

## SOME ANOMALIES IN THE DEVELOPMENT OF THE SEED OF PINUS.

ALMA MARIE BELL, Indiana University.

A very brief study of the formation of the seed in certain species of *Pinus* is almost sure to reveal one or more unusual phenomena, or anomalies, which pertain to the pollen tube, to the presence and location of more than the normal number of archegonia, to more than a single endosperm, and to aborted megaspores, as well as to other details.

The Department of Botany of Indiana University has preserved a number of unusual occurrences in seed development in *Pinus* and the slides have been turned over to the writer for study.<sup>1</sup>

### HISTORICAL.

**Structure within pollen tubes.** In each of three species of *Pinus*, Ferguson ('04, p. 134) observed an instance of an extra nucleus in the male gametophyte. She interpreted them as follows: In *Pinus austriaca* Höss. two stalk cells had been formed; in *P. Strobus* and *P. rigida* probably the smaller nucleus had been cut off from the generative cell. More than the normal number of nuclei have been reported in other genera of Pinaceae. Arnoldi ('00, p. 56) concluded that two tube nuclei had been formed in a pollen tube of *Cephalotaxus Fortunei*. In *Podocarpus*, Coker, ('02, p. 93) decided that both the first and second prothallial cells may divide amitotically. However, several cases, in which more than two male nuclei are formed from one body cell in *Juniperus communis*, were noted by Nichols ('10, p. 215). He also says that Norén ('07) found in the same species one instance in which a large body cell contained three nuclei.

**More than one functioning megaspore.** The occurrence of two prothallia, probably derived from two megaspores in an ovule of *Pinus sylvestris* has been described by Farmer ('92), and Hofmeister ('51) as recorded by Ferguson ('04), and by Bartholomew ('09) as reported by Coulter and Chamberlain ('10). In *Pinus rigida*, Ferguson ('04, fig. 260, plate XXIII) figured one case of two megaspore mother cells in an ovule. The pollen tube of *Pinus austriaca* which contained super-numerary nuclei as described by Mottier ('08) was later found to be not a pollen tube but a second megaspore. This same slide is discussed below under the observations of the writer. Other instances in which two or more megaspores in one ovule may be inferred are as follows: Two megaspores in an ovule of *Taxus baccata* by Hofmeister ('51) as reported by Coker, and in the same species another instance of the same thing by Jäger ('99, p. 249); two megaspores in an ovule of *Podocarpus* and a case in *Taxodium* by Coker ('02, p. 97 and '03, p. 15);

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two megaspores in an ovule of *Juniperus communis* by Sludsky ('05, p. 212); in the same species three dividing megaspore mother cells in one ovule by Nichols ('10, p. 219); and two megaspores in one ovule of *Sequoia* by Arnoldi as reported by Sludsky ('05).

**Number and position of archegonia.** One ovule in *Pinus Strobus* showed 23 archegonia in the upper part of the endosperm, according to Ferguson ('04, p. 136). Some of the archegonia had developed from jacket cells so that part of the archegonia were just below others. She figures one instance in *Pinus resinosa* in which a small archegonium is apparently budding from the larger. Two cases of archegonia having a similar relationship were observed for *P. resinosa*, and one for *P. rigida*. However, the archegonia in each of these had developed further than in the first instance mentioned. No neck cells were observed for any of these archegonia. Another variation in the arrangement of the archegonia was described for *P. montana uncinata*. This ovule contained nine archegonia which extended from the micropylar end down along one side of the endosperm. When this form of arrangement is present, funnel shaped openings lead down from the exterior to the neck cells.

As many as three groups of archegonia in one ovule of *Pinus austriaca* have been reported by Mottier ('08). These groups were located as follows: one in the normal position at the micropylar end, one at the chalazal end, and one at one side. A few instances were observed in which there was a group near the center. In such cases there was no connection with the surface. He has also found archegonia which budded in a manner similar to those described by Ferguson ('04).

One ovule of *Pinus maritima* showed archegonia arranged along the side. An instance in which the archegonium had developed far down on one side in *Tsuga canadensis* was noted by Murrill ('00, p. 592). As a result of placing *Callitris quadrivallis* in a warm room, Juel ('04, p. 57) secured some ovules which were abnormal. One ovule contained a group of archegonia on one side in addition to the one at the micropylar end.

In *Abies balsamea* and in *Picea excelsa*, Miyaki ('03, a, p. 138, '03, b. p. 364) observed several instances of two archegonia lying one above the other.

In *Cedrus atlantica*, Saxton ('23) says that the neck generally becomes multinucleate.

