THESIS.

THE EMBRYOGENY OF PASTINACA SATIVA.

BY

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THE EMBRYOGENY OF PASTINACE SATIVA L

During the last half-century our knowledge of the embryogeny of the Angiosperms has been extended to include a great many species well distributed among numerous families of both Monocotyledons and Dicotyledons. It is to be expected that our knowledge will advance most rapidly in those families in which the material proves to be most favorable. Numerous attempts have been made to trace the development of various Species representative of the family Umbelliferae, but the small size of the ovule, together with certain mechanical difficulties incident upon embedding and sectioning, have prevented any of these investigations reaching a stage which warrants publishing, with the exception of a recent work by Jurica. (16)

The present investigation was begun in 1916 in the Botanical Laboratory at Indiana University under the direction of Dr. D. M. Mottier, and for the past six years has been continued in the Botanical Laboratory at the University of Cincinnati under the direction of Dr. H. M. Benedict. I wish to acknowledge my indebtedness to Dr. Mottier for suggesting the problem and for his generous aid in the pursuit of the study, and to Dr. Benedict for his invaluable aid during the latter years of the investigation.

Pastinaca Sativa, which is a species introduced from Europe but widely escaped from cultivation, was chosen for material, supplemented by other species available in Southern Ohio. Flemmings solution was employed as a fixative and corrosive sublimate acetic acid fixative, gave good results in a few cases. Sections were cut six to ten micra, and stained on the slide with triple stain.

FLORAL DEVELOPMENT

The individual flowers arise as club-shaped masses of meristematic cells from the broad surface of the developing axis of the umbel. The rounded end of this structure soon broadens, and the primordia of petals, stamens and carpels appear in the order named. (Sepals are obsolete, but are occasionally seen in the material as in Fig. (3). The order of their appearance could not be determined). The two carpels begin their development as outgrowths from the meristematic surface. Each carpel is semilunar in shape, and as they unite along their inner margins, they form an arch over a single ovarian cavity. Prior to the union of these carpels their inner edges are sharply reflexed so that union is accomplished by the outer surface some distance from the edge. Thus the cavity of the ovary contains the edges of the carpels which by growth, chiefly from the base, finally divide this single cavity into two cells, each containing the two reflexed edges of a carpel. From each of these edges an ovule begins to develop, a fact reported by Coulter and Rose in 1888. (8). All of these four ovules frequently develop to the condition in which the archesporium is distinguishable, but one in each cell degenerates before setting up that series of changes which leads to the production of the megaspore.

ARCHESPORIUM

When the archesporium is first distinguishable, the ovule consists of a club shaped organ attached by a rather slender funiculus to the inner wall of the ovarian cavity, in its apical portion, the ovule extending backward. The mature ovule is completely anatropous. It begins its development straight and as growth continues, it turns outward toward the exterior wall of the ovary and continues to a position of complete anatropy in which the integument on the side next to the funiculus is scarcely recognizable.

The archesporium consists of a group of cells numbering from three to seven, which are easily distinguished by their staining reactions, their large nuclei and especially by their form. They completely occupy the end of the nucellus, forming a mass almost spherical in shape, in which each cell borders upon the nucellar wall and also upon the adjacent cells of the nucellus which lie in the line of food conduction from the chalaza. As a result of this arrangement, each cell is elongated in the direction of the long axis of the nucellus, those in the center being longer.

The sporogenesis in both the anther and the ovule of Angiosperms has now been investigated in a great many genera. While the archesporial tissue in the microsporangium is quite constantly a row, a plate, or a mass of cells, it was for a long time reported to consist of a single cell in the megasporangium. Strasburger (28) seems first to have reported a multicellular archesporium in the genus Rosa in 1879, although several other cases were reported the same year by Johnson (15). Since this time a considerable number of genera have been investigated, in which the archesporium sometimes consists of more than one cell.

Our present knowledge of this important matter is summarized in the accompanying table.

