Seed development of Picea abies and Pinus silvestris

Fröets utveckling hos gran och tall

by

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Preface

Professor Artur Håkansson, Institute of Genetics, Lund, has made a study of the embryological side of seed development in Scots pine and Norway spruce. His investigation was carried out in collaboration with the Department of Genetics of the Forest Research Institute of Sweden, the personnel of which collected and ~~fixed~~ the material. The investigation, which forms a link in a series of studies from the Forest Research Institute concerning seed and embryo development in conifer species, has been economically supported by «Fonden för skogsvetenskaplig forskning", Stockholm 51. This also generously met the printing costs.

Stockholm, January 4th, 1956.

Manfred Näslund

Introduction

The aim of this investigation was to establish time of fertilization and to follow embryo development in Norway spruce and Scots pine during summer and early autumn. Ovules were collected in 1954, a year with rather late though very rich flowering. Investigated were a number of trees growing at "Skogshögskolan", Experimentalfältet, in the vicinity of Stockholm. The earliest fixations were made on 12/6, stages of seed development such as macrosporogenesis and the forming of the young prothallus in the surviving macrospore were not investigated. It is a well-known fact that in *Pinus* the pollen tube and the young female prothallus hibernate in the ovule, continuing growth next spring. Thus fertilization is delayed about one year. In *Picea* pollination and fertilization occur in the same year, there is thus a more modest delay of fertilization, similar to that in our amentifers.

In all *Pinaceae* the cone scale carries two ovules on the upper side. The ovules are near the base and have a large nucellus and one integument forming a micropyle. The ovules are inverted, the micropyles being directed downwards. In many conifers pollination is facilitated through a so-called pollination drop.

The function of such a drop has recently been studied in *Callitris* (Baird 1953, p. 266). It was established through experiments that the drop after pollination gradually diminishes in size and after 10—20 minutes has contracted into the micropyle. "The grains remain in the surface layers of the liquid and are only drawn into the micropyle as the drop sinks." In the adjacent unpollinated ovule the drop is not changed, it slowly evaporates and a new drop may here appear next day. But in *Pinus* and *Picea* no pollination drop has been observed. In such conifers the micropyle generally shows certain structures facilitating entrance of pollen grains and retaining the grains within the micropyle or on the top of the nucellus until their germination. The apical part of the nucellus is large, forming a many-layered nucellar cap, while the tissue of the more basal parts of nucellus is largely destroyed by the expanding prothallus. The pollen tubes grow through the cap to the apex of the prothallus which contains the archegons.

Development of the spruce seed

The investigation of three spruces was supplemented with material from the South of Sweden, Holmsjö in Northern Blekinge.

Miyake (1903) has closely studied seed development up to the formation of the proembryo in *Picea abies*. Embryo development of *Picea* species has been investigated less closely (Buchholz 1942, see also Johansen 1950).

24/5 1954. — Pollination.

12/6. — Now, nearly three weeks after pollination, the most advanced pollen tubes had grown 1/10 of the way through the nucellus cap. As has been shown by Miyake, the pollen tubes contain tube nucleus, stalk cell and generative cell, the latter dividing when the tube has grown half-way through the cap. There were always several or many grains in the micropyle, in certain ovules pollen grains were only germinating. There is much starch in the cells of the nucellar cap. — The female prothallus was now completely cellular. It showed strong mitotic activity. The archegons had been formed, each consisted of one layer of neck cells and a large central cell. The nucleus of the latter, the so-called central nucleus, had in most cases moved close to the neck cells. Miyake counted the number of archegons, about one half of the ovules had four, the variation being 2—7. Here their number was similar (but compare p. 11).

15/6.—The pollen tubes now had grown 1/5—1/4 of their way through the nucellar cap. The generative nucleus had not divided. The nucellar cap contains still more starch, which may as a sheath surround the tubes. There are also starch grains within the pollen tubes.—The prothallus of the ovule and its archegons are enlarged; the so-called jacket layer, a layer of prothallus cells surrounding each archegon is more distinct. The nucleus of the central cell is still undivided.

19/6.—The pollen tubes have grown more rapidly and may have penetrated the whole nucellar cap, but were never in contact with the archegons or the archegon chamber of the prothallus. The generative nucleus is divided, two male nuclei being close together near the end of the pollen tube. The neck cells of the archegons now divide forming two layers.

