$  in diameter — with a reticulum consisting of granular masses of chromatin united by finer strands.

As noted by Coulter and Chamberlain (1901) in *Pinus*, the division just described may be either tetrahedral or bilateral (figs. 33, 34). The former method is more prevalent, but bilateral division is of frequent occurence. The successive development of different microspore mother cells in the same sporangium, observed by these authors and others in various conifers, is very noticeable in *Juniperus*, especially during the stages intervening between synapsis and the formation of tetrads where one step follows another with comparative rapidity (fig. 11). The sporangia at the apex of a cone are invariably further along in their development than those at the base.

Development of the microspores. — Miss Ferguson (1904) has made some very interesting observations in connection with the formation of the microspores in *Pinus*. She finds that "the wall of the microspore mother-cell increases markedly in thickness, and its protoplasmic contents is separated into four compartments by prominent cross walls which are continuous with the inner portion of the mother-wall. The microspores are then developed, each in its own particular chamber of the mother-cell." Norén (1907) reports that in *J. communis* a division of the

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protoplasm of the mother cell into four parts takes place, and the cytoplasm which surrounds each of the nuclei encloses itself with a thin cell membrane, but that no thick walls, like those described by Miss Ferguson, are formed. From the following account, however, it will be seen that the conditions in var. *depressa* are remarkably similar to those in *Pinus*. That these features have not been more frequently observed may be accounted for by the fact already pointed out by Miss Ferguson that the thickened walls usually react very poorly to stains.

The structure of the microspore mother cell at the completion of the homotypic division is shown by fig. 35. The four nuclei of the tetrad are connected with one another in all directions by kinoplasmic fibers. Some of these represent connecting fibers which were in the spindle of the homotypic division, and which have not yet disappeared. Others, however, arise de novo from the cyto-plasm, and to all appearances these are exactly like the true spindle. fibers. The fibers soon become thickened at the equatorial planes, giving rise to the six cell plates which separate the four nuclei (fig. 36). Another important change, however, has already taken place in the mother cell. During the anaphase of the homotypic division there begins to appear in the peripheral region a transparent, homogeneous layer which stains bluish with gentian violet (figs. 33-35). This becomes thicker, and when seen in section appears as a broad band entirely surrounding the protoplasmic contents of the cell. That this layer represents merely the swollen inner wall of the mother cell seems doubtful, for it appears very intimately related to the enclosed protoplasm, which by this time has shrunk away from the mother wall. It seems rather to be an entirely new wall developed in anticipation of the formation of microspores. The cell plates now split, and walls are laid down in the usual manner (fig. 37). These walls then apparently swell, become continuous with the thick enveloping wall (fig. 38) and assume the same reaction toward stains. In this manner the cells of the tetrad become separated from one another and from the outside by a thick, transparent wall, while the original mother cell membrane, already very thin and distorted, gradually disorganizes. The cells of the tetrad, while still enclosed by this thickened layer, begin to form the walls which are present in the mature microspore. These are sometimes evident in places where an enclosed cell has become separated from the thickened wall. The pollen grains are eventually liberated by the dissolution or breaking down of the enveloping wall.

There can be little doubt that the peculiar structure described here, and found in *Pinus*, is in the nature of a cell wall and is not merely a viscid or liquid substance, and an examination of living material of *J. communis* fully confirms the observations made on fixed and stained preparations. Yet while in *Pinus* it "is left behind as a definitely outlined wall after the escape of the spores" and "the empty mother-cell with its four chambers is often met with", in *Juniperus* it is not of such a permanent character

but disintegrates rapidly after the release of the pollen grains, conveying the impression that it is composed of some gelatinous substance. Incidentally it may be noted that similar thickened walls have been observed by the writer in the living pollen mother cells of *Picea excelsa*, and, as Miss Ferguson intimates, it is probable that these structures are of general occurrence throughout the higher plants.

During the time which intervenes between the formation of tetrads and pollination the spores round out and increase in size at the expense of protoplasmic material derived mainly from the disorganizing tapetum and inner layer of the sporangium wall. The mature pollen grain (fig. 39) is approximately spherical, about 20  $\mu$  in diameter, and possesses a thin, transparent intine and a slightly thickened, pigmented exine. The cell contains a single centrally situated nucleus, and scattered through the cytoplasm, often nearly obscuring the nucleus, are an abundance of large, usually compound starch grains.

In regard to the presence of prothallial cells, such as occur in many Gymnosperms, it may be stated with almost positive certainty that these structures are never formed in the Cupresseae. That this is true of Juniperus was first pointed out by Strasburger (1892). The material studied by the writer shows two peculiar features which at first sight suggest the presence of a prothallial cell. It is observed that shortly after their formation many of the spores contain two nuclei. A thorough search, however, fails to reveal any indication of mitotic figures, such as would be expected had both nuclei arisen in the pollen grain by the division of the primary nucleus. Moreover the two are apparently alike in every respect, while the spores themselves are invariably somewhat larger than the normal and are frequently constricted at the middle. In view of these facts it seems probable that the binucleate condition is brought about by the failure of a cell wall to develop between two nuclei of a tetrad, and that a consequent twinning of two microspores results. In the same sporangia with these abnormal pollen grains are found others in which there appears what might easily be mistaken for a disorganizing prothallial cell, - a dark, lenticular structure, seemingly closely appressed to the wall of the cell. But there is little doubt that this is merely an artificial condition induced by a slight invagination of the spore wall.

The primary nucleus of *Juniperus* undergoes no division until after pollination. In this respect *Taxus*, *Cupressus* and *Juniperus* differ from all other Conifers, so far as is known.

The wall of the mature microsporangium is composed of a single layer of prismatic cells which are elongated in a direction parallel to the slit by which the anther dehisces. A thin layer of cytoplasm lines the cell walls, and the nucleus in many cases has not yet disorganized. The cell walls are comparatively thin, but on the lateral walls are rib-like bands of thickening (figs. 13, 14) which stain bluish with cyanin, while the wall itself is erythrophilous.

# Pollination.

At the time of pollination the micropyle of the ovule is wide open (fig. 1), and "from each orifice there is exuded a minute globule of clear, shining liquid which rests like an irridescent bubble on the tip, and serves to catch the pollen and conduct it to the nucellus within" (Jack 1893). Here the microspores usually lodge in the irregular, saucer-shaped depression produced by the partial breaking down of the superficial cells of the nucellus, and within a few days the micropylar canal is almost completely closed by the growth of the inner layer of cells of the integument at the region near the tip of the ovule (fig. 2). Norén (1907) is of the opinion that this closure is incited by the entrance of the pollen, and he finds that in cases where extraneous pollen is present, but none of *Juniperus*, the micropyle remains open, an observation which the writer can confirm.

# The male gametophyte.

Development of the pollen tube. — As first noted by Hofmeister (1851), a little over twelve months elapses between pollination and fertilization. A similar prolonged period of growth is characteristic of the male gametophyte in *Pinus* and *Cephalotaxus*, but in the majority of Gymnosperms, among them *Juniperus virginiana*, only a few weeks or months intervene between pollination and fertilization. Sludsky (1905) maintains that in *J. communis* also the development of the male gametophyte is completed in a single season, but the investigations of Norén (1907) and of the writer prove conclusively that this is not the case.

In the material studied, no microspores were found which showed the primary nucleus in the act of dividing. This division takes place within a week after the pollen reaches the nucellus and results in two nuclei slightly different in size from one another, the smaller of which — the generative nucleus — immediately becomes invested with a dense layer of cytoplasm and is separated from the protoplasm of the cell by a thin plasma membrane or 'Hautschicht'. Soon after this division the exine of the microspore is ruptured, usually on the side toward the nucellus, and the intine is pressed outward (figs. 40, 41). The growth of the pollen tube proceeds rather slowly throughout the summer months and then ceases altogether until the following spring. During this period of activity the tube presses into the tissue in the upper part of the nucellus, disorganizing the cells with which it comes in contact, and branches somewhat, thus anchoring itself firmly (figs. 42, 43). Frequently the tube wanders across the top of the nucellus before penetrating it, and in many cases, after forcing its way into the nucellar tissue, the tube turns sharply and grows for some distance in a horizontal direction. During the first season's growth the vegetative nucleus occupies a position a short distance from the growing end of the tube, while the generative cell lies close against

the wall of the spore (figs. 40-42). This cell is at first lenticular, but toward the close of the summer it rounds out and becomes spherical, thus separating more or less from the spore wall. The male gametophyte passes the winter in the condition represented by fig. 43.

Activity recommences early in the spring when the tube nucleus moves down toward the tip of the pollen tube (fig. 44), where it becomes surrounded by a dense mass of cytoplasm. The lower portion of the pollen tube now undergoes a marked increase in size, enlarging in all directions at the expense of the adjacent sporophytic tissue, but there is no marked growth in length until after the division of the generative cell which occurs late in April.

