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PARTHENOGENESIS IN THALICTRUM PURPURASCENS

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(DEPARTMENT OF BOTANY)

BY
JAMES BERTRAM OVERTON

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PARTHENOGENESIS IN THALICTRUM PURPURASCENS.

INTRODUCTORY.

THE well known experiments of Loeb in inducing by artificial methods the segmentation of the unfertilized eggs of some of the lower animals, and the formation of embryos, suggested similar experiments with the eggs of plants. Experiments of like nature have been made upon lower plants, but the eggs of the higher plants do not lend themselves readily to experiment. Nevertheless, it was concluded to make the attempt with some angiosperm. It seemed best to select a dioecious plant, and one suspected of exhibiting parthenogenesis. The clue was furnished in a paper read by David F. Day at the Buffalo meeting of the American Association for the Advancement of Science in 1896. It was entitled "Parthenogenesis in *Thalictrum Fendleri*," and the following abstract was published in the Botanical Gazette (22: 241 S 1896):

In 1883 a seedling of *T. Fendleri* was sent home from Colorado for cultivation. In late May it flowered and proved to be pistillate. About the last of August it presented abundant and good seed, although no staminate plants of any species of Thalictrum were in the neighborhood. The seeds were planted and yielded abundantly staminate and pistillate plants. Staminate plants have been artificially prevented from maturing flowers almost every year since. At least eight times in thirteen years the pistillate plants have produced good seed in abundance. Plants were sent to Meehan, Missouri Botanical Gardens, and Orpet of S. Lancaster, Mass., and all report in 1896 perfect seed from pistillate plants. This seems to be a clear case of parthenogenesis. *T. dioicum* does not show a similar habit.

This might have been a case of parthenogenesis or of vegetative apogamy, not to be determined without careful morphological study. In any event, it suggested a species for study, and the allied *T. purpurascens*, abundant in the vacant lots in Chicago, was selected.

The work was begun early in the summer of 1900 and carried

on at the Hull Botanical Laboratory of the University of Chicago. Acknowledgments are due to Professor John M. Coulter for much suggestive advice during the prosecution of the work, and also to Dr. Charles J. Chamberlain and Dr. Burton E. Livingston for assistance in collection, technique, and interpretation. Mr. Andrew C. Moore, now of the University of South Carolina, also gave much assistance in collecting material.

METHODS.

A compound microscope was taken into the field in order to determine whether the flowers were pistillate or staminate. Only such plants as were found by this means to be pistillate were used. In fact, the flowers were all too young to be determined in any other way. A dozen such pistillate plants were isolated in the greenhouse of the laboratory, nine of which survived. The plants were numbered, and watered with solutions of various salts. This mode of treatment was kept up until after the time fertilization would have taken place normally and until the stigmas ceased to be receptive. Each week some of the developing flowers were killed in a one per cent. solution of chrom-acetic acid and kept in 70 per cent. alcohol for further study. In the autumn these plants were dried and their rootstocks preserved in pots over winter. These were forced about the first of April, and all produced abundant pistillate flowers long before those out of doors had blossomed. This made it certain that pollination did not occur, as these flowers were mature and their stigmas had ceased to be receptive long before those out of doors had even begun to bloom, much less to produce pollen.

Fifteen or sixteen other pistillate plants were chosen at the same time by the same means and transplanted into the garden of the laboratory. The inflorescences of these were securely covered or capped with paper bags so as to prevent pollination. The flowers developed in these bags from the time the pistils had to be determined by means of the microscope until the seeds matured. Each week a head was removed, the flowers being preserved as above for future study.

All plants that survived under all the conditions, in the greenhouse, or in the garden, or as rootstocks, produced abundant and fully developed seeds. Of course in such a case it seemed unlikely that the treatment with the solutions produced the effect. Therefore the problem resolved itself into an investigation of the embryos, to determine whether or not there was parthenogenesis, the embryo developing from the unfertilized egg, or vegetative apogamy. Abundant and good material had been preserved, which it was hoped would show all stages required to answer this question. The material after fixing and killing was brought gradually into 70 per cent. alcohol and there kept until used. The xylol-paraffin method was used entirely, and sections were cut with a microtome from 5–15μ thick as the case required. Sections were stained with Delafield's haematoxylin and also with Flemming's safranin, gentian-violet, and orange method. All drawings were made with a Zeiss camera and a $\frac{1}{12}$ Bausch and Lomb oil immersion.