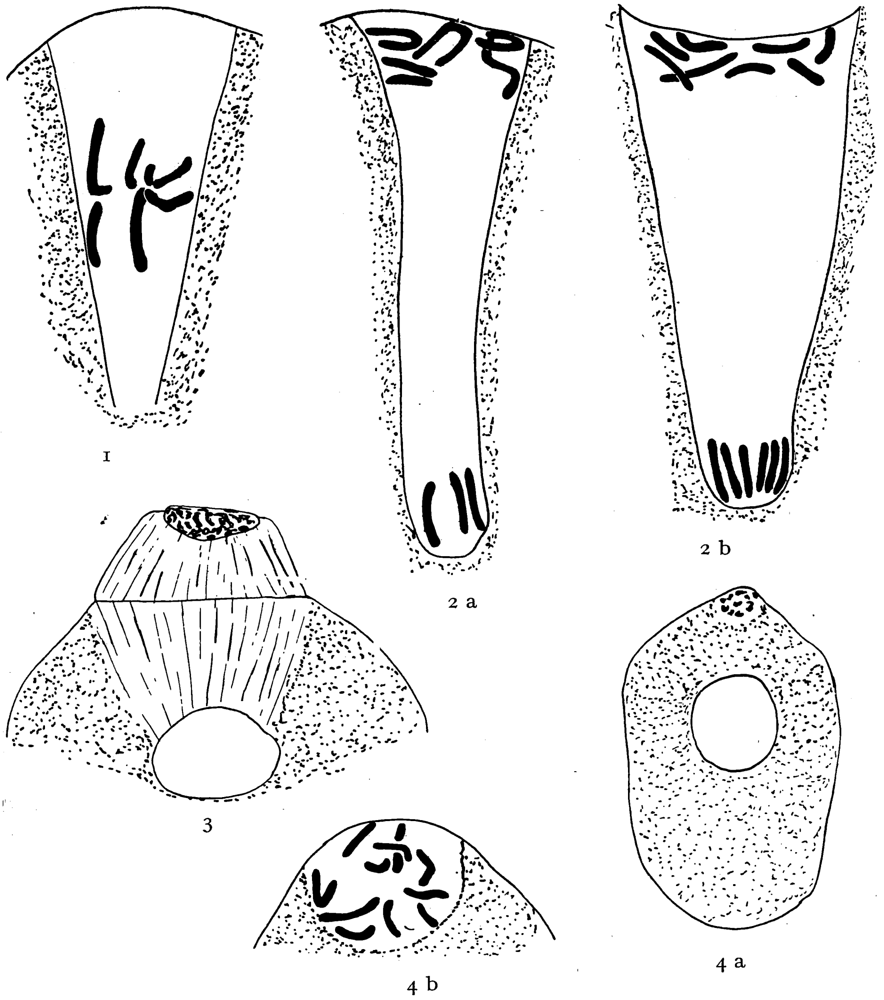
The division of the central nucleus occurred this day. It has been described by Miyake but some of my own observations may be reported. Miyake found this division five days or one week before fertilization. However, it occurred here still more close to fertilization, about two days. The large central nucleus is close to the neck of the archegon and, as has been described by Miyake, the first spindle fibres appear in the cytoplasm outside the nucleus at its inner side. The spindle formed is described as "more or less pointed at the lower end and somewhat blunt on the upper side". However, the apparent asymmetry of the spindle is still more pronounced than the cited words indicate. As is seen in Fig. 1 the spindle may seem unipolar, the outer half-spindle occupying a large part of the cell surface. The presence of an equatorial plate proves the functional bipolarity of the spindle. After anaphase the spindle elongates considerably, at the inner pole the egg nucleus is formed. Later this nucleus enlarges and moves to the centre of the archegon.

At the outer pole the chromosomes are scattered, the stage designated "tassement polaire" is often absent (Fig. 2 a, b). The ventral canal nucleus formed here is often broad and usually very ephemeral. The ventral canal cell has variable appearance: it may be dome-shaped (Fig. 3) or very low and broad. In most archegons the ventral canal cell was partly or completely severed from, often lying at a distance from the egg. This I only observed in spruce, perhaps it was caused by the fixation liquid. In the egg cytoplasm a large so-called pollination vacuole is formed (compare Fig. 9).

Occasionally the chromosomes at the outer pole were too scattered to allow the forming of a common nuclear membrane (Fig. 4), this was also observed in pine. In such cases the chromosomes do not form micronuclei, they remain unchanged, but once three of them had divided more than one week after mitosis. The absence of a ventral canal cell seems to make the penetrance of the pollentube into the archegon impossible, archegons with a chromosome group but no cell being unfertilized (Fig. 4 a).

In the prothallus mitotic activity still is frequent. Peculiar is, that the nuclei of the jacket cells now very often show prophase or, more rarely, metaphase. The chromosomes here are larger than in undifferentiated prothallus cells.

23/6.—Fertilization had probably in most ovules occurred two days earlier



1

2 a

2 b

3

4 a

4 b

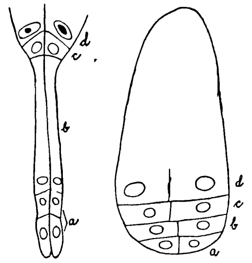
Figs. 1—4. Formation of the ventral canal cell. 1 : metaphase of the central nucleus of the archegon, the spindle is asymmetrical. — 2 a and b : after anaphase the two chromosome groups have a different appearance. — 3 : the ventral canal cell is formed. — 4 a : no ventral canal cell has been formed after the division of the central nucleus (compare the text). — 4 b : the chromosome group at the basal end of this archegon more highly magnified. — 1—3 spruce, 4 pine.

and thus was not observed in spruce. Only once the male nuclei were observed outside the archegons. After the entrance of the pollen tube the archegonic neck often was open. Photomicrographs of Juel showing two gametic nuclei in contact have been reproduced in Dahlgren (1931) and in Svensson (1936). A few times the first and second division of the zygote nucleus was observed, these stages, however, showed faulty fixation. Most ovules contained a more advanced stage, four or eight proembryo nuclei already being formed.

The formation of the proembryo of *Picea abies* has been described by Miyake, his observations have been confirmed here. As in other *Pinaceae* four nuclei first formed move to the distal (apical) part of the archegon. After mitosis the proembryo consists of two tiers each with four cells. The inner tier first divides, then follows the division of the apical tier. The proembryo then is complete counting 16 cells in four tiers, the cells of the innermost tier being incomplete with no wall against the interior of the archegon.

Generally the ovules contain more than one embryo. As many as four archegons may be fertilized owing to very favorable pollination conditions in the year 1954. Thus so-called primary polyembryony was almost the rule in this material; cleavage polyembryony does not occur in spruce. Mitotic activity now was weak in the prothallus which, however, expands owing to the increases of cell size. In rare cases the first starch had appeared in the prothallus, usually near the distal ends of the archegons.

28/6.—The two last divisions have now occurred in the proembryos which thus are complete. In most ovules proembryos had, however, undergone certain changes. Thus the cells of the second layer (c)—counted from the interior of the archegon—now are large and vacuolate. These cells are called rosette cells, in spruce they never divide. The cells of the third layer (b), the suspensor cells, are more and less elongated and have broken through the distal end of the archegon. The apical cells (a) thus have been pushed more or less high up in the prothallus. The first division of the four apical cells was sometimes observed. Buchholz (1942) has stressed that this division in the genus *Picea* is rather early, while in *Abies* which has a similar embryology it occurs later, after the suspensor has attained considerable length. The prothallus now contains more starch grains, these are assembled near the embryo.