Fig. 45 shows the spirem of the division by which the stalk and body nuclei are produced, but none of the later phases were seen. The division is consummated very rapidly. Fig. 46 is drawn from a pollen tube in the same pollen chamber as that from which fig. 45 is taken and represents the two resulting nuclei. The smaller of the two is the nucleus of the body cell, the larger is the stalk nucleus. The phenomena which follow the division of the generative cell in the conifers present certain differences, as described by various writers. Belajeff (1893) and Strasburger (1892) report that in J. communis and Thuja respectively two cells of unequal size are formed, the larger of which, the stalk cell, degenerates. Coker (1903b) describes a similar condition in Taxodium. In Sequoia (Lawson 1904a) and in Saxegothaea (Norén 1907), on the other hand, immediately after the division of the generative cell the nuclei of the stalk and body cells lie free in a common cytoplasm. In Sequoia the body nucleus soon becomes invested with a dense zone of cytoplasm and develops a distinct membrane, but at no stage is there a distinct stalk cell. The observations of the writer show that in the species studied the two nuclei likewise at first lie free in the cytoplasm of the tube, and that a true stalk cell is not formed. Doubtless a closer examination of this phase in other Cupresseae will show similar conditions.

Very soon after their formation the stalk and body nuclei pass down toward the tip of the tube, the stalk nucleus usually in advance. The tube nucleus, which has meanwhile wandered back toward the upper end of the tube, meets the two nuclei about midway, and they pass down the tube together (fig. 47). The three are easily distinguished at this period, the stalk nucleus being somewhat larger than the body nucleus and slightly smaller than the tube nucleus, while there is usually a similar difference in the size of their nucleoli. By the time they have come to rest in the swollen tip of the tube, the body nucleus has become surrounded by a dense zone of cytoplasm and is cut off from the surrounding protoplasm by a definite membrane (fig. 48). Concomitantly with this condition in the male gametophyte, the megaspore, which has begun to germinate, is in the four nucleate stage.

For a time the further growth of the pollen tube toward the female gametophyte proceeds very slowly, so that four weeks later it has penetrated scarcely more than a third of the distance from the tip of the nucellus to the embryo sac. Simultaneously with the appearance of the archegonium initials in the female prothallium, however, the pollen tube commences to force its way rapidly through the nucellar tissue, crushing and disorganizing the cells of the nucellus with which it comes in contact, and in a few days enters the archegonial chamber where its tip presses up close to the archegonium complex (fig. 91). Lawson (1907b) states that in the Cupresseae the contents of the various tubes are discharged into a common archegonial chamber. While this may be true in some cases for J. communis, repeated observations of instances where more than one pollen tube have entered the archegonial chamber show that as a rule the male elements continue to be enclosed in their respective tubes until the discharge of the male cells into the egg. Throughout the period which has just been described the body cell and the two vegetative nuclei are found close together near the lower end of the tube (figs. 48-51). The stalk and tube nuclei enlarge somewhat and become so nearly alike that it is impossible to distinguish one from the other, while the body cell increases enormously in size and, just previous to the formation of the male cells, attains a diameter of about 60  $\mu$ .

The presence of a distinct delimiting membrane about the body cell in gymnosperms was apparently first noted by Hofmeister (1851) in Juniperus sibirica. It has since been described in J. communis by Belajeff (1893), Norén (1904, 1907), and Sludsky (1905), and appears to be a characteristic feature in all the Cupresseae thus far studied. A similar structure is found among the Taxeae, and in Sequoia. Among the Podocarpeae, on the other hand, a membrane is present about the young body cell of Dacrydium (Young 1907), but soon disappears, while such a structure is apparently entirely absent in Podocarpus (Coker 1902), Saxegothaea (Norén 1908), and Phyllocladus (Kildahl 1898). Among the Abieteae the body nucleus is surrounded only by a dense zone of cytoplasm which may include also the stalk and tube nuclei. In the cycads and Gingko a membrane is always present.

The demonstration of blepharoplasts in the body cells of the Gingkoales and Cycadales suggested the possibility that some traces of cilia-forming organs might exist among the Coniferales and Gnetales. A careful study, however, of a large number of body cells in *J. communis depressa*, in all stages of development, has failed to reveal any structures which appear to be definitely homologous with blepharoplasts. As in the other *Cupresseae*, the body cell is densely packed with starch. A radiate structure of the cytoplasm has been described by Coker (1903 b) in *Taxodium*, and by Norén (1907) in *J. communis*, but has not been observed by the writer.

The division of the body cell. - The division of the body cell to form the male cells takes place about four days after the pollen tube enters the archegonium chamber, and less than three days before fertilization. As pointed out by Coker (1903b) in *Taxodium*, this division usually occurs almost simultaneously with that of the central cell of the archegonium. Ordinarily two male cells, equal or nearly so in volume, and bounded by definite membranes, are formed, as described in the European form by various observers. These cells are at first hemispherical and lie close together (figs. 58, 52, 91), but after separating they become approximately spherical (figs. 53, 94). A slight inequality in their size is sometimes noticeable, but there is no doubt that they are physiologically equivalent, as will be proven later. The formation of two functionally equivalent male cells is of uniform occurrence among the Cupresseae and in Sequoia. On the other hand, but one male cell (or nucleus) appears to be functional in the Taxeae, Podocarpeae, and Abieteae. It would seem, as suggested by Juel (1904) and Coker (1907), that the formation of two functionally equivalent male cells (or nuclei) is restricted to those genera in which one pollen tube comes in contact with, and therefore has the opportunity to fertilize, more than one archegonium.

Juel (1904) describés in Cupressus Goweniana a peculiar condition which has occasioned considerable discussion. Here he finds that, in contrast to the usual conditions among the Cupresseae, several (as many as twenty) male cells are formed in a single pollen tube. Lawson (1907b) examined two other species of Cupressus and finds that there more than two male cells are never produced. He therefore regards the condition observed by Juel as "simply an interesting abnormality". Juel, however, considers this phenomenon a reversion to a primitive type and, coupling it with the fact referred to above that in this group it is possible for a single pollen tube to fertilize several archegonia, he concludes that the phylogeny of the Cupresseae has been different from that of the other conifers. Juel's opinion that the Cupresseae are descended from an ancestor in which a complex of male cells was formed is strengthened by the results of Caldwell (1907), who finds that in Microcycas such a condition actually exists, and that there the male gametophyte normally develops sixteen male cells. Moreover Norén (1907) describes the occurrence in the pollen tube of J. communis of a large body cell with three nuclei, while the writer has noted several cases in the form under consideration where more than two male cells have been formed by the division of the body cell. Fig. 54 represents an instance where four male cells have been thus produced. To be sure, such conditions are not normal, and it is probable that two of these cells always develop at the expense of the others, so that eventually only two are functional, as might be inferred from the conditions shown in fig. 94. But although the writer is not prepared to discuss the question of the probable ancestry of the Cupresseae, he ventures the opinion that the unusual conditions

found in *Cupressus Goweniana*, as well as those in *Juniperus* communis, are not without significance.

Since *Juniperus* is dioecious the number of pollen tubes found in the ovule depends primarily on the proximity of the staminate and pistillate plants. In the European form Belajeff (1893) and Norén (1907) find four or five pollen tubes in a single nucellus. A specimen prepared by the writer shows seven pollen tubes, all of which have reached the archegonial region and have formed male cells, so that in this case there are present at the time of fertilization fourteen male nuclei, each of which is capable of uniting with an egg.

The cytological phenomena which accompany the division of the body cell (or nucleus) to form the male cells (or nuclei) have been carefully studied in only a few Gymnosperms. Webber (1901) finds that in Zamia the spindle is entirely of nuclear origin, and, judging from the figures, a similar intra-nuclear derivation of the spindle would seem to be characteristic of Taxus (Robertson 1907) and Ephedra (Land 1907). A peculiar mode of spindle formation is described by Miss Ferguson (1901) in the body nucleus of Pinus, where the spindle is "extra-nuclear and unipolar in origin". According to Miyake (1903a) the same type of spindle formation occurs in Picea, and it is perhaps characteristic of the Abieteae. In none of the Cupresseae has this phase been fully worked out. Thus far the most complete observations are those of Coker (1903b), who establishes the fact that in Taxodium the spindle fibers are of nuclear origin. In J. communis Sludsky (1905, fig. 2) figures in the anaphase of this division a sharply bipolar spindle, the poles of which are situated near the periphery of the cell. Norén (1907) describes a condition seen shortly before the orientation of the chromosomes at the equator, but finds no indications of spindle fibers. An unsuccessful effort was made by the writer to secure a complete series of the various stages of spindle formation in var. depressa, but several interesting phases of this division were found which prove that, even if the spindle is not intra-nuclear throughout its history, as Sludsky's observations would indicate, it at least originates entirely within the nucleus.