Family and Species.	No. of Arch. Cells.	Investigator.
Monocotyledoneae		
Butomaceae		
Butomus umbellatus	2-4	Holmgren, I. (13)
Araceae		
Aglaonema	2-3	Campbell, Douglas H. (3)
Arisaema Triphyllum	2-4	Mottier, D.M. (23)
Nephthytis liberica	2-many	Campbell, Douglas H. (3)
Liliaceae		
Lilium philadelphicum	3-5	Coulter & Chamberlain (7)
Ornithogalum pyrenaicum	2	Guignard, L. (12)
Smilacina racemosa	2	McAllister, F. (21)
Orchidaceae		
Calopogon pluchellus	2	Pace, L. (27)
Gastrodia	2	Kusano, S. (18)
Dicotyledoneae		
Casuarinaceae		
Casuarina suberosa	numerous	Treub, M. (31)
Casuarina rumphiana	numerous	Treub, M. (31)
Casuarina glauca	numerous	Treub, M. (31)
Piperaceae		
Chloranthus chinensis	numerous	Armour, Helen M. (1)
Chloranthus officinalis	numerous	Armour, Helen M. (1)
Peperomia hispidula	numerous	Johnson, D.S. (14)
Salicaceae		
Salix glaucophylla	2-3	Chamberlain, C. J. (4)
Populus tremuloides	2-3	Chamberlain, C. J. (4)
Juglandaceae		
Juglans cordiformis	numerous	Karsten, G. (17)
Juglans regia	several	Bensen, Margaret (2)
Betulaceae		
Betula alba	numerous	Bensen, Margaret (2)
Carpinus betulus	numerous	Bensen, Margaret (2)
Corylus avellana	several	Bensen, Margaret (2)
Fagaceae		
Castanea vulgaris	5-8	Bensen, Margaret (2)
Fagus sylvatica	numerous	Bensen, Margaret (2)
Quercus velutina	numerous	Conrad, Abram H. (5)
Loranthaceae		
Loranthus sphaerocarpus	2	Treub, M. (30)
Viscum articulatum	4-many	Treub, M. (30)
Santalaceae		
Thesium	several	Coulter & Chamberlain (7)
Phytolaccaceae		
Phytolaca decandra	2	Lewis, I.F. (20)
Ranunculaceae		
Caltha palustris	numerous	Mottier, D. M. (24)
Delphinium tricorne	2-many	Mottier, D. M. (24)
Helleborus cupreus	3	Coulter & Chamberlain (7)
Ranunculus abortivis	2	J. M. Coulter (6)
Ranunculus multifidus	2-3	J. M. Coulter (6)
Ranunculus septentrionalis	2-13	J. M. Coulter (6)

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Family and Species	No. of Arch. Cells	Investigator
Cruciferse		
Capsella byrsa-pastoris	2	Guignard, L. (12)
Saxifragaceae		
Astilbe japonica	several	Webb, J. E. (35)
Rosaceae		
Alchemilla acutangula	numerous	Murbeck, S. (25)
Alchemilla alpina	numerous	Murbeck, S. (25)
Agrimonia	numerous	Fisher, E. (9)*
Cydonia	numerous	Fisher, E. (9)
Eriobotrya japonica	3	Guignard, L. (12)
Geum	numerous	Fisher, E. (9)
Rosa livida	several	Strasburger, E. (28)
Rubus	numerous	Fisher, E. (9)
Sanguisorba	numerous	Fisher, E. (9)
Linaceae		
Linum usitatissimum	3	Jonsson, B. (15)
Euphorbiaceae		
Euphorbia procera	4-5	Modilewski, J. (22)
Cistaceae		
Helianthemum	several	Fisher, E. (9)
Orangraceae		
Lopezia coronata	4-5	Tackholm, G. (29)
Oenothera lamarckiana	2	Greets, T. H. (11)
Umbelliferae		
Pastinaca sativa	3-8	Beghtel, F. E.
Asclepiadaceae		
Asclepias tuberosa	3-more	Frey, Theodore C. (10)
Caprifoliaceae		
Adoxa moschatellina	several	Lagerberg, T. (19)
Campanulaceae		
Lobelia syphilitica	2	Jonsson, B. (15)
Compositae		
Chrysanthemum leucanthemum	several	Jonsson, B. (15)
Pyrethrum balsaminatum	3	Ward, H. M. (34)*

* Original paper not available.

It should be stated that perhaps not all of the papers referred to in connection with this list represent equal care in determining the exact limits of the archesporium. These limits are not always sharply marked out, and some of the works referred to, date back to a time when the exact meaning of the term archesporium had not yet been carefully defined. However, only those species have been included in which the descriptions or drawings leave little doubt of the existence of more than one archesporial cell.