**Fusion of the archegonia.** Instances in which a cytoplasmic union had taken place on the adjacent sides of two archegonia, were figured for *Larix dahurica* and *L. europaea* by Wóycicki ('24). Some of the archegonia seem to attack the endosperm cells and extend out into the endosperm. Wóycicki ('24) also reports that some of the endosperm cells contain two nuclei.

**Supernumerary nuclei in the central or egg cell.** Ferguson ('04, p. 138) noted several instances of the fragmentation of the egg nucleus and figured one in *Pinus Strobus*. One ovule of *P. austriaca* showed an immature archegonium which contained two daughter nuclei in the central cell according to Mottier ('08). In two instances in *Abies*

*balsamea*, Miyaki ('03, p. 138) found two egg nuclei in one archegonium. Sometimes an extra nucleus was observed in the upper part of an egg in *Pseudolarix*, by Miyaki and Yasui ('11, p. 643). From one to three supernumerary nuclei were often seen in the egg cells ready for fertilization in *Cedrus atlantica* by Smith ('23). In some instances he finds the ventral canal cell giving rise to the supernumerary nuclei.

**Behavior of the nucleus of the ventral canal cell.** Sometimes the ventral nucleus of *Pinus Laricio* Poiret approaches the size of the egg nucleus according to Chamberlain ('99, p. 217). He also reports ('10) that Blackman ('98) and Haydon ('07) describe similar cases in *P. sylvestris*. In both *P. Strobis* and *P. austriaca*, Ferguson ('04, p. 137) observed two instances in which the ventral canal nucleus approached the size of the egg nucleus. There was no membrane, in either case, cutting off a ventral canal cell. In *Cephalotaxus Fortunei* an egg which contained two nuclei but no ventral canal cell was noted by Arnoldi ('00, p. 54). In *Podocarpus*, Coker ('02, p. 99) observed that in one case both the ventral canal nucleus and the egg nucleus divided amitotically into a number of fragments. Hutchinson ('15, p. 458) says that the ventral canal nucleus in *Abies balsamea* slips back into the egg cell and unites with one of the male nuclei. The behavior of the ventral canal nucleus in *Cedrus atlantica* has already been referred to in connection with supernumerary nuclei in the egg cell (Smith, 23).

**Parthenogenesis.** Occasionally Saxton ('09) noticed that in some ovules of *Pinus Pinaster* containing proembryos in all stages of development, there were no pollen tubes in the nucellus or they extended only a short distance in the nucellus and no nuclei were found in them. In one ovule he observed that the egg nucleus had begun to divide before the pollen tube had reached the archegonium while the pollen tube still contained both sperm nuclei. These phenomena were interpreted by Saxton as representing parthenogenesis. Smith ('23) found that in *Cedrus atlantica* a proembryo had formed but the ventral canal cell and neck cells of the archegonium were still uninjured, and there was no trace of a pollen tube in the nucellus or in the archegonium. Many eggs ready for fertilization contained from one to three small supernumerary nuclei which suggested parthenogenesis. However, the author did not regard the evidence as sufficient to conclude that parthenogenesis took place.

**Behavior of pollen tube.** Ferguson ('04, p. 138), in *Pinus Strobis*, found a pollen tube which had conjugated with the egg by forcing its way through the sheath cells at one side of the archegonium. Four nuclei of the proembryo had formed indicating that fertilization had taken place. In *P. austriaca*, Mottier ('08) noted two instances in which the pollen tube had reached the archegonium before the ventral canal cell had been cut off, or before the archegonium had attained adult size. One of these tubes had penetrated the archegonium but had not discharged its contents. One tube started down along the side of the endosperm. In *P. Pinaster*, Saxton ('09) also observed a pollen tube penetrating laterally through the prothallus instead of entering the

canal leading to the archegonial neck. The lateral penetration of the endosperm by the pollen tube in *P. sylvestris* has been reported by Haydon ('07).

#### OBSERVATIONS.

Unless otherwise stated the anomalies which are reported in the following occurred in *Pinus austriaca* Höss.