RESULTS.

It is not the purpose of this paper to describe the development of the megaspore in detail, for it differs in no way from that usual among angiosperms. In the very young ovule the archesporial cell is distinguishable (*fig. 1, a*), later enlarging (*fig. 2*), and dividing unequally to form the so-called tapetal cell (*fig. 3, t*) and the larger primary sporogenous cell (*fig. 3, s*). The tapetal cell may or may not divide further. *Fig. 4* shows a division. The primary sporogenous cell gives rise to the usual row of four megaspores (*fig. 5*), the innermost spore of the tetrad functioning (*fig. 5, fm*).

The megaspore germinates in the usual way, enlarging at the expense of the surrounding cells until it occupies a large part of the nucellus (*fig, 6*), and the nuclear divisions resulting in the usual groups of nuclei at each extremity of the sac.

Fusion of the polar nuclei takes place immediately, and this seems to act as a stimulus for the rapid enlargement of the sac (*fig. 6, pp.*). The fusion nucleus also enlarges very rapidly and

staining shows it to be rich in chromatin. It may lie near the oosphere, or in the center of the sac, or close to the antipodals (*figs.* 7, 8, 11). It is always surrounded by abundant cytoplasm, which is connected by strands to the mass about the egg and the antipodals, and often contains numerous nucleoli. It remains very large and active while the sac is enlarging to four or five times the size it had when the polar nuclei fused (*fig.* 11).

The synergids present the usual appearance. They are vacuolated at the lower end, with the nuclei above the vacuoles, the upper end presenting a striated appearance (*fig.* 11 *syn*).

The oosphere is not much longer and very often shorter than the synergids (*o* in *figs.* 6, 7, 8, 11), and is never very rich in stainable material, the nucleus taking only slightly the stain taken by the definitive nucleus very heavily. There is abundant cytoplasm surrounding the egg in all cases, with strands radiating to the mass about the definitive nucleus, as above mentioned. Although the egg is usually shorter than the synergids at the time of fusion of the polar nuclei (*figs.* 6, 7, *o*), after this fusion, while the sac is greatly enlarging in all directions (*fig.* 11) especially in length, the egg begins to reach down into the sac far below the synergids, becoming highly vacuolated and staining very feebly (*figs.* 3, 7, 10, *a*, 11, *v*), appearing to reach its fullest development just at the time the free endosperm nuclei become parietally placed.

One of the most notable features of the sac is the remarkably large size of the antipodal cells, which often reach almost to the center of the sac, their nuclei multiplying by fragmentation (*figs.* 7, 8, 11).

As before mentioned, the definitive nucleus is remarkably large, resting near the egg, or near the antipodals, but more frequently centrally placed in the sac. Free nuclear division takes place very rapidly, and in no instance did division of the egg take place before division of the endosperm had begun. This free nuclear division is so rapid as to produce in a remarkably short time a great number of nuclei, during which the sac enlarges very rapidly in all directions. It may be well to note here that

the first division of the egg takes place when the free nuclei become parietally placed, lining the whole sac. Coulter found in species of Ranunculus "occasional evidence of endosperm-formation before the fusion of gametes, and even before the entrance of the pollen-tube into the cavity of the sac." In my examination of normal material of *T. purpurascens* I have been unable to find any stages that show segmentation of the egg before the definitive nucleus divides, in all cases free nuclear division having begun before fertilization. In the material that I know to be parthenogenetic the free nuclear division began without any stimulus from fertilization, and always before the egg divides. Undoubtedly fertilization, when it takes place, may exert an influence upon the definitive nucleus, as it is known to do upon other adjacent structures, but it is not absolutely necessary to its division. The parietal placing of the free endosperm nuclei is followed by the formation of cell walls, and the endosperm gradually fills the cavity of the sac.

As described above, the egg becomes elongated far below the synergids, and there seems to be a great lack of stainable material in both nucleus and cytoplasm. In every case observed the cytoplasm of the sac was very dense about the egg, except the zone immediately in contact with it. This layer appears to be of a different consistency and stains very little, much resembling the zone of broken-down endosperm tissue so frequently found surrounding an embryo in the seed. It suggests that the egg is giving off an enzyme that digests the adjacent cytoplasm.