As the body cell approaches division, it usually loses its spherical shape and becomes ovoid. Up to this time the nucleus has exhibited no features of especial interest. It possesses a large nucleolus, which is frequently vacuolate, and a coarse, anastomosing reticulum in which no differentiation into chromatin and linin can be made out. The reticulum now resolves itself into a slender, uniformly distributed spirem, which at first, in contrast to the conditions described in the pollen mother cell nuclei, shows beautifully a distinction between linin and chromatin very similar to that described by Allen (1905) in the pollen mother cells of *Lilium canadense* (fig. 55). The chromatin granules are very clearly arranged in pairs and are distributed at fairly regular intervals along the lighter staining band of linin. In addition to the chro-

matin and linin proper, there is evident in the nucleus a delicate protoplasmic network. The presence here of these three seemingly distinct nuclear elements in not necessarily at variance with the conditions found in the microspore mother cells, where it is impossible to make out such a differentiation, since many writers, especially among the zoologists, maintain that both "nucleus and cytoplasm have arisen through the differentiation of a common protoplasmic medium" (Wilson 1900, p. 40), and that the various elements in the cell are merely different physiological expressions of the same substance (see also Chamberlain 1899, p. 277). Fig. 56 shows a later stage in the division. The spirem has segmented into twelve slender and often twisted chromosomes, while the distinction between chromatin and linin has disappeared. At the same time delicate granular fibers have arisen in the nucleus. The chromosomes rapidly become shorter and thicker and are oriented at the equatorial plane while the fibers give rise to a blunt, multipolar diarch spindle (fig. 57), and the nuclear membrane disappears. As shown in the figures, no indications of a fibrillar structure are as yet present in the cytoplasm outside the nucleus, although Sludsky's figure would lead one to look for them. Fig. 58 represents the late telophase of this division. A cell plate has been formed extending entirely across the cell, and the connecting fibers are still evident. The nuclei have reorganized, and the chromatin appears to be in the form of small granules the pseudonucleoli of Norén (1907) — suspended in a network of linin, as was described by Lawson (1904b) in *Cryptomeria*.

# The megasporangium.

Development of the megaspore mother cell. — Norén (1907) finds in the European J. communis that soon after pollination a group of several cells, the archesporium, becomes recognizable in the lower portion of the nucellus, one of which becomes the megaspore mother cell. The non-functioning archesporial cells give rise to the tapetum, which surrounds the developing embryo sac and persists until after the formation of the endosperm. The tetrad divisions take place early in the year following the appearance of the archesporium and generally give rise to three cells, one of which is the functional megaspore. During the first of these divisions a reduction in the number of chromosomes is effected in the same manner as in the microspore mother cells.

The buds which give rise to the pistillate flowers in var. depressa are formed during the latter part of the growing season which precedes pollination. They are borne in the axils of the leaves on branches of the same year. In material collected less than five weeks before pollination it is impossible to distinguish vegetative from flower buds, but four weeks later (May 19) the ovules have begun to develop and present the appearance shown

in fig. 1. The integument is well defined, and the nucellus is composed of a mass of cells among which a slight differentiation is already apparent. Those near the tip have ceased to divide and have become vacuolate. Before long their growth stops entirely and their walls become slightly thickened. In the basal portion of the nucellus, in marked contrast to the apical region, the cells continue to divide by periclinal walls, giving rise to longitudinal rows of prismatic cells (fig. 59). At the lower end of these rows several cells soon become prominent by reason of their large size, big nuclei, and dense cytoplasm. This group constitutes the archesporium, which it will thus be seen is organized over twelve months before the formation of the prothallium. During the summer the archesporium increases somewhat in size, yet at no time is there a sharp line of demarcation between the sporogenous cells and the other cells of the nucellus. Certain cells of the archesporium take the lead in growth, but it is impossible to state with



Fig. 1.

Longitudinal section through young ovule immediately before pollination. Micropyle open.  $\times$  66.

certainty which of these will become the spore mother cell. The vegetative cells surrounding the archesporium, as noted by Norén (1907), become somewhat flattened and form more or less concentric layers. It is of interest to note that in *Cephalotaxus* (Coker 1907), where pollination likewise takes place the year preceding fertilization, the development of the female gametophyte proceeds at the same rate as in *J. communis*, while in *Pinus* (Ferguson 1904), where similar conditions are found, the embryo sac starts to develop the first season and passes the winter in the thirty-two nucleate stage.

Tetrad division. — The writer has made no attempt to obtain a complete series of the various stages in spore formation, but they are doubtless similar to those described by Norén (1907). The megaspore mother cell can be positively identified for the first time early in April, when its nucleus enters synapsis (fig. 60). During this period the nucleus presents an appearance very similar to that described in the nuclei of the microspore mother cells, the

chromatic elements being heaped together in a compact mass which lies close to the nuclear membrane. The cytoplasm of the cell appears more or less alveolar, and in it are imbedded numerous minute starch grains. The megaspore mother cell is slightly larger than the microspore mother cell, measuring about 35  $\mu > 20 \mu$ , while its nucleus has a diameter of about 17  $\mu$ .

Tetrad formation takes place about April 20<sup>th</sup>. The first division of the mother cell nucleus, as observed by Norén (1907), gives rise to two nuclei containing the haploid number of chromosomes. The spindle of this division is represented in figs. 61 and 67. In the latter figure it will be seen that a cell plate has been formed, but a permanent membrane is rarely if ever laid down between the daughter nuclei. In this respect the heterotypic division of the megaspore mother cell resembles that of the microspore mother cell. Frequently a protoplasmic membrane separates the two nuclei, but often they lie free in the cytoplasm of the mother



Fig. 2.

Longitudinal section through young ovule about two weeks after pollination. Micropyle closed.  $\times$  66.

cell (fig. 63). Both daughter nuclei become more or less completely reorganized, but as a rule only the lower one of the two reaches a resting stage. In such cases the latter alone undergoes the homotypic division, and as a result there is usually produced a group of three cells (fig. 67), only two of which are morphologically megaspores. Where both nuclei divide, a true tetrad is formed. Figs. 64—66 represent instances in the species studied where both daughter nuclei are undergoing the homotypic division, and where four potential megaspores are thus being developed. It will be seen that the two spindles may lie either side by side or in 'tandem'. No especial attention was given to the formation of these spindles, but fig. 64 shows a multipolar diarch similar to that noted by Miss Ferguson (1904, fig. 142) in *Pinus*.

Norén (1907) states that he has never observed a case where more than one embryo sac has developed within a single nucellus. The writer, however, was fortunate enough to secure one preparation in which three megaspore mother cells had under-

gone division (fig. 67). In two of the cells figured, one of the daughter nuclei of the first division has redivided, while in the other the heterotypic division has just been completed. Whether more than one of the functional megaspores thus formed would have developed further cannot, of course, be stated, but it is not unlikely, since Sludsky (1905) reports that in the European form he twice found two endosperms in a single nucellus. In connection with these abnormal cases it is interesting to note that here, as in *Cryptomeria* (Lawson 1904b), two megasporangia occasionally occur within a single integument.

During the division of the megaspore mother cell there is present just below the nucleus the conspicuous kinoplasmic body (figs. 63, 66, 67) which Norén (1907) has described in the European form. Analogous structures have been noted in the megaspore mother cells of *Taxodium*, *Thuja*, and *Taxus* (Coker 1903, 1904), and *Torreya* (Robertson 1904a). Its significance is still a matter for discussion, but it may be worthy of mention that the writer has observed very similar bodies in the later stages of prothallial development (fig. 77).

Immediately following the homotypic division the functional megaspore becomes cut off by a membrane and enlarges until it fills the entire space originally occupied by the mother cell (figs. 68, 69), while the nuclei of the non-functional cells rapidly disorganize and are eventually absorbed.

# The female gametophyte.

Development of the prothallium. — The observations of Norén (1907) on the development of the female gametophyte, briefly stated, are as follows: The megaspore germinates rapidly and gives rise to an embryo sac containing a large central vacuole and numerous free nuclei imbedded in a peripheral layer of cytoplasm. Nuclear division takes place simultaneously throughout the sac. In the development of the prothallium open tubes are formed which grow in toward the center of the embryo sac in the manner first described by MIle. Sokolowa (1890). The nucleus of each tube then divides, and cross walls are laid down. The megaspore membrane consists of two distinct layers. Norén's observations are for the most part confirmed by those of the writer.