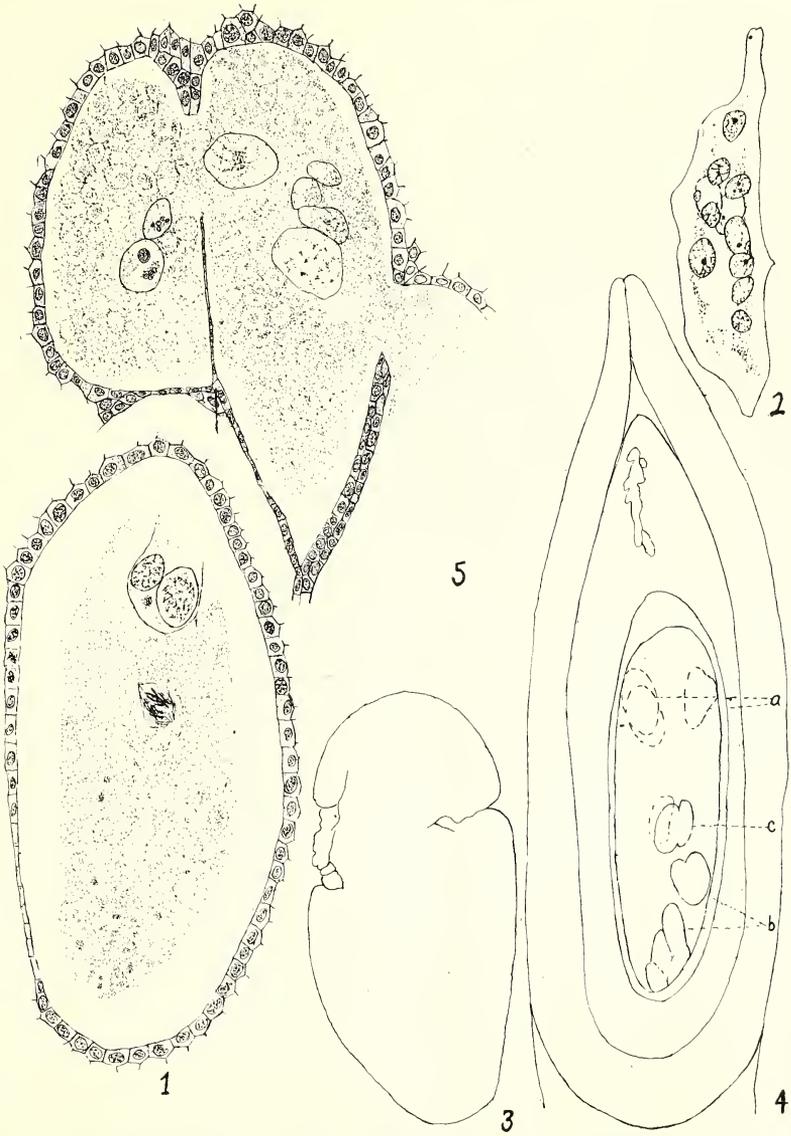
**Behavior of the pollen tube.**—In the preparations at my disposal, three instances were noted in which the pollen tube had arrived prematurely. Two of these were in *Pinus sylvestris* and one in *P. austriaca*. The archegonia in each case appeared to be quite young. Each had a nucleus in the upper part of the central cell as the ventral canal cell had not been cut off. The cytoplasm of this cell was very vacuolate. Most of these archegonia were immature and had not attained full size. One of the pollen tubes had reached the archegonium but had not discharged its contents. The other pollen tubes varied in the distance to which they had penetrated the endosperm; some had just reached the endosperm, while the more advanced touched the jacket layer. Two pollen tubes have arrived at one archegonium as shown in figure 1. A male nucleus from the first is uniting with the egg nucleus, while the other pollen tube has just entered the egg cell and still contains the two male nuclei and the remains of the tube nucleus.

The lateral penetration of the endosperm by the pollen tube in *Pinus austriaca* has been observed a number of times in the laboratory at Indiana University.

**More than one megaspore.** An instance of two megaspores was observed in one ovule. One megaspore had a well developed endosperm and contained two groups of archegonia, while the other, which was adjacent to the larger endosperm, was aborted and contained about twelve nuclei. The immature megaspore is shown in figure 2.

**Lobing of the endosperm.** A few ovules showed endosperms which were rather deeply lobed. The greatest degree of lobing is shown in figure 3. Here there is a lobe near the micropylar end which is so deep that in a few sections it seems to be altogether separate from the rest of the endosperm. Then just a little above this on the left side there are several smaller lobes varying in size. In other instances observed, the lobes varied in size from a very small lobe at one end to those which consisted of about one-third of the endosperm. Two of the ovules in which the endosperm was deeply lobed contained a group of archegonia in the lobe as well as another group in the rest of the endosperm.