No attempt was made to trace in detail the development of this embryo, as that was not the purpose of the work. Without fertilization the first division occurs, and is transverse (*fig. 12*) as usual. The next division is also transverse, and a row of three or four cells is formed, after which a longitudinal division takes place in the terminal cell, differentiating the embryo-proper from the suspensor. The persistence of the synergids is very noticeable, and this might be expected, since no pollen tube has entered the sac to draw upon them for food supply. The synergids in the normal material were not evident in all cases after

the embryo had begun to develop. After the first longitudinal division of the embryo, similar divisions may take place in the suspensor, resulting in a massive, rather short, much twisted, thick-walled suspensor (*fig. 15, su*). Finally, the parthenogenetic embryo becomes morphologically well developed, showing dermatogen, periblem, and plerome, exactly as in normal embryos. In fact no difference can be seen between the perfectly normal embryo and this parthenogenetically formed one (*fig. 16*). The endosperm continues to develop until it entirely fills the cavity of the sac and lies in a mass about the embryo. Abundant seed is produced by *T. purpurascens*, both from free and isolated pistillate plants. The percentage of seed produced in the parthenogenetic material is quite as great as under perfectly normal conditions in the field.

GENERAL DISCUSSION.

There have been described only two other cases of true parthenogenesis among spermatophytes. In 1898 Juel[1] described parthenogenesis in *Antennaria alpina*. No figures were given, and we have to depend upon his text for the facts. In 1876 Kerner[2] had noticed that plants of *A. alpina* were matured in the Botanical Garden at Innsbruck when no staminate flowers were present. It is the rule for plants of *A. alpina* to have only pistillate flowers, while staminate flowers are exceedingly rare, and are not necessary to the propagation of the species. The pollen grains are not fully developed, or very seldom so, even when there are staminate flowers present, being functionless according to Juel. Juel maintained that Kerner did not find parthenogenesis in the true sense of the word, but only seed-development without fertilization. A critical study of *A. dioica* showed that it behaved normally, fertilization occurring and the embryo coming from a fertilized egg. In *A. alpina*, however, he found that the egg forms an embryo without fertilization. In this case the polar

[1] Parthenogenesis bei *Antennaria alpina*. Bot. Centralbl. 74:369. 1898.

[2] Parthenogenesis einer angiospermen Pflanze. Sitzungsb. Acad. Wiss. Wien 74:469. 1876.

nuclei never fuse or even approach each other, dividing independently to form the endosperm, which is finally absorbed by the embryo. Juel explains the behavior of the polar nuclei on the ground that one of the polar nuclei and the egg have arisen through the same nuclear division, and as the egg nucleus is able to divide without fertilization, the polar nuclei can divide without fusion. Even if this explains the division of the upper polar nucleus, it does not apply to the lower one. Juel did not follow the chromosome reduction at that time, but later investigated it in *A. alpina*. He found[3] that tetrad formation does not take place, while in *A. dioica* there is the usual row of four. Juel concludes that there is no reduction in *Antennaria alpina*, so that the nuclei of the parthenogenetic embryo contain the normal number of chromosomes.

In 1895 Murbeck[4] suggested that certain species of Alchemilla are parthenogenetic. During the summers of 1892 and 1893 he observed in the neighborhood of Stockholm a form of Alchemilla which seemed to be intermediate between two forms that grew in the same locality. In order to determine whether the intermediate form was a hybrid, he made a comparative study of the reproductive power, and later of the formation of pollen. In all three forms he found the pollen impotent, and yet all set seed. Two other forms, growing in the Royal Botanical Garden of the Academy of Science of Stockholm, behaved likewise. Material was also collected from different parts of Europe in 1894 and 1895. Murbeck found that *A. alpina*, *A. sericata*, *A. pubescens*, and *A. vestita* produced no pollen whatever; that *A. acutangula*, *A. subcrenata*, and *A. alpestris* sometimes developed pollen, but the amount was very much out of proportion to the number of seeds produced. Of the great number of species examined only the oriental species *A. speciosa* showed normal pollen. He came to the conclusion, after mixing the plants and finding no variations in the species, that the pollen sparingly

[3] Botaniska Notiser 102. 1900.