The development of the embryo sac from megaspore to multicellular prothallium occupies about five weeks. During the few days following its differentiation the megaspore increases very little in size, but the primary nucleus undergoes its first division. The two resultant nuclei occupy a central position, and the cell contains several small vacuoles. With the advent of the four nucleate stage the small vacuoles flow together to form one large central vacuole, and the cytoplasm with its included nuclei comes

about the periphery of the young embryo sac, which to lie begins to enlarge and within three days has increased in volume about twenty-five times (fig. 71). In the embryo sac represented by this figure sixteen free nuclei were counted. The growth of the female gametophyte now proceeds rapidly, while nuclear divisions continue to take place simultaneously, and by May 25<sup>th</sup>, just previous to the formation of cellular tissue, its volumetric ratio, as compared with that of the megaspore at the time of the first nuclear division, is about 12500:1. The embryo sac has assumed the form of a prolate spheroid, the longitudinal axis of which measures about  $1400 \mu$ . A figure of the entire cell at the time of wall formation, if drawn to the same scale as figs. 68, 70, and 71, would be 1.5 m. in length. Fig. 72 represents probably the last free nuclear division, and in this preparation not only are all of the nuclei undergoing division, but all are in the same phase of mitosis. The axes of the spindles are for the most part parallel with the major axis of the embryo sac. No attempt was made to count the number of nuclei present after the last free division. The number has been variously estimated in different gymnosperms. Miss Ferguson (1904) writes that "about 2000 have been counted in *Pinus Strobus* at the time when the nuclei are being separated by the development of dividing walls", and according to Norén (1907) the number in J. communis appears to be scarcely smaller. Growth still continues after the formation of prothallial tissue, and at the time of fertilization the female gametophyte has acquired a length of fully 2800  $\mu$ .

The manner in which the cell tissue of the prothallium is organized agrees closely for the most part with the observations of Mlle. Sokolowa (1890) and Norén (1907) to which reference has already been made. Immediately after the last free nuclear division delicate anticlinal walls are laid down separating the nuclei, but no periclinal walls are formed, so that the cells "appear as uncovered boxes, the opening extending toward the center of the prothallial cavity" (Ferguson 1904). When viewed from their inner, open ends the cells appear as in fig. 75. Their nuclei lie slightly below the free inner margins of the walls, and from them arise delicate strands of cytoplasm which radiate toward the free edges of the walls. An optical section through the nucleus and parallel to the megaspore membrane (fig. 76) shows that these radiations are restricted to the open ends of the cells, the remainder of the cell cavity being intersected by coarser strands of cytoplasm. This relationship is even more clearly brought out in a radial section (fig. 74). Here it is seen that the outermost fibrillae are continuous from one nucleus to the next. As the walls are secreted these strands advance (fig. 77), so that, up to the time when the inner ends of the tube-like cells become closed in, all the nuclei of the embryo sac are connected with one another by kinoplasmic fibrils.

According to Mlle. Sokolowa (1890) and Norén (1907) no cross walls are formed until the tube-like cells meet at the center of the prothallial cavity. Miss Ferguson (1904), however, finds

that in *Pinus* the cells never reach the center "without having first divided by cell walls", and that "a ring of tissue composed of longer or shorter cells is formed rather early in the inward growth of the prothallium". This appears to be precisely what happens in the form under discussion, as may be seen from fig. 77. The further development of the prothallium was not followed in detail. Fig. 3, however, shows its appearance shortly after the cells have become closed in, and fig. 4 represents a similar section at about the time of fertilization.

The phylogenetic importance of the megaspore membrane in Gymnosperms has been recently emphasized by Thomson (1905a) who states that "the megaspore coat closely resembles that of a microspore both in its structure and its chemical composition, and



Fig. 3.



Radial section through lower portion of prothallium shortly after formation of cross walls.  $\times$  190.

Fig. 4. Section similar to fig. 3 at about the time of fertilization.  $\times$  190.

thus affords additional evidence of the free-sporing nature of the ancestral forms of the Gymnosperms". He finds that this structure is present in all the groups and sub-groups of these seed plants, except the *Taxeae*, from the ovule of whose forms it is entirely or almost entirely eliminated. Thomson describes the megaspore membrane in *J. sabina* and *J. virginiana*, while Norén (1907) describes that of *J. communis*, and the observations of the writer are in accord with those of these two authors. The coat is formed during the free nuclear period of the embryo sac and reaches its highest development at about the time of fertilization. It is  $2.5-3 \mu$  thick in the basal region of the prothallium and of fairly uniform thickness throughout, except at the micropylar end of the embryo sac where in the archegonial region it becomes quite thin. In section (fig. 73) two distinct layers are seen, the outermost of

which, the exosporium, is fibrillar and somewhat thicker than the inner, homogeneous endosporium. According to Thomson the exosporium is suberized, while the endosporium is composed principally of cellulose. When viewed from its outer surface the membrane presents a speckled appearance, and this in *Taxodium* led Coker (1903 b) to conclude that the coat was pitted. Thomson, however, shows that pits are not present.

Just as the megaspore membrane is homologous with the coat of the microspore, the so-called "spongy tissue" is now generally recognized to bear the same relation, on morphological as well as physiological grounds, to the tapetum of the microsporangium. Thomson (1905a) finds that in Gymnosperms "where the normal type of membrane occurs, there is present a more or less well defined tapetum". Norén (1907) describes this tissue in J. communis, and its history in var. depressa will be only briefly referred to here. As in the microsporangium the tapetum is derived from the non-functional cells of the archesporium. At the time of the tetrad division these cells are large and readily distinguishable from the surrounding nucellar tissue (fig. 62). They have large nuclei and are densely packed with cytoplasm in which are imbedded minute starch granules. As the megaspore develops the cells of the tapetum divide actively and continue to invest the young embryo sac (fig. 71). The layer is still present when the cellular tissue of the prothallium is being organized (fig. 74), but after this it rapidly disorganizes.

The archegonium. — According to Norén (1907) the archegonia are derived from the superficial cells in the upper part of the prothallium. The nucleus of the archegonium initial soon divides, cutting off a primary neck cell which by subsequent divisions forms usually a single tier of four neck cells. The archegonia vary in number from four to ten, and, as is characteristic of the *Cupresseae*, they are always grouped close together without intervening parenchyma, thus forming a single complex. During the development of the archegonia to their full size the vegetative cells at the upper end of the endosperm grow and divide rapidly, and as a result of this local activity the prothallial tissue surrounding the archegonium complex rises above the level of the neck cells, a depression in the tip of the prothallium being produced at the bottom of which lie the archegonia.

Fig. 78 of the present paper represents the archegonium chamber in var. *depressa* in the process of formation. At this stage, which is found about one week before fertilization, the central cell of the archegonium contains a relatively small amount of cytoplasm restricted to the periphery of the cell, thus enclosing a large central vacuole. The nucleus lies directly beneath the neck cells, and, while at first it scarcely differs from those in the surrounding prothallial cells, it soon becomes distinguishable by its large size.

Shortly before the division of the central cell nucleus to form the ventral canal nucleus and egg nucleus the cytoplasm of

the central cell begins to show the peculiar aster-like structures which Norén (1908) terms "Strahlungscentren", and for which the writer suggests the term Asteroid. Granular areas appear which occupy definite positions in the cell, and from these the cytoplasm radiates in all directions. These kinoplasmic radiations, as shown in figs. 80-85, present the appearance of granular fibers, and the whole structure assumes the form of an immense aster. One of these asteroids is invariably situated in close proximity to the nucleus, and a second one may frequently be seen directly below this, while one or more are present in the lower part of the cell (figs. 91, 95). They are most prominent during the division of the central cell nucleus, but the lower ones at any rate are still visible in the egg cell shortly before fertilization. The significance of the asteroids, especially of those in the lower portion of the arche-gonium, is not clear. Coker (1903b) suggests that the latter regulate the entrance of nutritive materials from the jacket cells, while Norén (1907) regards them as attraction centers, since, as the writer's investigations also show, granules of the cytoplasm are drawn toward them and accumulate at their centers (see Norén 1907, fig. 10). It may be worthy of note that the first appearance of the protein vacuoles follows shortly after the organization of the asteroids. Concerning the probable function of the upper asteroid mention will be made presently. Structures similar to these have been observed by Coker (1903b, 1902) in *Taxodium* and *Podocarpus*, by Land (1902) in *Thuja*, and by Lawson (1907 a) in Cephalotaxus. In several other Gymnosperms, viz., Cycas (Ikeno 1898), Dioon (Chamberlain 1906), Pinus (Blackman 1898, Chamberlain 1899, Ferguson 1904), Tsuga (Murrill 1900), Picea and Abies (Miyake 1903a, 1903b), kinoplasmic radiations of a more or less pronounced character have been either described or figured in connection with the division of the central cell nucleus or the development of the egg, but they are much less conspicuous than the structures found in Juniperus, where they form one of the most striking features of the archegonium.