2/7.—The suspensor has been much elongated, the embryo proper often being rather near the apical end of the seed. The primary suspensor cells first formed have been supplemented by two or three layers of so-called secondary suspensor cells or embryo tubes. These are formed of the apical cells of the proembryo. The suspensor parts of the embryos are lying rather free in a cavity which now has been formed in the longitudinal axis of the seed. The embryo proper is rather small consisting of 3—5 tiers and still only four cells in each tier; this part of the embryo is imbedded in prothallial tissue (Fig. 16). The seed often contains more than one embryo, as a rule lying at different levels in the prothallus, each embryo is surrounded of much starch.

Other parts of the prothallus have less starch which always occurs in the form of simple grains.

At the basal part of the seed certain changes occur. The content of the archegons is emptied, somewhat later jacket cells and neighbouring tissue also disappear. Cell divisions may be rather frequent here but there is no starch in these ephemeral parts of the prothallus. Isles of prothallial tissue may look similar to a young embryo.

8/7.—A more rapid development of the embryo has set in. It is now considerably broader than the suspensor, showing for instance eight cells along the diameter of the embryo. The length of the embryo was often 8—16 cell tiers (Fig. 17). Embryos with retarded development have often disappeared, one or more rarely two larger embryos being left. The prothallus of the seed is now being changed to an endosperm, true storage by degrees filling the prothallus replacing the simple grains of starch earlier visible in the cells. The apical part (top) of the prothallus is filled considerably later; there is often a sharp limit in the endosperm between parts with different cell content.

15/7.—A further increase in size of the embryo has occurred. There is a rather large variability among the seeds, more evident now than during earlier stages. Unfertilized ovules may have considerable size, but are always thin and opaque owing to the absence of storage in the endosperm. Other embryo-less ovules had simple starch grains of the kind which is the normal precursor of true storage; in such ovules fertilization probably had occurred though embryo development had been interrupted and the embryo had disappeared.

No traces of the archegons are now observed. The endosperm bordering the central cavity of the seed has no starch, then follows a zone with simple starch grains while the largest part of the endosperm has true storage. Thus the endosperm often has three zones, the largest with true storage, the inner ones ephemeral being absorbed later by the thickening embryo.

22/7.—The embryo may occupy 1/3 or sometimes more of the length of the seed. It grows in the direction of the basal end of the seed, filling gradually the cavity which contained the suspensor. A considerable thickening of the embryo occurs, and cotyledons appear at the apical end of the embryo (Fig. 18). There is now in most seeds only one embryo.

29/7.—The embryo fills most of the central cavity in more advanced seeds, the cotyledons are rather high and a small plumule may already be visible. In other seeds the cotyledons were just appearing. The endogenous formation of the root may have been initiated rather far from the basal end of the embryo (compare Fig. 19).

5/8.—The differentiation of the embryo into cotyledons, plumule and primary root is completed. The number of cotyledons was eight, they were united in pairs, at the base. Small grains of storage appear in the embryo. A small, undifferentiated second embryo may still exceptionally be observed at the basal part of the seed. Here curved suspensor cells persist.

12/8.—Further changes of the fullgrown embryo are the formation of provascular bundles in hypocotyle and cotyledons and the gradual increase of storage in the embryo.

The prothallus of unfertilized ovules is now showing degeneration. In large parts the nuclei had disappeared, the cells being completely empty.

19/8.—There is now much storage also in the cotyledons of the embryo.

26/8, 8/9, 23/9.—The content of storage in embryo and in endosperm seems to increase. Morphological and anatomical changes of the embryo hardly can be observed. Surely physiological changes occur, thus no doubt the water content of the embryo and the endosperm diminishes.

Spruce growing in Holmsjö.—Seed development was rather similar here. A difference, however, was the lower number of archegons in the prothallus. Three was the most common number, but many seeds showed only two archegons. The number of trees investigated was small, however, the difference observed may be due to chance.

Pollination had probably occurred at Ascension, that is 27/5, fertilization about Midsummer Eve (at its old date, 23/6). Fixations made on 24/6 showed beginning formation of the proembryo. On 28/6 the proembryo had 16 cells and in some ovules elongation of the suspensor cells had set in. Some starch grains were now visible in the prothallus. In 1/7 the four apical cells of the proembryo were observed dividing simultaneously. On 5/7 there was more starch grains in the prothallus. To the primary suspensor were now added secondary suspensor cells. The embryo proper was still not broader than the suspensor, it consisted for instance of four tiers. On 8/7 the length of the embryo could be 7—10 or in another tree 20 tiers, while the diameter was 5—6, respectively 10 cells. There is one or two embryos, one of them sometimes near the base of the seed. The embryos may more rarely be on the same level. On 17/7 a further development had occurred. In 3/8 the embryo had attained nearly its full length, the cotyledons were growing and the root was visible.

Conclusion.—The time between pollination and fertilization was found to be about four weeks. This is in conformity with observations of Miyake who investigated spruce from Ithaca, New York. He reports pollination about the second week of May, fertilization about the 15th of June. The growth of the pollen tube was at first very slow, but later, probably after the division of the generative cell, accelerated. About two days before the penetration of the pollen tube through the neck of the archegon the egg nucleus and the ventral canal cell are formed, the former enlarging considerably. The rather complicated formation of a proembryo lasts about one week, in a further week suspensor elongation and formation occurs, 2—3 weeks after fertilization embryo development becomes more rapid, but later it is still more accelerated, the period of filling the cavity of the seed and differentiation being about two weeks. It was often completed on 5/8. Further development during the month of August involved the inner anatomical differentiation of the embryo and the assembling of storage in the embryo.

Seed development during the summer also includes the changing of the prothallus into an endosperm, filled of storage. Simple starch grains appear in the prothallus during the formation of the proembryo. They are first seen along the path of the elongating embryo but later are also scattered in other parts of the prothallus. These simple starch grains are more of the nature of precursors to the true and definite storage substances which later fills that part of the prothallus tissue, which persists in the mature seed.