The occurrence of an archesporium of several or many cells in the ovule has come to be looked upon as a primitive character of the Angiosperms, while the single hypodermal archesporial cell terminating an axial row in the nucellus is considered a derived character, reaching its greatest expression in the more highly specialized groups of both Monocotyledons and Dicotyledons. Thus Coulter and Chamberlain in 1903 (7) state; "The temptation is strong to consider the many celled archesporium as a primitive feature of the Dicotyledons." Campbell in 1905 (3), in a paper dealing with the Araceae, states, "We may conclude, then, that the Araceae are really a primitive family of Monocotyledons.----- The not infrequent occurrence of a multicellular Archesporium --- points to such a conclusion." Also Pace in 1909 (27), in discussing the multicellular Archesporium says, "It seems best to regard it as a primitive character that has been retained, or at least not entirely eliminated."

A study of the accompanying table indicates that the accumulated information on the question of a multicellular archesporium necessitates a careful reconsideration of the value of this character in determining the relative position of large groups in any phylogenetic scheme. It is still true that the majority of species reported belong to families recognized as among the more primitive, but the occurrence of an archesporium of more than one cell in such highly specialized families as the Compositae, Campanulaceae, Carifoliaceae, and Umbelliferae cannot be disregarded. As the number of genera reported from these families increases the character in question certainly becomes less significant phylogenetically.

Another fact of considerable importance which has not been indicated in the table is the relative frequency of the occurrence of a multicellular archesporium in those genera where it has been observed. In Calopogon it was observed but once, in Lilium philadelphicum twice, in Phytolacca, Gastrodia, Salix, and Asclepias it is reported as occasionally met with, while in Quercus, Pastinaca, and many genera of the Rosaceae its presence seems to be the rule. In Pastinaca the author has examined a great number of ovules in the archesporial condition, and no instance of a single archesporial cell was found among this number. Any extensive comparisons of this kind are impossible as there is great variation among the investigators as to the number of individuals examined and records are very incomplete.

PARIETAL TISSUE

Parietal tissue has been entirely suppressed in Pastinaca and the cells of the archesporium are potentially spore mother cells. The failure of the archesporial cell or cells to divide in the production of a parietal layer has previously been pointed out in the family by Coulter and Chamberlain (7) in the genus Sium; and Jurica (16), in a recent publication, has figured Sium cicutaefolium and Eryngium yuccifolium. Although no statement is made in the discussion, the figures indicate clearly that no parietal tissue is developed in these species. It becomes increasingly evident, therefore, that the suppression of the parietal tissue, long known to be common among certain of the less highly specialized groups, is also common in the Umbelliferae which is representative of the higher Archichlamydeae.

The failure to produce parietal cells which commonly contribute to the wall of the nucellus, accounts, at least in part, for the meager development of this nucellar wall. At the time that the archesporium is first distinguishable, it is surrounded by a single layer of cells constituting the nucellar wall. These cells divide anticlinally only, and thus keep pace with the development of the sporogenous tissue, without increasing the number of layers of the wall. This development, however, is not extensive and the wall soon begins to break down. By the time the embryo-sac is mature it has entirely disappeared, leaving the embryo-sac to lie within the integument. The nucellar cap often seen in the micropylar end is quite wanting.

The layer of tapetum often found in the megasporangium is the product of the parietal tissue and is consequently wanting in Pastinaca. Its nutritive function seems to be taken over by a mass of cells which remain of the nucellus back of the developing sporogenous tissue. This we may call the nutritive tissue. Fig.(20). This nutritive tissue continues to degenerate as the development in the spore chamber proceeds, and no doubt contributes largely to the nourishment of the female gametophyte.

FATE OF THE MEGASPORE MOTHER CELL.

The multicellular condition of the archesporium of the megasporangium is of peculiar interest because it represents a type of natural selection among cells each of which is potentially a megaspore mother cell, and one or more of which is destined to produce the megaspore and subsequently play an important part in the ultimate development of the new sexually formed individual.

Unfortunately few investigators have followed the development far enough to determine the fate of the megaspore mother cells in those cases where more than one archesporial cell is produced. In those species where this feature is but rarely met with this would be difficult, however where it seems to be constant, further study is very desirable.