The occurrence of "Hofmeisters Körperchen", or protein vacuoles, in the archegonium of *J. communis* has already been demonstrated by Norén (1907, fig. 65). In var. *depressa* they appear shortly before the division of the central cell nucleus and are seen to best advantage in the lower part of the egg cell just previous to fertilization. Immediately after the fusion of the male and female nuclei the protein vacuoles begin to disappear and at no time are they as conspicuous as in the *Abieteae*. Their significance has been the cause of considerable controversy, and for a full review of the literature the reader is referred to the excellent paper of Stopes and Fujii (1906). These authors suggest that the protein vacuoles "may be digestive vacuoles comparable in origin and function with the digestive vacuoles of the lower organisms". Whatever their function, it is now generally agreed that they arise within the central cell and are in some way concerned with the nutrition of the egg.

Division of the central cell nucleus. — About three days before fertilization the nucleus of the central cell divides, giving rise to the ventral canal nucleus and the egg nucleus. Strasburger (1879) figures the spindle of this division in *J. virginiana*, and it has recently been described in *J. communis* by Norén (1904, 1907) and Sludsky (1905). Up to this time the central cell nucleus has exhibited no unusual structural peculiarities It possesses a delicate reticulum in which dark staining masses and irregular, light staining threads are discernible and has a well defined nucleolus (fig. 79). As the nucleus approaches division more or less continuous, comparatively thick threads arise in which a distinction between chromatin granules and linin is apparent (figs. 80, 81), and at the same time the nucleolus tends to stain less deeply and reveals a vacuolate structure (fig. 93).

Coincident with these changes within the nucleus the nuclear membrane on the surface toward the asteroid becomes pressed or drawn inward in the manner shown by figs. 80 and 81, while delicate granular radiations extend between the center of the asteroid and the invagination thus formed. The first impression is that these radiations represent fibers pressing into the nuclear cavity, but the careful examination of a large number of preparations has failed to reveal any actual connection between them and the spindle fibers eventually formed. Similar phenomena have been variously interpreted by different writers. Murrill (1900) finds that in Tsuga the spindle fibers, arising within a fibrous mass beneath the nucleus "grow upward against and press in the nuclear membrane", and that the membrane then "disappears below, and the spindle fibers press into the nuclear cavity". Miyake (1903 a) reports that in Picea "the spindle fibers first arise from a clear court along the lower side of the nucleus and grow into the nuclear cavity", pressing in the nuclear membrane in the manner described by Murrill. Miss Ferguson (1901) also describes a clear region with delicate granular threads along the lower half of the nucleus in *Pinus*, and an irregular indentation of the upper and lower surfaces of the nucleus, but she fails to ascertain whether any of the threads enter the nuclear cavity and contribute to the formation of the spindle, while Coker (1903b) concludes that in Taxodium the spindle fibers are almost entirely of nuclear origin. Of course, if such is the case, as Miss Ferguson remarks, "the cytoplasmic activity in connection with this division would be inexplicable". Nevertheless, in the light of the writer's observations, the asteroid does not appear to contribute to the formation of the spindle, and its only apparent use in Juniperus is to form a support for the free lower pole of the spindle, a function already suggested by Murrill (1900).

The division of the central cell nucleus is consummated rapidly and takes place almost simultaneously in all the archegonia of a group. In one ovule, for example, eight such nuclei were found undergoing division. With the formation of a continuous spirem the distinction between chromatin and linin disappears, and the

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nuclear thread appears as a slender, deeply staining band of uniform thickness (fig. 82). The spindle is at first multipolar, but it soon becomes a multipolar diarch, the upper extremity of which is very broad, while the lower bundles of fibers converge toward the center of the asteroid, thus giving to the whole figure somewhat the form of an inverted, truncated cone (fig. 83). With the segmentation of the spirem and the orientation of the chromosomes at the equator the nuclear membrane disappears, leaving the spindle surrounded by the cytoplasm of the central cell, and the multipolar diarch soon becomes bipolar. The axis of the spindle may be parallel to that of the archegonium, but more frequently it is inclined at an angle. Frequently the spindle is some distance below the neck cells.

The daughter chromosomes, as they approach the poles, are U or V shaped (figs. 84, 85). At the poles the chromosomes rapidly draw together, becoming separated from the surrounding cytoplasm by membranes, and two resting nuclei are developed. No cell plate is formed, nor is there any indication whatever that a wall is ever developed between the ventral canal nucleus and the egg nucleus.

The two nuclei are at first very similar in appearance, but the egg nucleus matures rapidly, while the ventral canal nucleus develops slowly and as a rule disintegrates before fertilization. Usually the ventral canal nucleus lies above the egg nucleus, but one case was found (fig. 91) in which these relations were reversed, a condition previously described by Coker (1903 b) in *Taxodium*. Figs. 86 and 87 show two unusually well developed ventral canal nuclei. No evidence was found to support the theory (cf. Chamberlain 1899) that this nucleus is the homologue of the egg nucleus, but very often, as noted by Coker (1902, 1903 b) in *Podocarpus* and *Taxodium*, by Land (1902) in *Thuja*, and by Norén (1907) in *J. communis*, the ventral canal nucleus persists for a long time. Figs. 88 and 89 show such nuclei in archegonia where the egg has been fertilized and the development of the proembryo has begun. Sometimes they undergo division (fig. 90) and this division is mitotic.

Incidentally it may be noted that in this species archegonia occasionally are found superposed one above the other (fig. 91) or more or less deeply imbedded in the prothallium. Similar cases are described in *Picea* and *Abies* (Miyake 1903a, 1903b), and in *Pinus* (Ferguson 1904), while analogous conditions occur in the Bryophytes (Coker 1903a).

Surrounding the archegonium complex is a fairly well defined layer of jacket cells (fig. 95), while the walls of the egg cells are thin, except in the upper part, and no structures are visible which can be interpreted as pits. Stopes and Fujii (1906), however, have recently shown that even in those Gymnosperms where the thick wall of the egg is perforated by pits, the latter are closed by definite membranes which are pierced only by delicate threads of cytoplasm, so that the actual transfer of solid substances

from the jacket cells into the egg cell is impossible. They suggest that "the jacket cells are glandular or secretory and render the storage food of the endosperm soluble and available for the developing egg". According to Chamberlain (1906), in many cycads the egg "receives food material through haustorial projections which are in direct contact with the cytoplasm of the jacket cells". But among the *Cupresseae* the conditions are very different from those found in such groups as the Cycadales and Abieteae. There each archegonium is completely enveloped by a layer of jacket cells, while among the Cupresseae only the outermost archegonia of the group are in contact with the jacket layer. Yet, as Lawson (1907b) clearly points out, "the cytoplasm of the centrally situated egg cells shows very little difference in the character and quantity of food granules from that of the egg cells in contact with the jacket cells". Lawson therefore believes that "all food substances carried into the egg are translocated in soluble form", and that "the transference of food substances from egg cell to egg cell is the same as that from jacket cell to egg cell". During their later history the jacket cells in J. communis, as noted by Norén (1907), are frequently binucleate (fig. 95), and while in some cases the nuclear division which gives rise to this con-dition may be amitotic, as Norén affirms, the frequent presence of mitotic figures in the jacket cells as late as the time when the proembryo is being developed would indicate that these nuclei are ordinarily produced in the usual manner.

The nucleoli and pseudonucleoli. — Probably no structures in plant or animal cells have been the subject of more discussion, yet withal are more incompletely understood, than nucleoli. The term nucleolus has been applied so indiscriminately by different writers to various structures both inside and outside of the nucleus that it has come to have a very vague meaning, and a thorough comprehension of the different bodies thus designated and of their relation to the metabolic activities of the cell would doubtless illuminate many cytological problems which are at present inexplicable. It is not the purpose of the writer to enter upon a discussion of the nucleolus, except in so far as it directly affects the more general phenomena under consideration, but, in view of the large number of nucleolus-like bodies which are found in the egg nuclei of gymnosperms, it seems best at this point to consider these structures briefly as they appear in J. communis depressa.

In the microspore mother cell there is always present a conspicuous nucleolus which takes the chromatin stains and seems to be more or less intimately associated with the reticulum. During the nuclear changes which precede the heterotypic division this body gradually loses its affinity for dyes and at diakinesis has literally faded from view, apparently without having undergone any change in shape. Coincident with the separation of the daughter chromosomes toward their respective poles there appear in the region of the spindle minute droplets, the so-called extranuclear nucleoli, which react slightly to stains, but which usually

disappear after the reappearance of nucleoli in the resting nuclei. Later, after the homotypic division, small nucleolus-like bodies are frequently found lining both surfaces of the cell plates. These, however, will be referred to presently.