Development of the pine seed

A beautifully illustrated description of earlier stages of seed development in *Pinus* one finds in the classical paper of Ferguson (1904). She has investigated several species though not *P. silvestris*. The excellent photomicrographs of H. O. Juel of fertilization and proembryo formation of the latter species are reproduced in an essay of Dahlgren (1931), some also in Svensson (1936). Embryo formation in *Pinus* has mostly been studied by Buchholz. It is described in the books of Johansen (1950) and Wardlaw (1955). Here ovules from four different trees were investigated.

End of May 1953.—Pollination.

12/6, 1954.—The pollen tube had grown through about 1/3 of the nucellar cap. There are large masses of starch in the cap and also the pollen tubes contain much starch.—The female prothallus is completely cellular, each archegon being surrounded by a distinct jacket layer. The archegonial neck consists of two cell layers, the central nucleus lies close to the neck. The number of archegons was higher than in the *Pinus* species investigated by Ferguson, five seemed the most common number. The prothallus was large but still showed strong mitotic activity.

15/6.—The pollen tubes now had grown half-way through the nucellar cap. The actual division of the generative nucleus was not observed, only prophase. —The archegons are enlarged, their form is more spherical than in spruce. The archegons are now rather deeply imbedded in prothallial tissue, more than in spruce, though their neck always is in open communication with the surface of the prothallus.

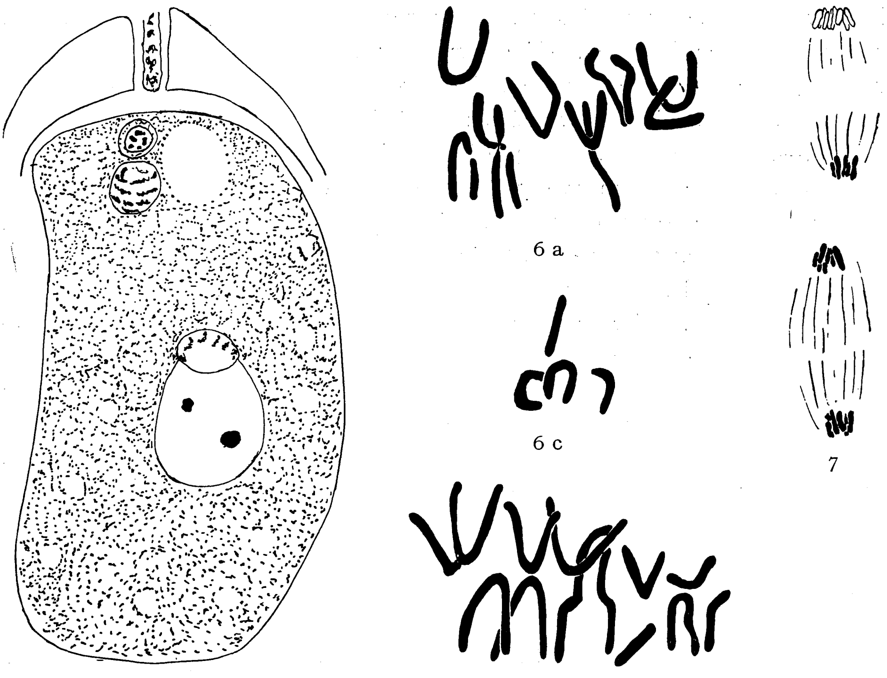
19/6.—Pollen tubes now had grown through the nucellar cap, they may have rached the prothallus but their penetration to the archegonial neck never had been initiated.—The female prothallus now showed decreased mitotic activity. In the archegons the large central nucleus was still observed close to the neck. Thus a ventral canal cell had not been formed.

23/6.—In two of the investigated trees fertilizations were common, while in two trees the proembryo may have attained the eight-nucleate stage, fertilization here being probably the day before. At the time of fertilization the mitotic activity in the prothallus had ceased except in the jacket cells which often divide. The form of the archegon is somewhat changed, but the archegons are generally broader than in spruce. The mitosis leading to the formation of the ventral canal cell was not observed here, it is profusely figured in the paper of Ferguson. The ventral canal cell was in pine broad and low, always lying close to the egg.

As is shown in an often reproduced figure of Ferguson the pollen tube does not grow into the archegon, only emptying its content. Slightly earlier a vacuole (a so-called pollination vacuole) is formed in the egg-cytoplasm near the neck, this vacuole persists after fertilization. The broad, empty pollen tube I often observed sticking in the micropyle-like opening to the eggcell (Fig. 5 and 8), certain archegons with a contracted cytoplasm showed the growth of the tube in the archegon, but it never had penetrated into the cytoplasm of the egg. In the basal (proximal) part of the egg cytoplasm soon the two male gametes are visible, they have, as often has been shown, somewhat different size (Fig. 8). There is also a small chromophilous nucleus from the pollen tube, presumably the stalk nucleus (Fig. 5). The tube nucleus seems more difficult to observe. The larger male nucleus soon is in close contact with the egg nucleus. The latter is here still larger than in spruce.

As was shown by Ferguson and other investigators, fusion of the gametic nuclei is delayed until the forming of the spindle, but her contention that the male and female chromosomes keep separate during the whole division I am not able to confirm (Fig. 6). The spindle is, as is seen in the figure 9, intranuclear. The chromosomes are rather large, having about ordinary somatic size. At the second division of the zygote nucleus the two spindles are rather close together, often forming right angles (Fig. 7), with a tetrahedral arrangement of four nuclei as the result.

These four nuclei move to the distal end of the archegon, there forming a tier and enlarging considerably. A new phase of proembryo formation has thus been initiated. The movement of the nuclei must have been rapid, not being observed. Johansen (1950) states that it is effected through special attraction fibres. This seems to be accepted by Wardlaw but seems a rather curious statement. The second phase of proembryo formation ends with the formation of the usual proembryo of *Pinaceae,* four tiers each consisting of four cells,



5 6 a 6 b 6 c 7

Figs. 5—7. Fertilization, and first division of zygote nucleus. — 5: archegon with male nucleus and egg nucleus in contact, at the basal end of the archegon one observes the second male nucleus, the stalk cell from the pollen tube and the pollination vacuole; the pollen tube is seen in an opening of the prothallial tissue. — 6 a, b, c,: the chromosomes of the mitosis in Fig. 9. — 7: the second division of the fertilized egg nucleus. Pine.

the proximal cells are incomplete, that is they are open against the archegon. The divisions have several times been described. At mitosis the chromosomes are very slender.