In a number of species where the multicellular archesporium has been found only a single embryo-sac is formed if we may judge from the information at hand. i.e. Chloranthus, Caltha, Lopezia, Pastinaca and Phytolacca. In some other species the condition varies widely, thus in Smilacina two instances of two fully formed embryo-sacs in an ovule were found; in Casuarina a number of tetrads are formed and several embryo-sacs begin to develop but only one persists; in Salix more than one embryo-sac develops; in Quercus one instance of two four celled embryo-sacs was found; in Ranunculus several may reach the four celled stage; and in Astilbe several may begin development. Alchemilla matures as many as four embryo-sacs none of which are typical while in Astilbe several mother cells may begin development. In Euphorbia four tetrads may be produced while in Nephthytis more than one embryo-sac matures and in Gastrodia each of two mother cells produces a normal embryo-sac. These examples will serve to indicate the great irregularity in the time at which the selection among archesporial cells or their progeny is carried on as well as to show that in certain species, at least, the selection is never completed.

A number of genera have been reported in which the condition in the archesporium is unknown but in which two or more embryo-sacs are known to develop in one ovule, or two or more embryos in one seed. It must be borne in mind that these facts do not necessarily indicate that the condition is the result of incomplete selection among previously existing archesporial cells as the accessory embryo-sac or embryo may have arisen in some other way. It is highly probable, however, that in these genera farther study will reveal an archesporium of more than one cell. A more complete investigation of such genera is very important.

SELECTION IN PASTINACA.

In Pastinaca, when the first changes leading to the heterotypic division in the mother cells are first noticable, it is evident that processes of selection are already under way. Certain cells, usually the larger and more centrally located ones, first show mitotic activity and the majority of the others may never institute such changes. There can be little doubt that the chief determining factor here is access to the available food supply. Those cells that are first able to reach a condition of food storage and organization which enables them to enter upon mitosis maintain a decided advantage over the others. In a large number of ovules examined never more than three cells were found to have entered upon mitosis and in such cases one cell is always considerably in advance of the others. Fig. (15) represents a cross section of a nucellus in which four of six archesporial cells have reached an advanced stage of deterioration while of the two remaining, one is still in the resting condition while the other has entered upon nuclear division. A very large number of ovules containing the nature embryo-sac has been examined and in no case has more than one embryo-sac been found in an ovule. It is evident, therefore that selection is speedily carried to the limit and that all but one of the elements are eliminated.

The first division of the megaspore mother cell is in every way typical of the heterotypic division. The synaptic knot is very compact and is followed by segmentation of the spireme thread. The chromosomes are short and thick occuring in pairs. The spindle is quite complete. Fig. (17). Subsequent steps follow the usual course of the reduction division. The small size of the chromosomes and spindle, togather with the difficulty of obtaining these stages which limits the number of good preparations, makes counting of the chromosomes very difficult and uncertain. The haploid number is probably eight.

The second division follows rapidly after the first and subsequent development to the maturity of the embryo-sac is accomplished in a remarkably short time. Great difficulty was experienced in obtaining preparations showing these steps and, although a large amount of material was examined, it has been impossible to demonstrate beyond a doubt the production of a complete tetrad. It is known, however, that both daughter cells, which are approximately the same size, set up second division at about the same time. It is possible that the division in the inner daughter cell is never completed, for very soon after the appearance of the spindles, the entire sporogenous cavity of the ovule presents a condition very difficult to fix in such a way that clear preparations are possible. The tissue of the nucellus degenerates rapidly below the spore chamber and several large vacuolate cells appear to occupy the cavity. Among the degenerating cells of the nucellar tissue at the chalazal end of the megaspore chamber ~~is~~ are frequently to be seen three or more darkly staining, irregular masses believed to be the remains of the three inner megaspores together with those cells which have originated from other mother cells which did not degenerate before division. It should be borne in mind that at this time there is present in the spore cavity a great amount of degenerating tissue derived from numerous mother cells and the whole nucellus is so much clouded that repeated efforts have failed to produce clear preparations.

It appears that the outer megaspore invariably functions, a condition that is an exception rather than the rule. Numerous cases have been reported in which the outer of three or four megaspores may function at times. Such a condition has been reported by Vesque (32), Treub. (30) and Oliver. (26). In Pastinaca no exception to the development of the outer megaspore has been noted among a great number of cases examined.