The body cell and the male cells to which it gives rise also present some interesting problems in connection with the nature of the so-called nucleoli, and it is evident from a study of these cells that the structures are of at least two sorts. The body cell possesses a large vacuolate nucleolus, which is probably homologous with the "plastin nucleolus" described by Coker (1903 b) in Taxodium. During the division of the nucleus, however, in contrast to the behavior of the nucleolus of the microspore mother cell, this body appears to shrink in size, although still maintaining its spherical shape, and is recognizable as late as the time when the chromosomes are becoming oriented at the equator (figs. 56, 57). Subsequent to this division prominent nucleoli of the same type reappear in the daughter nuclei. In addition to these structures there are present in the male cells small nucleolus-like bodies, the 'pseudonucleoli' of Norén (1907). These are distributed throughout the nucleus, and in view of the absence of any visible chromatin are regarded by Lawson (1904, 1907) and others as chromatin granules. Outside the nuclei of the male cells, immediately after their formation, and lining the cell plate on both sides there may be found in favorable material granular or lumpy masses which likewise take the chromatin stains, and which are probably of the same nature as those referred to in the microspore mother cells. These masses are frequently very conspicuous in the young male cells (fig. 58) but usually disappear soon after the laying down of the dividing wall. They are doubtless homologous with the "plastin granules" described by Coker (1903b) in Taxodium, and it is doubtful whether they ought to be interpreted as nucleoli.

In the egg nucleus the nucleolus-like structures are still more numerous and are exceedingly difficult to interpret. Frequently the entire chromatic content of the nucleus seems to have resolved itself into nucleoli and pseudonucleoli, yet even after the study of a large number of preparations one is unable to formulate any satisfactory conclusions as to the nature of these structures. That the pseudonucleoli are different from the typical nucleoli, however, seems obvious. Some at least of the former appear to be definitely associated with the reticulum, while in the mature egg nucleus they are the only parts which take the chromatic stains with avidity. It is not improbable that, as Wager (1904) suggests, the pseudonucleoli "form a part of the nuclear network in which chromatin or chromatin substance may be stored and possibly to some extent elaborated".

There is little doubt that many features in these nuclei which appear to be normal, and which have been described as such by various writers, are artefacts, and that the appearances seen in fixed and stained material differ greatly from the structures present in the living nucleus. Chamberlain (1906), after a study of the

living egg nucleus in *Dioon*, writes that his results are far from satisfactory, that "a nucleolus is visible, but otherwise the contents are nearly homogeneous. There are few globules and the network could not be identified". He adds: "It is possible that most of the globules and the network are coagulation products due to fixing".

Maturation of the egg nucleus. — The egg nucleus grows rapidly, usually becoming slightly ovoid in shape, and shortly before fertilization has acquired a length of about 40  $\mu$ . Figs. 86 and 87 show two of the various aspects which it presents during its development, while fig. 92 represents the mature oosphere nucleus. At the time of fertilization the reticulum appears as a network of almost colorless, granular threads which ramify throughout the nucleus and exhibit slight indications of a differentiation into chromatin and linin, while sometimes there is also visible in the nuclear cavity a faint protoplasmic meshwork (fig. 87). Coincident with the growth of the egg nucleus the vacuole of the egg cell diminishes in size (figs. 91, 95).

Fertilization. — Fertilization stages were found in material collected June 15<sup>th</sup>, 1906 and June 29<sup>th</sup>, 1907. In 1908 daily collections from June 6<sup>th</sup> to 13<sup>th</sup> showed that, although fusion is almost simultaneous in all the archegonia of a single ovule, a week or more may elapse between conjugation in different flowers on the same plant. In material collected June 7<sup>th</sup>, for example, the body cell in many pollen tubes had not yet divided, while in other cases fertilization had taken place and the development of the proembryo had begun.

Preparatory to fertilization the membrane of the pollen tube is dissolved or ruptured directly above the neck of an archegonium, and one of the male cells squeezes through the neck into the egg, carrying the broken-down neck cells with it. There is no receptive vacuole in the oosphere, such as occurs in the Abieteae. The wall of the male cell is cast off during its entrance into the egg, or immediately afterward, and is frequently seen lying either outside the neck or, together with the disorganized remains of the neck cells and vegetative nuclei of the male gametophyte, in the upper part of the egg. Reference has already been made to the equality in the size of the male cells, and ample proof has been found that both may be functional. Numerous cases were noted where the number of developing proembryos is greater than that of the pollen tubes present, while fig. 95 shows one instance where two male cells from the same pollen tube (the only pollen tube present in this ovule) are in the act of entering different archegonia. In the event of more than one male cell entering the same archegonium only one functions, the superfluous cell disintegrating in the upper part of the egg cell.

The male nucleus, accompanied or followed by its mantle of cytoplasm and starch, rapidly approaches the female nucleus which has come to lie slightly above the center of the egg. Figs. 96—101 illustrate the general appearance of the conjugating nuclei,

and it will readily be seen that there is considerable diversity in their relative size. Norén (1907) states that in the European form the two are nearly equal, and, as figs. 97—99 show, this is often the case, but it is by no means the rule. The volumetric ratio, for example, of the nuclei represented in fig. 100 is about 4:1. The mass of cytoplasm and starch derived from the male cell (indicated in the smaller figures by the dotted line) gradually surrounds the conjugating nuclei, so that there is never any possibility of mistaking the fusion nucleus for an unfertilized egg nucleus. After the union the fusion nucleus gradually migrates toward the base of the egg.

The two nuclei apparently fuse while in a resting condition (fig. 102). Previous to the dissolution of the membranes between them there sometimes appears to be a condensation of the substance of the male nucleus near the surface of contact, but, although more than thirty archegonia were examined which showed the male and female nuclei in contact with one another, in none of them was there any indication that the spirems of the first segmentation division are formed before the dissolution of the dividing membranes, as is the case in *Pinus* (Ferguson 1904). A still larger number of conjugating nuclei were found in which fusion had taken place, yet in none of these was the writer able to distinguish the male from the female elements until the organization of the definite spirems just previous to the first segmentation division.

The nucleus of the fertilized egg presents various appearances which, in the light of present knowledge, it is impossible to interpret satisfactorily. Norén's figures (1904, fig. 4; 1907, figs. 76, 77) show the structure very well, so far as it can be made out. As a rule a faintly staining network is visible, but sometimes the entire content of the nucleus, with the exception of the various nucleolus-like bodies, appears almost homogeneous. The most conspicuous structures are the nucleoli and pseudonucleoli which are usually indistinguishable from one another. These may be bunched together or distributed through the nucleus; they may appear intimately connected with the reticulum or entirely disassociated from it; and their affinity for stains also varies greatly, — in some cases all stain deeply with iron-haematoxylin, while again some stain scarcely at all.

Development of the proembryo. — Blackman (1898) observes that in the fusion nucleus of *Pinus* "the chromosomes of the male and female nuclei could be distinguished into two groups at the time when the first segmentation spindle was in the multipolar condition", while Chamberlain (1899), working independently, reports the presence within the oospore nucleus in this genus of two distinct spirems. The results of these two writers are amply confirmed by the extensive investigations of Miss Ferguson (1904), while Woycicki (1899) and Murrill (1900) find similar phenomena in *Larix* and *Tsuga* respectively. Under such conditions, as Blackman points out, the process of fertilization

cannot be considered as completed until the "half chromosomes derived from the male and female nuclei respectively fuse together at the poles of the first segmentation spindle". In the European *J. communis* Norén (1907, p. 43) describes a rather peculiar condition. He writes: "Die kettenförmig mit einander verbundenen Pseudonucleolen verschmelzen schließlich mit einander (l. c., fig. 78), wodurch dicke, unregelmäßige Fäden entstehen. Auf diesem Stadium können noch die den resp. Kernen zugehörigen Chromatingruppen deutlich unterschieden werden, also auch noch nachdem die Membranen zwischen den Kernen völlig verschwunden sind".

The behavior of the chromatin in J. communis depressa is very similar to that observed by the above mentioned writers, but no indications were found that the spirems originate, in the manner described by Norén, from the "melting together" of the pseudo-nucleoli. For one or two days after the union of the male and female nuclei the fusion nucleus to all appearances continues in a resting condition. Then there begin to appear in the nucleus numerous more or less connected, moniliform threads which exhibit. a beautiful differentiation into chromatin and linin (fig. 103). These strands run all through the nuclear cavity, and, while it is im-possible to make out with certainty whether two spirems are present at this time, such is presumably the case. The threads soon draw away from the nuclear membrane, become more closely coiled, and in the stage represented by fig. 104 it can be clearly seen that two distinct spirems have been organized. At the same time the whole band comes to stain uniformly, and the nucleoluslike structures disappear. The infrequency with which the condition described here has been observed is easily accounted for by the difficulty with which suitable preparations are obtained. Fig. 104 represents the only instance, out of over 2000 archegonia examined in which this stage might be looked for, where it is possible to distinguish the male and female elements in the fertilized egg.