28/6.—In some advanced ovules the elongation of the four suspensor cells of the proembryo had been initiated pushing the apical cell tier into the prothallus. More often the archegon had an unchanged proembryo. Only one or two proembryos are formed in the ovule, a high percentage of the archegons remain unfertilized. In such archegons the egg nucleus rather increases in size and may be lobed; on the egg lies a degenerating ventral canal cell. The chief cause of fertilization failures was lack of pollen tubes owing to inadequate pollination. A second cause, of rather small importance, however, was incapability of a pollen tube to penetrate into the archegon, a few times a degenerating tube with small nuclei being observed.

Now some starch may appear in the prothallus, usually near the distal ends of the archegons where the proembryo grows into the prothallus. Jacket cells with large chromosomes may have invaded the archegons. Several investigators have previously reported this phenomenon, which, however, has been denied by other authors. Perhaps it is an artefact caused through pressure at the fixation of the ovules.

2/7.—The primary suspensor cells generally are elongated (Fig. 10). The apical cells of the proembryo often have divided one or two times. Through these divisions subsidiary suspensor cells are formed which contribute to the elongation of the embryo suspensor. The form of the end-cells facilitates the penetrating of the embryo into prothallus tissue. There is now more starch near the embryo parts sticking in the tissue.

8/7.—Though the suspensor has been elongated, it generally is shorter than in spruce, the embryos being less near the apical end of the seed. The division of the embryo into four, each consisting of a row of cells has often occurred. This so-called cleavage or secondary polyembryony occurs regularly in *Pinus*. The four embryos are rather near one another and soon increase in diameter (Fig. 11 and Fig. 12). But the cleavage may fail, one finds ovules with only one large embryo. At the basal part of the seeds formation of so-called rosette embryos may be initiated through the division of the cells of the rosette tier of the proembryos. They never attain larger size.

The seeds now show large changes. The archegons and the prothallial tissue at the basal part of the seed have disappeared leaving a funnel-shaped cavity which is continued through the central part of the seed owing to dissolution of the prothallial tissue surrounding the embryo suspensors. The changing of the prothallus to an endosperm has now set in, storage appearing in the cells. The endosperm nearest to the central cavity remains free from starch or has a few single starch grains.

15/7.—The embryos now show increased diameter. Their length is very different. It may be about 20 cells but more often about 7—10. Sometimes the seed has only one comparatively large embryo; thus cleavage of the primary embryo had failed. The endosperm shows three concentric zones. The innermost has no starch or only simple grains, the middle zone has starch masses, while the cells of the peripheral zone have a dense content but no solid storage (Fig. 15).

22/7.—Larger seeds often contain only one embryo. The largest embryo had attained 1/4 of the length of the seed and a very rapid embryo growth now is setting in. The cavity of the seed is extended in apical direction and the growing pine embryo has room to elongation also in the latter direction.—Rosette embryos and other embryos with retarded or interrupted development occur. In exceptional cases no embryo attains the stage of differentiation.

29/7.—In larger seeds the embryo may fill most of the cavity of the seed, also absorbing starch-less parts of the endosperm. The cotyledons are often visible. Accessory small embryos may still be observed near the base of the seed.

5/8.—The embryo may now have attained almost full size. The cotyledons are large, the differentiation of a primary root has started. The endosperm may be more homogeneous, the outer zone now being filled of solid storage. Scattered starch grains may be observed also in the embryo.

12/8.—The embryo has plumule and root. In the hypocotyle and the cotyledons one observes provascular bundles. The quantity of storage gradually increases.

19/8.—Much storage is now also accumulated in the cotyledonal part of the embryo. The endosperm is very packed with storage, epidermis being filled later than the rest of the endosperm.

8/9.—The plumule of the embryo consists of very large cells showing no mitosis. Mitotic activity in the embryo now is weak.

23/9.—There was still no storage in the plumule or the root tip.

7/10.—The seeds still show certain differences in degree of development.

21/10.—The morphological and anatomical differentiation of the embryo seems always complete though in the root cap, that is the tissue between root and the end of the embryo, mitotic activity still continues. The plumule consists of rather few very large cells which may contain storage.

Conclusion.—The seed development in pine rather closely corresponded to the development in spruce, although pollination of the ovules was a whole year more early. The earliest collection of pine ovules investigated showed that the pollen tubes only had grown through 1/3 of the nucellar cap while fertilization was one or two days delayed compared with spruce which had been pollinated in the year 1954. At the vicinity of Uppsala fertilization about Midsummer has been observed (Juel). The development of the proembryo seems to consume the same time in spruce and pine, but the process of embryo cleavage in pine induces a delay which may be estimated to one week. Buchholz has discussed the problem of embryo cleavage in *Pinaceae,* he has stressed, that it is a primitive character and an adaptive character leading to a competition of embryos and a survival of the fittest embryo. This period of seed development in *Pinus* he calls the period of embryo selection. His observations on the later development are best summarized with the words of Wardlaw "once prominence has been attained by one embryo it grows rapidly, and differentiates all its organs in 10—12 days, seed ripening is completed in a few weeks with little change in seed size" (Wardlaw p. 194).