[4] Skandinaviska former af Alchemilla vulgaris. Botaniska Notiser 265 (Fussnote). 1895.

produced by some forms had no fertilizing power. These results he published in 1897,[5] with the statement that he hoped later to discover whether there is actual parthenogenesis or whether the embryo arises from the nucellar tissue. With this purpose in view, Murbeck examined numerous species of the section EUALCHEMILLA, but his important results are mainly derived from *A. alpina*. He has traced every stage of development from the archesporial cell to the formation of the embryo, and published his results in 1901.[6] He found a central mass of archesporial cells, and that the numerous primary sporogenous cells may each give rise to a row of three or four megaspores, one or all of which may give rise to embryo sacs. In regard to chromosome reduction, he observed in a pollen mother-cell of *A. arvensis* that the reduction number was 16, but he claims that in parthenogenetic species there is no reduction of chromosomes. He shows that there is no direct relation between endosperm-formation and embryo-formation. A noticeable fact is that before the egg divides the whole egg-apparatus stretches far into the sac, and then division takes place without fertilization, a perfect embryo being developed. The first division takes place while the flower is still in developmental stages. Murbeck also found two embryos in a single sac, one developed from the egg and the other from a synergid.

The two cases described by Juel and Murbeck, and *Thalictrum purpurascens*, described in this paper, are the only cases of true parthenogenesis thus far recorded among seed-plants, though of course the phenomenon is common enough among certain lower plants. The so-called cases of parthenogenesis among seed-plants, as *Coelobogyne ilicifolia*, *Mercurialis annua*, etc., were long ago shown by Strasburger[7] to be cases of vegetative apogamy.

[5] Om vegetativ embryobilding hos flertalet Alchemillor och den förklaring öfver formbeständigheten inom slägtet som densamma innebär. Botaniska Notiser 273. 1897.

[6] Parthenogenetische Embryobilding in der Gattung Alchemilla. Lunds Universitets Årsskrift **36** : 1–40. *6 pls.* 1901.

[7] Ueber Polyembryonie. Jenaisch. Zeitschr. Naturwiss. **12** : 659. 1878.

In *Balanophora elongata* Treub[8] has shown that there is no fertilization, but that a "pseud-embryo" is developed apogamously from the endosperm. We see no reason why this whole "pseud-embryo" structure with the endosperm in which it develops may not be considered an embryo developed apogamously from the micropylar polar nucleus, which later organizes a growing point as does a normal embryo. Lotsy[9] found exactly the same state of affairs in *Balanophora globosa*, a species with no staminate flowers. In the case of *Rhopalocnemis phalloides*, which Lotsy[10] also recently investigated, no seeds are ever produced. He could not discover a pollen tube, nor could he induce pollen tubes to develop by artificial pollination. In a few cases he secured some seeds, but was certain the embryos had developed from the eggs, probably after fertilization.

It is shown that an embryo may be produced from any cell of the embryo sac, and in this sense they may all be regarded as potential eggs. But since the same fact is true of cells of the nucellus, the statement has little significance. Experimental work done within the last five or six years upon the eggs of marine animals has thrown some light upon the causes of the segmentation of unfertilized eggs. Mathews[11] showed that the unfertilized eggs of star-fish could be made to extrude polar bodies by violent shaking. Morgan[12] found that eggs placed in sea water of a higher osmotic pressure than normal sea water divided upon being returned to the latter. Mead[13] showed that eggs of Chaetopterus could be made to divide by placing them in sea water to which KCl had been added. Morgan[14] confirmed the results obtained by Hertwig as to the action of strychnin sulfate. He expressed the opinion that eggs are in a

[8] L'organe femelle et l'apogamie du *Balanophora elongata*. Ann. Jard. Bot. Buitenzorg 15: 1–22. 1898.

[9] Balanophora globoso. Ann. Jard. Bot. Buitenzorg 16 : 26–29. 1899.

[10] Rhopalocnemis phalloides Jungh., etc. Ann. Jard. Bot. Buitenzorg II. 2: 73–101. 1900.

[11] Anat. Anz. 9: 150. 1894.

[12] Archiv. für Entwicklungs. mechanik der Organismus 8 : 448. 1899.

[13] Lectures at Woods Hole. 1898.