Segmentation into chromosomes takes place rapidly and apparently in the usual manner, the chromosomes become oriented at a common equatorial plane (fig. 105), and a broad, multipolar diarch spindle is organized. At this period coarse, granular threads are sometimes seen in the protoplasm of the nucleus outside the spindle, but their relation, if any, to the formation of the spindle in obscure (cf. Ferguson 1904, figs. 228-230). The multipolar diarch quickly becomes bipolar, and the manner in which the chromosomes split and separate toward their respective poles is to all appearances identical with the process as described in the division of the central cell nucleus. Fig. 106 represents the telophase of this division. The chromosomes have drawn together at the poles, and nuclear membranes are about to be formed. By this time the membrane of the fusion nucleus itself has disappeared, although the nuclear cavity still remains clearly delineated, while the connecting fibers are still visible between the massed chromosomes. After the development of membranes about the two

nuclei the outline of the original nucleus disappears, and the surrounding layer of cytoplasm and starch presses in and invests the two daughter nuclei. The latter grow rapidly and enter upon a brief period of rest (fig. 107).

Shortly after the completion of the first segmentation division the daughter nuclei divide (fig. 108), giving rise to four resting nuclei which are usually arranged in a tetrad, as shown in fig. 109. There is no rule as to the position of the spindles of this division with respect to the axis of the archegonium, and in this, as well as in the following division, the spindle is of intra-nuclear origin. Fig. 110 represents the third free nuclear division (the fourth dividing nucleus appears in the next section). In this figure the chromosomes have obviously split longitudinally, although they are just being oriented at the equator. After the organization of the eight nuclei which result from this division wall formation usually takes place, as already described by Norén (1907) in the European form, and in this respect Juniperus agrees with the Abieteae and Cupresseae thus far investigated. Occasionally, however, sixteen free nuclei may be formed before the separating membranes are laid down (fig. 111), thus approaching the condition found in Podocarpus (Coker 1902) and Cephalotaxus (Coker 1907, Lawson 1907a) where sixteen are normally produced.

Of the eight cells which are usually cut off by the formation of walls the three or four which will give rise to the embryo lie at the base of the archegonium, while the remaining cells form a well defined tier above these. The latter are not entirely enclosed by walls, being exposed above to the cytoplasm of the egg, and they soon divide, giving rise to two tiers of cells (figs. 112, 113). Of the cells thus formed those of the upper tier constitute the rosette, and, as in most conifers, they never become enclosed above by a wall. The cells of the lower tier are the suspensors, by the elongation of which (fig. 114) the embryo is pushed deep into the endosperm. Simultaneously with the cell division just described the cells of the embryo proper usually undergo division (fig. 112). The further development of the embryo has not been followed by the writer.

# Summary.

The buds which give rise to the staminate cones are formed during the summer of the year preceding pollination.

The archesporium originates from one or more hypodermal cells at the base of the sporophyll.

The tapetum and the inner layer of the sporangium wall are both derived from the outermost cells of the archesporium, while the outer layer of the wall — the only layer present in the mature sporangium — is developed from epidermal cells. Thus the inner layer of the wall is more closely correlated to the tapetum than to the outer wall-layer.

The microspore mother cells enter synapsis about May 1<sup>st</sup>. Preparatory to this period a pairing of different portions of the chromatin becomes evident.

At the completion of the heterotypic division two resting daughter nuclei are formed, but a wall is never developed between them. The second division may be either tetrahedral or bilateral.

Preparatory to the formation of microspores the cavity of the mother cell becomes divided into four chambers by thick, transparent walls. Within these compartments the spores are developed, and they are eventually set free by the breaking down of the enveloping walls.

Pollination occurs about May 25<sup>th</sup>.

The elapsed time between pollination and fertilization is about twelve and a half months.

During the first season's growth the pollen tube penetrates a short distance into the nucellus and frequently branches, while the primary nucleus divides, giving rise to the tube nucleus and the nucleus of the generative cell. The latter immediately becomes surrounded by a 'Hautschicht'.

Soon after the renewal of activity the following spring the nucleus of the generative cell divides, forming the stalk nucleus and the body cell nucleus. The latter becomes invested with a definite membrane, but a true stalk cell is never formed.

By the time the pollen tube enters the archegonium chamber the body cell has attained a diameter of about 60  $\mu$ .

The division of the body cell takes place about three days before fertilization and usually results in the formation of two male cells equal in volume and bounded by definite membranes. The spindle of this division is of intra-nuclear origin.

Several instances were found where three or four male cells had been produced by the division of one body cell, but it is probable that no more than two are ever functional.

The ovulate buds first become distinguishable from vegetative buds a few weeks before pollination.

The archesporium is derived from the cells in the lower portion of the nucellus and is recognizable at the time of pollination, but it is impossible to distinguish the megaspore mother cell until the following spring.

Generally only one of the archesporial cells becomes a true mother cell, but occasionally as many as three may function as such. The non-functional cells give rise to the tapetum.

Tetrad formation takes place late in April. As a rule but one of the nuclei resulting from the heterotypic division undergoes the homotypic division.

The nucleus of the functional megaspore becomes separated from the other nuclei in the mother cell by a membrane.

The development of the female gametophyte occupies about six weeks.

The megaspore membrane consists of two distinct layers.

The tapetum persists until after the formation of prothallial tissue.

From four to ten archegonia are organized, and, as in the other *Cupresseae*, these form a single complex which is surrounded by a layer of jacket cells.

Peculiar aster-like structures, termed asteroids, are conspicuous in the central cell of the archegonium and persist until after fertilization. Their function is obscure.

The division of the central cell nucleus takes place about three days before fertilization and is approximately simultaneous in all the archegonia of a group. The spindle of this division is apparently entirely of intra-nuclear origin.

A true ventral canal cell is never formed. The ventral canal nucleus usually disintegrates, but sometimes persists for a long time, and may divide mitotically.

In the mature egg nucleus the most conspicuous features are the nucleoli and pseudonucleoli. Some of the latter are presumably related in some manner to the chromatin.

Fertilization takes place in June, and an interval of a week may elapse between conjugation in different flowers on the same plant.

Both male cells may be functional.

The entire male cell may enter the egg, but frequently the cell membrane is cast off outside.

The male nucleus and the egg nucleus apparently fuse while in a resting condition, and the fusion nucleus becomes surrounded by a mantle of starch derived from the male cell.

No distinction between male and female chromatin is apparent until the organization of the spirems of the first segmentation division, when two separate masses of chromatin may be clearly distinguished.

The spindles of the first division of the fertilized egg and of the following divisions are intra-nuclear.

Usually eight free nuclei are formed before the appearance of walls, but in exceptional cases wall development is delayed until after the fourth nuclear division.

Cell divisions in the upper tier of cells of the proembryo give rise to the suspensors and the cells of the rosette.

Note: Since the completion of this work there has appeared a paper by Miss A. M. Ottley on "The development of the gametophyte and fertilization in *Juniperus communis* and *Juniperus virginiana*". (Bot. Gazette 48: 31-46.) In the main her results are in accord with those of the writer. She describes, however, the formation of a distinct stalk cell (p. 34), a structure which the investigations of the writer have failed to demonstrate. Also the fact should be noted that the mature male cells are spherical, not hemispherical (l. c. p. 35).

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# Explanation of plates VIII-XVII.

All figures drawn with the aid of a camera lucida.

## Plate VIII.

Sections of microsporophylls showing successive stages in development of microsporangia. Sections cut radially through staminate cones.

Fig. 5. Archesporial enitials.  $\times$  285.

Fig. 6. First periclinal division of archesporial cells.  $\times$  285.

Fig. 7. More advanced stage in development of archesporium.  $\times$  285.

Fig. 8. Differentiation of archesporium into primary sporogenous cells, tapetum, and inner wall layer.  $\times$  285.

Fig. 9. Sporangium just before synapsis.  $\times$  285.

Fig. 10. Sporangium during synapsis.  $\times$  285.

Fig. 11. Sporangium during first heterotypic division.  $\times$  285.

Fig. 12. Sporangium after formation of tetrads.  $\times$  285.

Fig. 13. Sporangium shortly before its dehiscence.  $\times$  285.

Fig. 14. Cells in wall of mature microsporangium. Surface view.  $\times 75$ .

#### Plate IX.

Successive stages in tetrad division of microspore mother cells.

Fig. 15. Nucleus of mature spore mother cell.  $\times$  1280.

Figs. 16, 17. Nuclei preparing to enter synapsis. A pairing of the chromatic material is taking place.  $\times$  1280.

Fig. 18. Nucleus during synapsis.  $\times$  1280.

Fig. 19. Nucleus emerging from synapsis.  $\times$  1280.