**Number and position of the archegonia.** Among the ovules studied the abnormal location of the archegonia was the most common anomaly. From one to four groups of archegonia were found. These groups were located in almost all parts of the endosperm. One ovule showed a single archegonium which was situated near the center of the endosperm. In some instances another group of archegonia was present in addition to those normally occurring at the micropylar end. One case was noted



Figs. 1-5—All figures for *Pinus austriaca* Höss. 1. Longitudinal section of archegonium showing a stage in fertilization. Second pollen tube at the top of the archegonium. The place at which the tube entered the archegonium lies in a different section. X 250. 2. Aborted megaspore containing twelve nuclei. X 250. 3. Lobed endosperm. X 50. 4. Longitudinal section of an ovule showing three groups of archegonia, a, b, c. X-50. 5. Longitudinal section through two archegonia and a part of the third showing the cytoplasmic connection. X-250.

for *Pinus austriaca*. The position of this second group varied from the sides to near the chalazal end; and there were a few which occurred near the center of the endosperm. A few ovules contained a third group of archegonia which was about midway between the micropylar and chalazal ends, either near the geometric center or on one side. This arrangement was noted for one ovule in *P. sylvestris* and one in *P. austriaca*. One instance showed four groups of two archegonia each. In this case besides the normal group at the micropylar end there was a group on either side at the chalazal end and a fourth a little above these two groups on the left side. Figure 4 shows an ovule in which three groups of archegonia are present. It contains a total of twelve archegonia which are arranged in the following manner: one group of three at the micropylar end, *a*, a second group of six near the chalazal end, *b*, and a third group of three a little below the center near the chalazal group, *c*. The group at the micropylar end has two archegonia on the left side and one on the right. The archegonia in group *b* vary in size. Of the four lower ones the two upper archegonia are larger than the other two. The two upper archegonia and the lower one on the left side have partially fused, a cytoplasmic union having taken place at the adjacent sides of the two archegonia on the right with the archegonium on the left; and the adjacent ends of the two archegonia on the right have fused. The two remaining archegonia of this group are to the right and somewhat above the other four. They are separated from the lower ones by one layer of cells. A cytoplasmic union has taken place between the contiguous sides of these two. Group *c* is above the archegonia just described and almost centrally located. The jacket cells of the adjacent sides of two of these have disappeared, and here also a partial cytoplasmic union has taken place. The third archegonium is just a little above these two in another plane. Some of the archegonia in the lower groups of figure 4 have jacket cells which are very much enlarged. These cells contain very large nuclei, and the cytoplasm is similar in character to that of the egg. The greatest number of enlarged jacket cells observed for one archegonium was in the larger archegonium on the right side at the chalazal end. Another ovule showed archegonia in which the jacket cells were quite large and contained large nuclei; but here the cytoplasm was more dense than usual. There were no neck cells for the two lower groups of archegonia. The absence of necks was frequent in archegonia which were found at or near the chalazal end and in those near the center.

One archegonium showed both the egg nucleus and the ventral canal nucleus in the spirem stage before fertilization had taken place.

**Supernumerary nuclei of the egg or in the central cell.** A few of the archegonia studied showed from two to six supernumerary nuclei. Save in one instance, each archegonium containing supernumerary nuclei in the egg cell had a cytoplasmic connection with the adjacent archegonium. The ovule in figure 4 has four archegonia in each of which there are from two to three nuclei. Five of the six nuclei found in one egg cell are shown in figure 5. The sixth nucleus is behind the lower one. In this case the archegonium has a cytoplasmic connection with the

one to the left and the one below. The archegonium to the left also has two nuclei. Some of the archegonia which showed supernumerary nuclei were very young and contained but little cytoplasm in the central cell. The nucleoli were very distinct in these instances. There was, in most instances, very little difference in the size of the nuclei, except in the case shown in figure 5.

#### SUMMARY.

A few instances were observed in which the pollen tube had arrived prematurely.

In one case two pollen tubes had arrived at one archegonium.

One ovule contained two functioning megasporangia, one with a well developed endosperm, and the other with about 12 free nuclei.

Some ovules showed an endosperm which was deeply lobed.

The groups of archegonia in one ovule varied from one to four. These groups were found in nearly all parts of the endosperm. However, many of those which were in any other than the normal position at the micropylar end showed no necks. This was altogether the case with those near the center. In certain cases a few of the jacket cells were quite large.

One archegonium showed both the ventral canal nucleus and the egg nucleus in the spirem stage.

Except in one instance in which there were supernumerary nuclei in the egg or central cell, the archegonia were united by cytoplasmic connection.

Lateral penetration of the endosperm by the pollen tube was observed in *Pinus austriaca*.

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