[14] Science N. S. 2: 176. 1899.

state of unstable equilibrium, and would react to various stimuli by division just as other cells would react in other ways to the same stimuli. Loeb[15] found that exposure of eggs of Arbacia for a short period to sea water to which alkali or acid had been added induced cell division. He has arrived at certain conclusions by further experiments upon the unfertilized eggs of echinoderms.[16] He has shown that such eggs can be made to develop into normal embryonic forms through a certain increase in the osmotic pressure, produced either by electrolytes or non-electrolytes. He suggests the probability that parthenogenetic development is caused by loss of a certain amount of water from the egg. Mathews[17] found that karyokinetic divisions in the eggs of Arbacia could be induced by the lack of oxygen, by heat, by exposure to ether, alcohol, and chloroform. Loeb[18] also produced artificial parthenogenesis in eggs of other animals than echinoderms by increasing the osmotic pressure, as Chaetopterus, etc. His experiments have convinced him that the essential feature in increasing the osmotic pressure of the surrounding medium is a loss of water on the part of the egg. He also states that if we assume the spermatozoön starts the development of the egg in the same way as in the case of artificial parthenogenesis, it must follow that it possesses more salts of a higher osmotic pressure than the egg. He has also suggested that the spermatozoön may bring about the same condition in the egg as is produced by loss of water.

These experiments suggest an explanation of parthenogenesis as observed in Thalictrum. The egg is invested by a dense sheath of cytoplasm, and that there is some reaction between the two is evidenced by the change in the structure of the cytoplasmic layer immediately in contact with the egg. Whether or not the egg excretes an enzyme that digests the cytoplasm is a matter of detail. The fact remains that physical changes are

[15] Jour. Phys. 3 : 447. 1899.

[16] Further experiments on artificial parthenogenesis and the nature of the process of fertilization. Jour. Phys. 4 : 178. 1900.

[17] Jour. Phys. 4 : 341. 1900.

[18] Science N. S. 2 : 70. 1900.

evident in the cytoplasm in contact with the egg, which then divides. Such changes may well vary the osmotic pressure within the egg, and lead to nuclear division as shown by the experiments referred to above.

While *Thalictrum purpurascens* is parthenogenetic under artificial conditions, parthenogenesis also takes place in plants grown under perfectly natural conditions if pollination be prevented, as is shown by plants setting seed in the gardens when the flowers are covered with paper bags. All such plants showed quite as many seeds per plant as those which had been fertilized and grew in the field. Many flowers were cut from natural specimens in order to compare them with the parthenogenetic material. In many cases a pollen tube could be detected, but in far the greater number no such tube or any evidence of a tube having been present could be seen. One can tell by even a casual inspection of the micropyle whether a tube has been present or not. I am led to conclude that many seeds are produced parthenogenetically under normal conditions if for any reason fertilization fails. So far as I could determine, there seems to be no real necessity for pollination in order to propagate the species. The plant is getting towards the habit of complete parthenogenesis, when pollen will become impotent, a condition apparently attained by *Antennaria alpina* and several species of Alchemilla. Even though the number of recorded parthenogenetic genera among dicotyledons be so small, the genera are rather widely distributed. It would seem as if parthenogenesis must be of much more common occurrence among angiosperms than is at present known.

SUMMARY.

1. The development and germination of the megaspore is that usually found among angiosperms.

2. Fusion of the polar nuclei is early, always before fertilization in normal material, and before the division of the egg in parthenogenetic material.

3. Fertilization is not necessary to embryo-development or to endosperm-development.

4. Embryos were produced parthenogenetically under all conditions, and normal material showed the phenomenon to be general in nature.

5. The cytoplasm of the early stages of the sac is closely packed about the egg. Later the egg becomes surrounded by an area much resembling a vacuole, which may affect the osmotic pressure and indicate a withdrawal of water, causing the oosphere to divide.

6. The development of the embryo in parthenogenetic material is the same as found in normal material.

7. Parthenogenesis is becoming fixed in Thalictrum.

ILLINOIS COLLEGE,
Jacksonville, Ill.

EXPLANATION OF PLATES I AND II.

FIG. 1. Section of young ovule showing archesporial cell (*a*).

FIG. 2. The same, showing enlarging archesporial cell (*a*).