Concerning *Pinus ponderosa* Buchholz (1950) states that embryonic selection covers nearly six weeks while the differentiation of the embryo into root, stem and cotyledons occurs during a very rapid growth covering only 10 days or 2 weeks. In *P. silvestris,* however, the period of embryo selection is shorter and hardly gave evidence of a struggle for life among embryos. Most embryos cease growth and disappear early, one or more rarely two continuing development. Only one embryo usually attains the stage of differentiation, in most seeds the one with the longest suspensor. No doubt the cleavage of the primary embryo entails a developmental delay in comparison with spruce but the delay is hardly more than one week. The following stage of growth and outer differentiation is still more rapid than in spruce. As a last period of seed development may be considered formation of storage in the fullgrown embryo. This continues long, complete seed dormancy was not attained near the end of October.

General conclusions

The seeds of conifers are very similar to angiospermous seeds of the more primitive type with endosperm. An endosperm is formed which partly is used to the nutrition and formation of the embryo, but mainly is a storage tissue which is emptied during the germination of the seed. However, the seeds in the genus *Callitris* (fam. *Cupressaceae*) show resemblance to so-called endosperm-less seeds in angiosperms, the mature embryo almost filling the seed, the endosperm being reduced to a thin layer (Baird l. c.). As is well-known the similarity of endosperms does not imply morphological homology, the endosperm of conifers is a haploid prothallial tissue. This tissue becomes filled of storage some weeks after fertilization. Thus it seems somewhat ambiguous to describe the meaning of suspensor elongation in *Pinus* with the wording "the embryo has been thrust into the richly stored cells of the prothallus" (Wardlaw p. 192). It is a general interpretation that the nutrition of the coniferous embryo is facilitated through this suspensor elongation, but it must mainly be through the absorption of cells containing liquid carbohydrates or simple starch grains. The storage tissue is only of importance at germination. It may be suggested that a primary factor of suspensor elongation is the necessity to give the developing embryo sufficient room to growth, the mature embryo being very large. It must be appropiate to move the embryo before the prothallus is changed to a hard storage tissue. It might be objected that in many angiosperms the embryo grows and fills the seed. However, in angiosperms the forming of endosperm is initiated later, after fertilization, storage is formed in proportion later, thus the embryo here grows into a still soft endosperm.

Striking differences between *Picea* and *Pinus* are the occurrence of very early pollination and of cleavage polyembryony in the latter genus. In *Pin us silvestris* the interval between pollination and fertilization is more than a year. A similar interval has been found in the third conifer native in Sweden, *Juniperus communis,* Norén (1907) observing pollination at the end of June, fertilization during the first week of July next year. Usually the interval is constant for the species. In *Callitris robusta,* however, Baird on the same tree observed cones that after pollination were dormant until the following year, as well as cones growing in the same season. In some *Callitris* species the interval is 20 months. In all coniferous families long intervals occur but except in *Araucariaceae* all families also have species with shorter intervals. These two types of pollination may occur within the same genus, in *Juniperus virginiana* pollination and fertilization are only separated through an interval of two months (see Schnarf 1933). Schnarf remarks, "die genannten grossen Zeitunterschiede — ein Jahr und darüber — sind wohl sicher keine ursprünglichen Charactere sondern hängen sicher mit tiefeinscheidenen Wirkungen des Wechsels der Jahreszeiten . . . zusammen" (l.c. p. 265).

Cleavage polyembryony Buchholz considers a primitive character in conifers. This view has been widely accepted (but compare Baird l.c. p. 292). This does not hold true, however, of his hypothesis that cleavage polyembryony is a mechanism effecting competition among embryos, the most fitted being selected. It seems curious that such an embryo also should be the best in nature. The objection that cleavage embryos from the same zygote must be genotypically identical, Buchholz (1950) has accepted concerning nuclear factors, but he is of the opinion that "differences in these embryos are due to variations in the amount of male cytoplasm or substances diffused from the male, at the time of fertilization". This reasoning is highly hypothetical. The theory of embryo selection may appear elegant but it seems improbable. The embryological evidence of a competition is, as was mentioned on p. 16, small in *P. silvestris.*

Thus, the significance of cleavage polyembryony seems mysterious. It may be that an inherent high embryo mortality in early conifers was compensated through a multiplication of the primary embryos, but cleavage polyembryony is probably no adaption being a separation of four perhaps loosely connected cell rows, each growing through an apical cell. The four apical cells divide rather synchronously before separation.

Miyake, Ferguson and other investigators have stressed that the time relations of seed development are somewhat different in different years, probably chiefly owing to different conditions of growth and different time of pollination. During the summer 1954 fertilization and embryo formation in spruce and pine ran parallel. The embryo was morphologically and anatomically rather complete before the end of the summer. In higher latitudes with later pollination there obviously often but not always is time to completed embryo growth. Simak and Gustafsson (1954) have through grafting proved that the incomplete development and low germination of pine seeds from northern regions is due to poor ripening conditions and not to any special genetical constitution of North Swedish populations. "The problem of seed development in northern regions is thus chiefly a question of growth and maturity" (l. c. p. 72). Some observations of Norén (1907) indicate a very slow development of the juniper seed on northern latitudes. Investigation of material collected on 60°36′ showed a delay of one month, compared with the material of the main investigation collected on 58°22′. The material from 68°23′ showed a delay of two months.

Summary

Seed development in Norway spruce and Scots pine in the summer 1954 was investigated. Though pollination in pine was about one year more early fertilization in both species occurred near Midsummer. Embryo development was rather parallel and was in the vicinity of Stockholm completed before the end of August.

Sammanfattning

Fröets utveckling hos gran och tall

Befruktning och embryoutveckling hos gran och tall under 1954 har studerats. De undersökta fröämnena hade samlats från några träd vid Skogshögskolan, varvid de tidigaste fixeringarna utfördes den 12/6.