Fig. 20. Nucleus fully recovered from synapsis. The spirem is slender and uniformly distributed, while indications of a fibrillar structure are already apparent in the cytoplasm of the cell.  $\times$  1280.

Fig. 21. Spirem shortened and thickened. Nucleus surrounded by cytoplasmic "felt".  $\times$  1280.

Fig. 22. Thin tangential section of nucleus to show longitudinal splitting and transverse segmentation of spirem.  $\times$  1280.

Fig. 23. Diakinesis.  $\times$  1280.

Fig. 24. Multipolar spindle organized.  $\times$  1280.

Fig. 25. Multipolar diarch spindle. Chromosomes are oriented at the equatorial plane.  $\times$  1280.

Fig. 26. Bipolar spindle. Separation of daughter chromosomes about to take place.  $\times$  1280.

Fig. 27. Same stage as that shown in the preceding figure. Section cut perpendicular to axis of spindle and showing twelve chromosomes.  $\times$  1280.

Fig. 28. Anaphase of heterotypic division. A longitudinal fission of the daughter chromosomes apparent.  $\times$  1280.

Fig. 29. Late anaphase, showing the massing together of the chromosomes at the poles.  $\times$  1280.

Fig. 30. Late telophase of heterotypic division, showing reconstruction of daughter nuclei.  $\times$  1280.

Fig. 31. Resting daughter nucleus of heterotypic division.  $\times$  1280.

Fig. 32. Metaphase of homotypic division. Separation of the granddaughter chromosomes about to take place.  $\times$  1280.

Figs. 33, 34. Telophase of homotypic division. First indications of a thickened wall enclosing the protoplasm of the mother cell.  $\times$  1280.

Fig. 35. The same. Nuclei connected by cytoplasmic fibrils. Thickened wall well developed.  $\times$  1280.

## Plate X.

Figs. 36-39. Development of microspore.

Fig. 36. Reconstruction of granddaughter nuclei and formation of cell plates.  $\times$  1280.

Fig. 37. Laying down of walls separating nuclei of tetrad.  $\times$  1280.

Fig. 38. Separating walls have swollen, and have become continuous with the thick, enveloping wall.  $\times$  1280.

Fig. 39. Mature microspore packed with starch grains.  $\times$  1280.

Figs. 40-44. Development of male gametophyte.

Figs. 40–42. Early stages in growth of pollen tube.  $\times$  480.

Fig. 43. Pollen tube at conclusion of first year's growth, showing winter condition.  $\times$  480.

Fig. 44. Pollen tube soon after renewal of activity the following spring.  $\times$  480.

#### Plate XI.

#### Development of male gametophyte (cont.).

Fig. 45. Spirem of division of generative cell.  $\times$  640.

Fig. 46. Body nucleus (b), and stalk nucleus (s) immediately after their formation.  $\times$  640.

Fig. 47. Body and stalk nuclei have passed down the tube, meeting tube nucleus (t) midway.  $\times$  640.

Fig. 48. Lower end of pollen tube, showing body cell with stalk and tube nuclei.  $\times$  640.

Fig. 49. The same, one week later.  $\times$  640.

Fig. 50. Body cell with stalk and tube nuclei during growth of pollen tube toward archegonia.  $\times$  640.

Fig. 51. Tip of pollen tube shortly after reaching archegonial chamber. Stalk and tube nuclei are indistinguishable from one another.  $\times$  480.

#### Plate XII.

#### Development of male cells.

Fig. 52. Tip of pollen tube shortly after formation of male cells.  $\times 450$ . Fig. 53. The same just before fertilization. The male cells have become spherical.  $\times 450$ .

Fig. 54. Abnormal case where four male cells have been formed by the division of one body cell.  $\times$  600.

Fig. 55. Division of body cell nucleus — early prophase. A more or less continuous spirem is present and the chromatin granules are paired.  $\times$  1200.

Fig. 56. Prophase. Formation of chromosomes and development of spindle fibers. Reconstructed from two adjoining sections.  $\times$  1200.

Fig. 57. Metaphase. The chromosomes are becoming oriented at the equatorial plane and have already split longitudinally. Reconstructed from two adjoining sections.  $\times$  1200.

Fig. 58. Late telophase. Nuclei of male cells have been organized and a wall is being laid down between the two cells.  $\times$  1200.

#### Plate XIII.

Development of the megaspores from the megaspore mother cell and the first nuclear division of the germinating embryo sac.

Fig. 59. Longitudinal section through nucellus shortly before pollination, showing archesporial region.  $\times$  160.

Fig. 60. Megaspore mother cell in synapsis — nearly a year later than the stage represented in fig. 59.  $\times$  800.

Fig. 61. Spore mother cell undergoing heterotypic division.  $\times$  800.

Fig. 62. Longitudinal section through base of nucellus to show tapetum.  $\times$  392.

Fig. 63. Daughter nuclei of heterotypic division. Reconstructed from two adjoining sections.  $\times$  800. (In this figure the megaspore mother cell is drawn on a larger scale.)

Figs. 64, 65, 66. Various phases in the homotypic division of the megaspore mother cell.  $\times$  800.

Fig. 67. Group of three mother cells which have undergone division. Reconstructed from two adjoining sections.  $\times$  800.

Figs. 68, 69. Functional megaspore shortly before the first free nuclear division. The shriveled remains of the non-functional cells are still visible.  $\times$  800.

Fig. 70. Embryo sac after first free nuclear division.  $\times$  800:

#### Plate XIV.

Development of prothallium and formation of archegonia.

Fig. 71. Section of embryo sac containing sixteen free nuclei.  $\times$  800. Fig. 72. Radial section of embryo sac shortly before the formation of prothallial tissue, showing nuclei dividing simultaneously.  $\times$  640.

Fig. 73. Section of megaspore membrane at the time of fertilization.  $\times$  800.

Fig. 74. Prothallium shortly after the development of primary walls. Radial section.  $\times$  640.

Fig. 75. The same. Surface view of inner, open ends of cells.  $\times$  640.

Fig. 76. The same. Optical section parallel to spore membrane.  $\times$  640.

Fig. 77. Radial section through prothallium at a later stage of development.  $\times$  640.

Fig. 78. Tip of prothallium after the formation of archegonia.  $\times$  80.

#### Plate XV.

Fig. 79. Mature central cell nucleus in tip of archegonium.  $\times$  640.

Figs. 80-85. Successive stages in division of central cell nucleus to form ventral canal nucleus and egg nucleus.  $\times$  640.

Figs. 86, 87. Egg nuclei and unusually well developed ventral canal nuclei shortly after their formation.  $\times$  640.

#### Plate XVI.

Figs. 88, 89. Ventral canal nuclei at the tips of archegonia in which the egg has been fertilized.  $\times$  306.

Fig. 90. Two nuclei resulting from the mitotic division of the ventral canal nucleus.  $\times$  306.

Fig. 91. Tip of prothallium shortly before fertilization showing group of archegonia and pollen tube in archegonium chamber.  $\times$  104.

Fig. 92. Mature egg nucleus.  $\times$  640.

Fig. 93. Nucleolus of central cell nucleus.  $\times$  1280.

Fig. 94. Tip of a pollen tube which contained one small and two large male cells. One of the latter has already entered an archegonium.  $\times$  306.

Fig. 95. Entrance of two male cells from one pollen tube into different archegonia.  $\times$  306.

Figs. 96—100. General appearance consugating male and female nuclei. The cytoplasm and starch of the male cell are indicated by the dotted lines.  $\times$  306.

## Plate XVII.

Fertilization and the development of the proembryo.

Fig. 102. Male nucleus and egg in contact and surrounded by mantle of starch.  $\times$  640.

Fig. 103. Fusion nucleus. More or less definite spirems have already been organized. Partly reconstructed from two of the three sections into which the nucleus was cut by the razor.  $\times$  640.

Fig. 104. Fusion nucleus just before first segmentation division. Two distinct spirems are present.  $\times$  640.

Fig. 105. Late prophase in first division of fertilized egg. The chromosomes are being oriented at a common equatorial plane.  $\times$  640.

Fig. 106. Telophase of segmentation division.  $\times$  640.

Fig. 107. Daughter nuclei of segmentation division.  $\times$  640.

Fig. 108. Second free nuclear division of proembryo.  $\times$  392.

Fig. 109. The four nuclei resulting from this division.  $\times$  392.

Fig. 110. Third free nuclear division of proembryo.  $\times$  392.

Fig. 111. An unusual instance where sixteen free nuclei are being developed before wall formation.  $\times$  306.

Fig. 112. First nuclear division following formation of walls.  $\times$  306.

Fig. 113. The same completed.  $\times$  306.

Fig. 114. Proembryo after slight elongation of suspensors.  $\times$  160.

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