The developing megaspore penetrates rapidly and deeply into the tissue of the nucellus in the direction of the chalaza. The single layer of epidermal cells that composed the wall of the sporangium also breaks down and the megaspore enlarges rapidly. When the eight nucleate stage of the embryo-sac is reached it occupies the entire space within the integument. There remains of the nucellar tissue only a small basal portion previously referred to as the nutritive tissue. Certain portions of this tissue still indicate by their staining reactions that degeneration is not yet complete.

<u>THE OVULE</u>.

It has been stated by Coulter and Chamberlain (7) that the development of a single integument is characteristic of the Umbelliferae. This statement is verified by Pastinaca as only one integument is produced. The first indication of the integument appears about the time that the sporogenous tissue is first destinguishable. It appears as a ridge like swelling about the base of the nucellus Fig. (14). As is common in ovules in which a single integument is produced that organ is thick and fleshy. The nucellus is completely enclosed by the developing integument at about the time that the tetrad of megaspores is produced.

THE EMBRYO-SAC.

The embryo-sac is of the usual eight nucleate type. The antipodals are small and lie within a depression of the tissue referred to as the nutritive tissue Fig. (20).

The flower of Pastinaca is completely epigynous and the fruit is defined by Warming and Potter (33) as a schisocarp composed of two mericarps. The fruit is very much compressed laterally bearing two free styles which unite at their base into a large nectary or stylar foot. This stylar foot develops early and by the time the anthers begin to mature it secretes a nectariferous fluid. At this time the styles are very short and have not yet developed stigmatic surfaces. It is about five days after the first stamens of a flower begin to dehisce that the stigmas of the same flower are ready to receive pollen. The outermost whorl of flowers in an umbel matures first. These are followed by the next whorl within, which shed their pollen at the time that the stigmas of the flowers of the outer whorl are receptive. In this manner cross pollination in the umbel is accomplished.

This type of cross pollination seems to be the rule, while that between umbels is not common. This condition is shown by the failure of the last whorl of flowers at the center of the umbel to develop because of failure to be pollinized.

Preparations showing fertilization of the egg were not secured although the pollen tube was traced through the tissues of the style and into the tissues beneath the stylar foot. There is no indication that the history of fertilization and the development of the embryo and endosperm vary in any way from the usual form described in other Dicotyledonous families.

SUMMARY

1. Four ovules begin development on the ovary of Pastinaca two of which early degenerate leaving one in each cell.

2. The archesporium consists of a mass of three to seven cells.

3. A review of our present knowledge of the multicellular archesporium in the megasporangium of Angiosperms indicates that this character is of little value in determining phylogenetic relationships.

4. No parietal tissue is produced.

5. The outer of three or four megaspores functions.

6. Only one embryo-sac natures and it is typical eight nucleate.

7. A single thick fleshy integument is produced.

EXPLANATION OF PLATES.

a, petals; b, stamens; c, carpels; c', carpels united along margin and developing from base of cavity; d, rudimentary sepal; e, archesporial tissue in anther; g, archesporial tissue in ovule; h, integument; i, degenerating mother cells; j, microspore tetrads; k, tapetum; l, young pollen grains; m, degenerating tissue; n, egg; o, synergids; p, polar nuclei; q, antipodals; and r, nutrative tissue.

Fig. 1. Longitudinal section of a young flower.

Fig. 2. Same as Fig. 1 but older.

Fig. 3. Same as Fig, 2 showing rudimentary sepal.

Fig. 4. Longitudinal section of a flower of same age as in Figs. 2 and 3 but cut with the plane of sectioning rotated fourty-five degrees.

Fig. 5. Cross section of a flower showing the arrangement of parts at the time of the appearance of the archesporium in the anther.

Fig. 6. Same as Fig. 5 but cut nearer to the base of the flower.

Fig. 7. Longitudinal section of the nucellus showing the archesporium and nucellus.

Fig. 8. Longitudinal section of the entire flower of the same age as that in Fig. 7. Dotted lines represent vascular strands and indicate lines of development of the flower. X 100.

Figs. 9, 10, 11, 12, 13, and 14, Examples showing multiple archesporium varying slightly in stage of development as indicated by the condition of the nuclei. x 950 excepting Fig. 13 x 1200.

Fig. 15. Cross section of nucellus showing degenerating mother cells.

Fig. 16 and 19. Stages in the development of the microspore.

Fig. 17. Spindle of the heterotypic division of the mother cell. x 1750

Fig. 18. Longitudinal section of nucellus showing germinating megaspore.

Fig. 20. The mature embryo-sac. x500

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