Granens pollinering hade skett 24/5, men 12/6 hade pollenslangen endast vuxit genom 1/10 av nucellus. Den växer senare snabbare och hade 19/6 vuxit genom nucellus. Samma dag delades arkegoniets s. k. centralkärna, vilket medför bildningen av äggkärna och bukkanalcell. Befruktningen kunde ej närmare studeras hos gran, den skedde ett par dagar senare och 23/6 var bildningen av proembryot inom arkegoniet igång. 28/8 var det 16-kärniga proembryot färdigt och i åtskilliga frön hade dess suspensorceller börjat förlängas. Den 2/7 hade suspensorn ofta nått sin fulla längd och det egentliga embryot bildas högt upp i prothalliet nära fröets spets (Fig. 16). Som följd av gynnsamma pollineringsbetingelser befruktas flera arkegonier och bildas flera embryoner (primär polyembryoni), men de övertaliga embryonerna sluta snart sin tillväxt och försvinna, äldre frön ha i regel endast ett embryo. 8/7 hade embryot blivit större och reservnäring börjar avlagras i prothalliets celler varigenom detta förvandlas till ett endosperm (fig. 17). 15/7 visar embryot något hastigare tillväxt och skillnaden i utvecklingen i olika frön är mera framträdande. Den 22/7 växer embryot hastigt mot fröets basala del medan hjärtbladen börjar synas i embryots spets (fig. 18), tillväxten blir nu så snabb att embryot 29/7 nästan nått fröets längd, hjärtbladen är utvuxna och roten anlägges. Den 5/8 hade embryot erhållit sin yttre morfologiska differentiering. Senare följer en inre, anatomisk differentiering och upplagring av reservnäring

i embryots celler. I fixeringar från slutet av augusti eller senare kan ytterligare förändringar knappast iakttagas.

Tallfixeringar hade utförts samma dagar som granfixeringarna. Ehuru tallen pollinerats ett år tidigare var utvecklingen av fröna parallell. Befruktningen, som ingående kunde studeras, skedde 23/6. Pollinationen hade varit mindre riklig och antalet obefruktade arkegonier var ganska stort men eftersom fröämnet innehåller flera arkegonier utvecklas nästan alltid embryo, ofta flera (primär polyembryoni). Tallen visar ofta sekundär polyembryoni: det unga embryot uppdelas i fyra celltrådar, som var för sig kan bilda ett embryo. Antalet embryoner blir därför större än hos gran, men de äldre fröna innehålla även här i regel endast ett enda, stort embryo. Bildningen av sekundära embryoner försenar utvecklingen högst en vecka jämfört med gran, embryots slutliga tillväxt och yttre-morfologiska differentiering är dock mycket snabb. De sista fixeringarna utfördes 21/10, mitoser kunde ännu förekomma i embryots »rotmössa», ehuru det egentliga embryot för länge sedan var färdigt.

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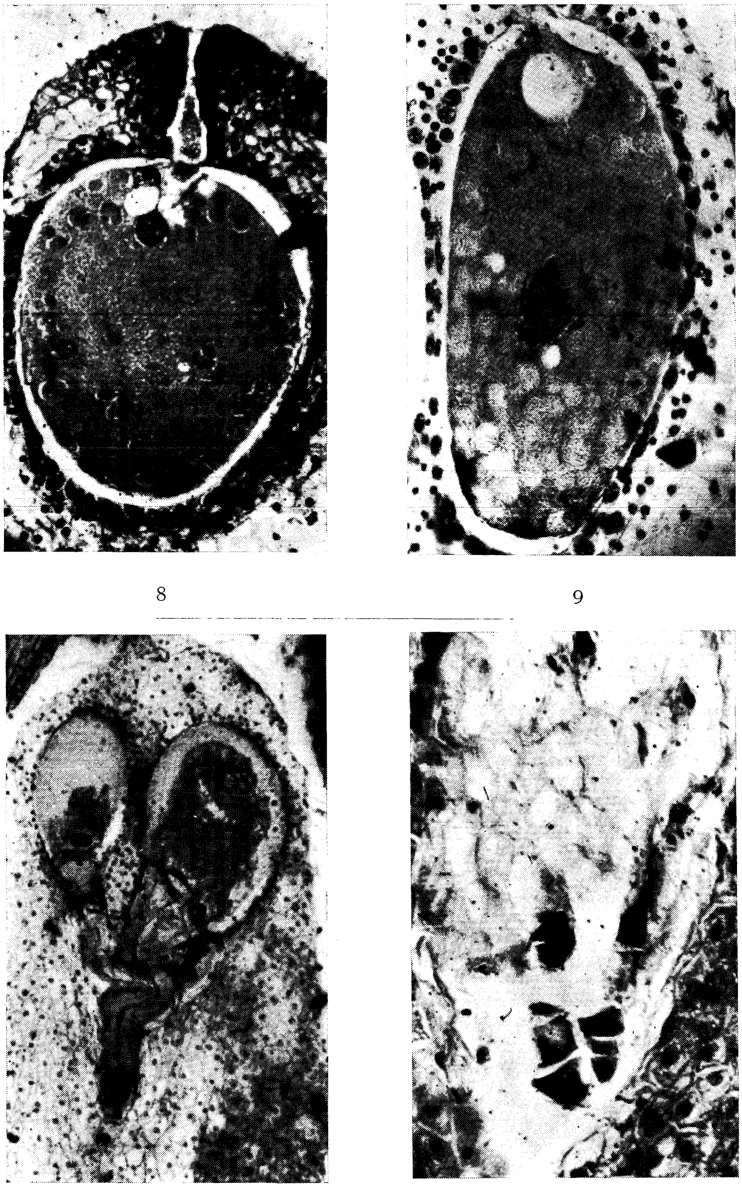
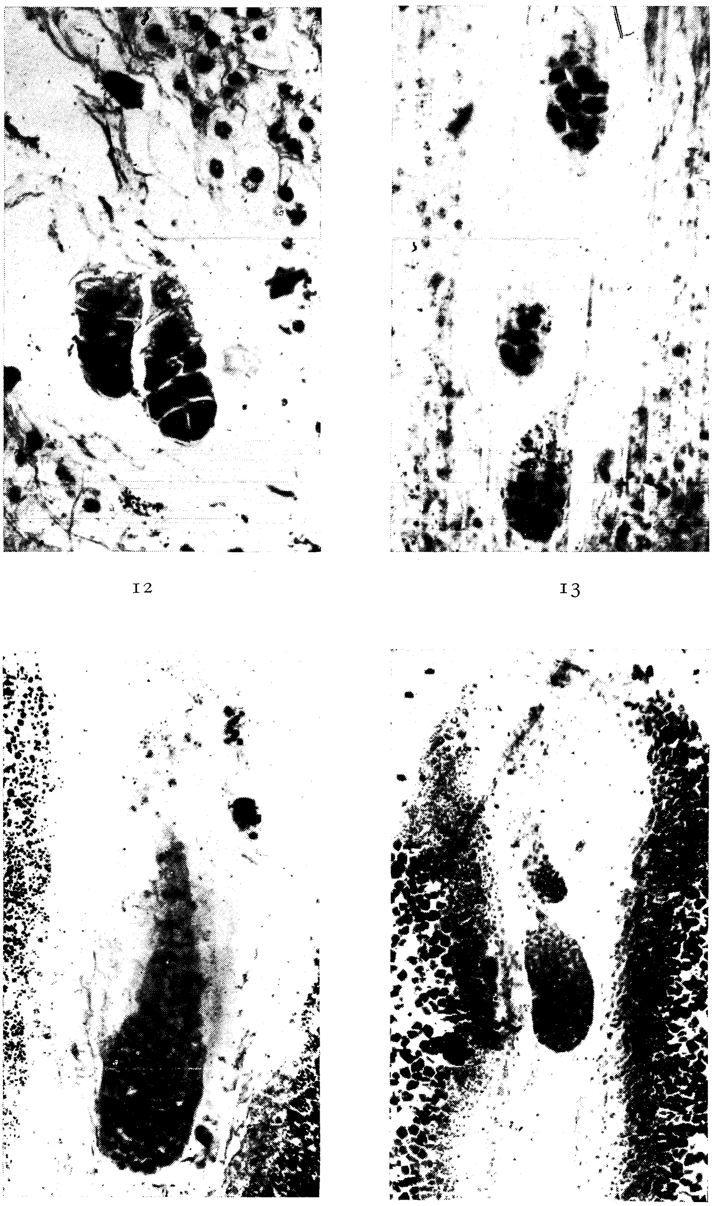