FIG. 3. Section of young ovule with integument forming, showing tapetal cell (*t*) and primary sporogenous cell (*s*).

FIG. 4. Anticlinal division of tapetal cell (*t*); the nucleus of the primary sporogenous cell also dividing.

FIG. 5. Young nucellus, showing tapetal cell (*t*), the three functionless megaspores (*m*), and the functional megaspore (*fm*).

FIG. 6. Young embryo-sac; *syn*, synergid; *o*, oosphere; *t*, tapetal cell, with the three functionless megaspores crowded between it and the embryo-sac; P_1 and P_2, upper and lower polar nuclei fusing.

FIG. 7. Young embryo-sac immediately after fusion of polar nuclei; *o*, oosphere; *v*, area or vacuole about the oosphere; *syn*, synergid; *nu*, definitive nucleus; *at*, antipodals.

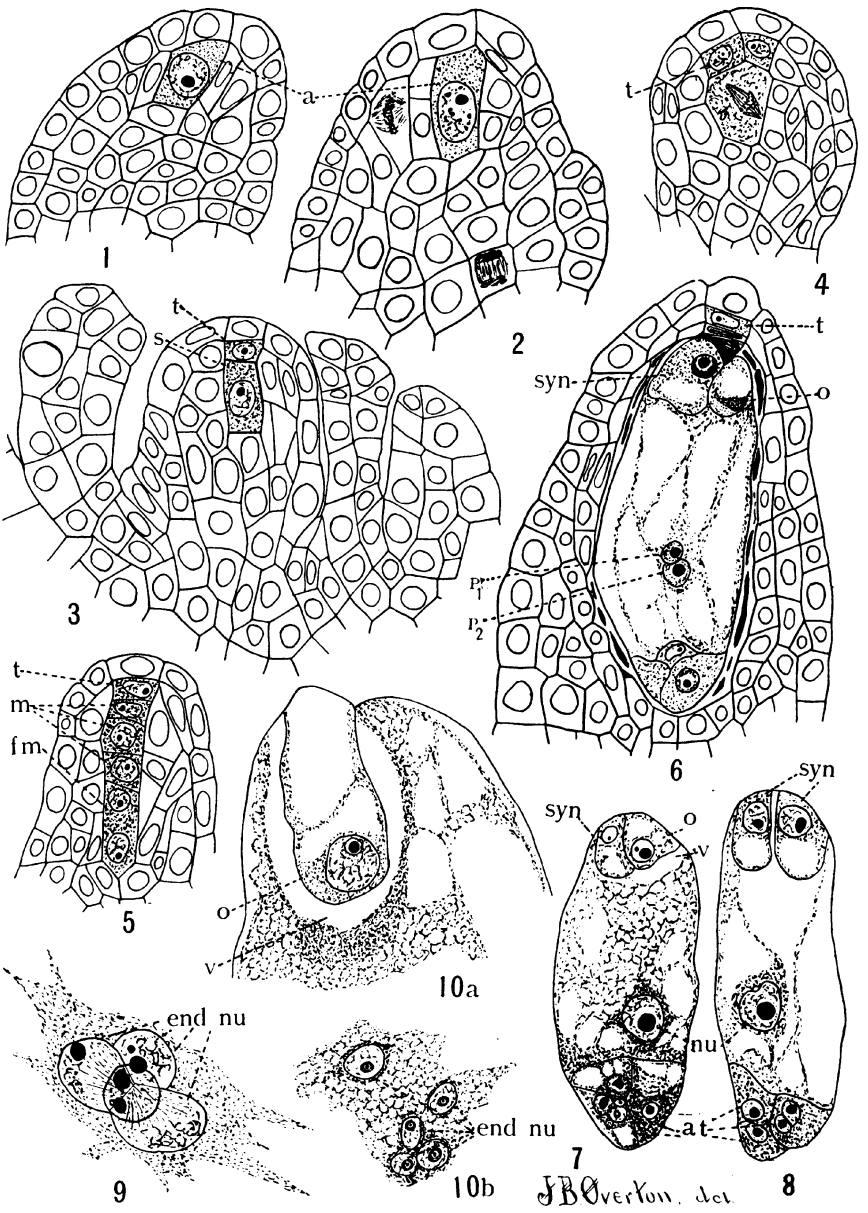
FIG. 8. Same as *fig.* 7, but showing both synergids (*syn*) without the oosphere.

FIG. 9. A group of endosperm nuclei resulting from the first divisions of the definitive nucleus.

FIG. 10*a*. The oosphere (*o*) beginning to extend into the sac, with the vacuole (*v*) surrounding it.

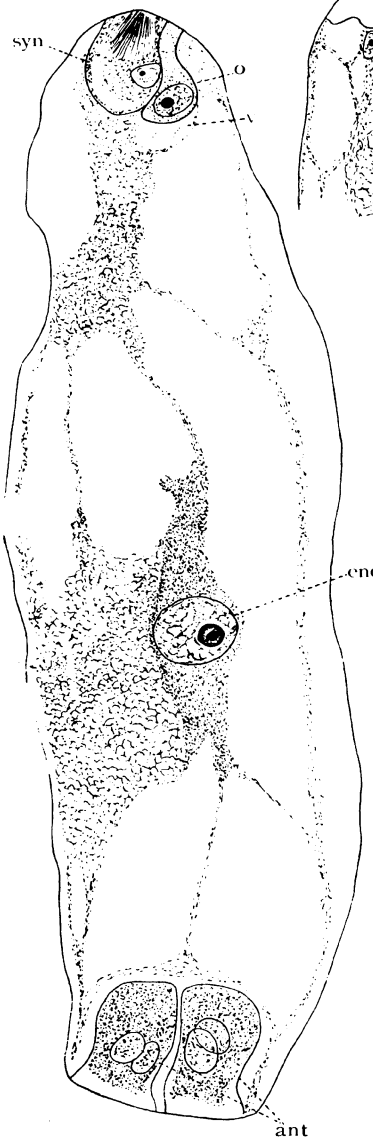
FIG. 10*b*. A group of free endosperm nuclei from same sac, showing free endosperm nuclei before egg divides.

FIG. 11. Embryo sac just before the division of the oosphere; *end nu*,



OVERTON on THALICTRUM

PLATE II



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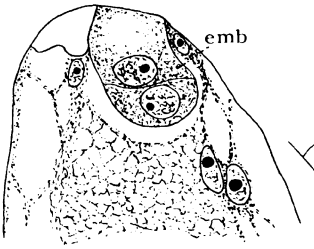

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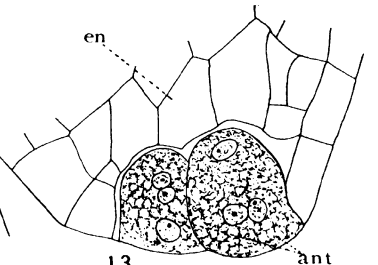

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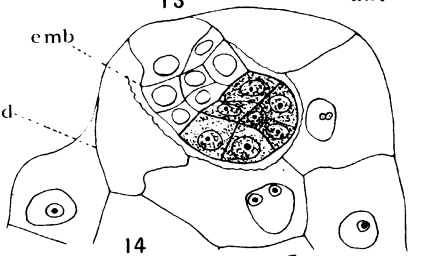

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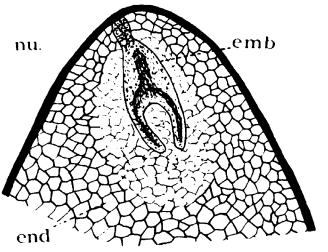

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large and active definitive nucleus; *syn*, synergid; *o*, oosphere before elongation into the sac; *v*, vacuole forming about the oosphere; *ant*, large multinucleate antipodals.

FIG. 12. Two-celled parthenogenetic embryo (*emb*) with the vacuole about it; a few free endosperm nuclei are seen scattered in the cytoplasm of the sac.

FIG. 13. Antipodals (*ant*) still present when the embryo sac is entirely filled with endosperm cells (*em*).

FIG. 14. A young parthenogenetic embryo (*emb*) at end of suspensor, and surrounded by endosperm cells (*end*).

FIG. 15. A still more advanced parthenogenetic embryo, showing the thick-walled twisted suspensor (*su*).

FIG. 16. Diagrammatic sketch showing general relation of the parthenogenetic embryo (*emb*) to the endosperm (*end*) in the seed.