8 9

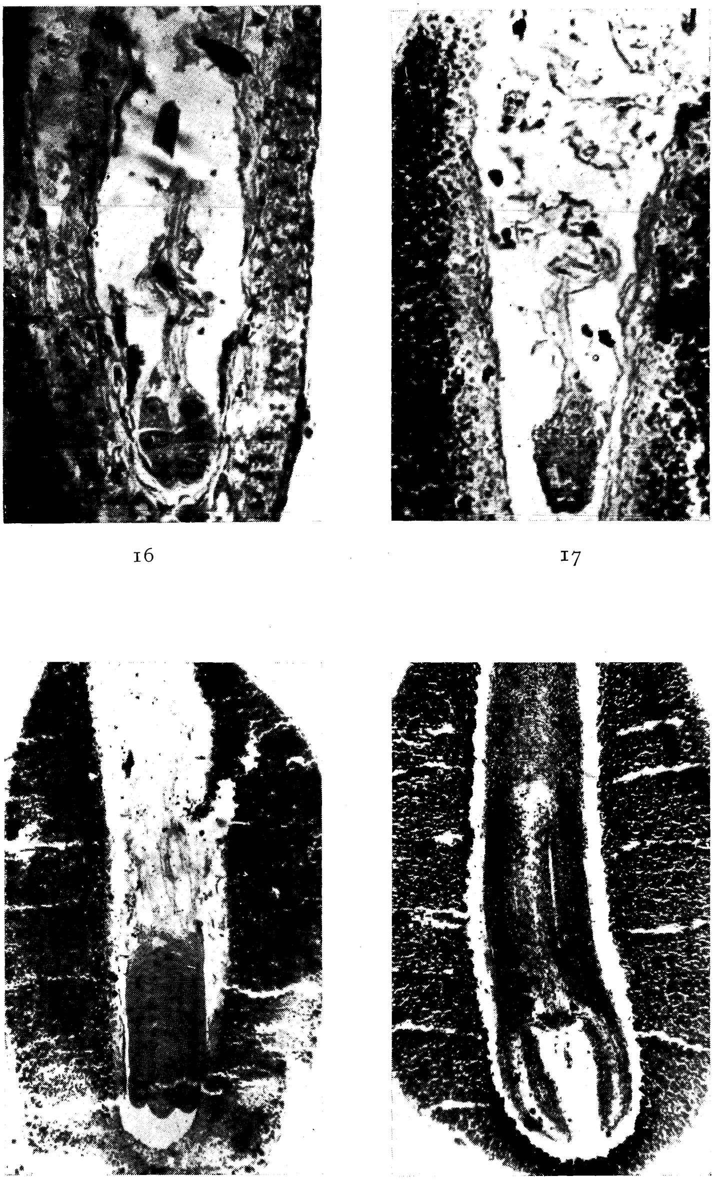
10 11

Figs. 8—11. Development after the entrance of the pollen tube into the ovule; pine. — 8: two male nuclei separated through the pollination vacuole at the basal part of the archegon. The egg nucleus was in another section. The male nuclei are of different size. —9: Division of the zygote nucleus, the spindle is intranuclear, the chromosomes are figured in 6 a—c. — 10: the suspensor cells have been elongated in two archegons: the apical cells of the proembryo are in the prothallus tissue and show prometaphase. —11: the primary embryo is divided into secondary embryos.



12 13 14 15

Figs. 12—15. Embryo development in pine. — 12: two secondary embryos (8/7). — 13: three secondary embroys (8/7). — 14: only one large embryo, remains of two aborted small embryos are seen (15/7). — 15: One large and one degenerating embryo (22/7).



16 17

18 19

Figs. 16—18. Embryo development in spruce. — 16: embryo from a seed fixed 2/7. — 17: embryo from 8/7. — 18: embryo from 22/7. — 19: mature embryo in pine. (8/9).