Suggested Short Title ;

FEMALE GAMETOPHYTE AND EMBRYO OF

SMILACINA RACEMOSA (L)Desf.

Development of the Female Gametophyte and Embryo in *Smilacina racemosa* (L) Desf.

by

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STATEMENT OF THE PROBLEM

The small number of berries reaching maturity in Smilacina racemosa, and their few-seeded condition, invited a reinvestigation of the embryology[1]. The embryo sac development had previously been reported by McAllister in 1913. Modern knowledge of fruit formation, of apomictic development, and of endosperm-embryo relationships, have enabled a more inclusive study than was possible in the previous investigation.

[1]. The word "embryology", as used in this thesis, follows Maheshwari's usage, including sporogenesis and gametogenesis as well as embryogenesis. Embryogenesis is the only phase of development which Johansen (1950) includes in the term "embryology".

DESCRIPTION OF THE PLANT

Smilacina racemosa (L.) Desf., "False Solomon's Seal" or "False Spikenard" is found frequently in shady woods and forests across southern Canada and most of the United States, passing into var. amplexicaulis (Nutt.) S. Wats. in the west. It is a perennial herb with an erect, simple stem from a creeping rhizome, alternate, mostly sessile leaves, and white flowers. The terminal inflorescence is a racemose panicle, ovoid to pyramidal. The perianth of the flower is six-parted, in two alternating whorls, widely spreading and persistent. There are six stamens with four-celled, introrse anthers, splitting longitudinally when ripe (Fernald, 1950). The compound pistil is composed of three carpels. The style is short and thick; the stigma is somewhat three-lobed. The superior ovary is tricarpellary with axile placentation, becoming unilocular near the base of the style, where it continues into the stylar canal. There are two hemianatropous ovules in each locule. The fruit is a one- to few-seeded berry, green mottled with red when young, and red when mature.

TAXONOMIC POSITION

The genus Smilacina, established by Desfontaines in 1807, was based on Convallaria racemosa, named by Linnaeus in Species Plantarum, 1753; it in turn went back to Polygonatum racemosum Cornut, 1635 (Emons, 1945).

The type species is Smilacina racemosa (L.) Desf. Several varieties and forms have been described (Marie-Victorin, 1929; Fernald, 1938) but Galway (1945) includes them all in the species, which is "very variable in the size, shape and surface texture of all its parts" and adds only one variety, var. amplexicaulis (Nutt.) S. Wats., which was treated formerly as a separate species.

Synonyms for Smilacina which have been used are Vagnera Morong, (Britton and Brown, 1913) and Tovaria Neck. (Engler and Prantl, 1930). However, the generic name Smilacina has been conserved by the International Rules of Botanical Nomenclature (Camp et al, 1947).

The nearest North American relatives of Smilacina are Convallaria L., Maianthemum Desf., Clintonia Raf. and Polygonatum Desf. These were grouped in one family Convallariaceae by Britton (1901). Engler and Prantl (1930) put all of these genera except Convallaria in the group Polygonatae, and Convallaria in the adjacent group Convallarieae, of the sub-family Asparagoideae, family Liliaceae. In this thesis Smilacina racemosa (L.) Desf. will be discussed with comparisons to Smilacina stellata Desf., Convallaria majalis L., Maianthemum canadense Desv. and the European species Maianthemum bifolium (L.) DC.

REVIEW OF LITERATURE

Types of Embryo Sac Development[1]

There are three major classes of embryo sac development: monosporic, bisporic and tetrasporic. Monosporic, called the Polygonum type or normal type, is found in most of the flowering plants. Of the four megaspores formed in reduction division, three degenerate. From the remaining megaspore, three mitotic divisions result in an eight-nucleate sac, which becomes organized into the mature female gametophyte.

The bisporic embryo sac, usually called the Allium type, arises by two mitotic divisions from one pair or dyad of the four megaspores. The other dyad degenerates. In most cases the chalazal dyad is functional, as for example in several species of Allium, Convallaria majalis (Stenar, 1941) and several of the Alismaceae (Johri, 1935, 1938b). The micropylar dyad is functional in a few plants, such as several species of Scilla (Hoare, 1934) and Smilacina racemosa. Older literature often refers to the Allium type as the "Scilla type". However this type of development was first described for Allium and only later Scilla; accordingly the type should be known as the Allium type.

There are several variations in the development of the tetrasporic sac. The simplest is the Adoxa type, in which the four megaspores

[1] Except where the source is cited, the information in this section has been obtained from a secondary source (Maheshwari, 1950), in which references to the original articles may be found.

by one mitotic division form an eight-nucleate sac. Since Lilium had been considered to have this type, it was known as the Lilium type. However, in 1928 Bambacioni discovered that the pattern of embryo sac development in Fritillaria and Lilium was actually quite different from that described in Adoxa. Since then, reinvestigations have resulted in the transfer of most species from the Adoxa type to the new Fritillaria type. Only five genera, including Adoxa and Sambucus are now known to show the Adoxa type of development.

In the Fritillaria type, the four megaspore nuclei all participate in the formation of the mature sac, but one megaspore remains at the micropylar end while the other three collect at the chalazal end. At the first mitosis the spindles of these three fuse, so that two nuclei are formed at the chalazal end instead of six, by this division. Two more mitoses result in an eight-nucleate sac.

Another variation of the tetrasporic sac is the Peperomia type. It includes those embryo sacs formed by two divisions of the four megaspore nuclei, resulting in a sixteen-nucleate sac. An egg and one synergid are formed at the micropylar end, six antipodals at the chalazal end, and the eight remaining nuclei fuse to form a secondary nucleus. While none of the species discussed in this thesis belongs in the above group, Stenar (1934) classed Maianthemum bifolium as a "modified Peperomia type"; it actually belongs in the Drusa type.

In the Drusa type which has four megaspore nuclei, one remains at the micropylar end while three move to the chalazal end. Two mitoses follow, resulting in a sixteen-nucleate sac with four micropylar and twelve chalazal nuclei. In the organization of the mature

gametophyte, a three-celled egg apparatus, two polar nuclei, and eleven antipodals are formed; some of the antipodals may degenerate at once.

Swamy (1949b) found in Maianthemum canadense that while most of the embryo sacs were of the Drusa type, about thirteen percent of the ovules were different in their development. The chalazal spindles of the last division fused in pairs, so that the mature sac had four haploid micropylar and six diploid chalazal nuclei. Swamy called this a modified Fritillaria type, and suggested that it formed a link between the Fritiallaria type and the Drusa type.

Historical Background

In Smilacina, both the Allium type and Drusa type are found. The embryo sac development in S. racemosa is of the Allium type. McAllister (1909) reported S. stellata as an Adoxa type (modified Lilium type). Stenar (1934) questioned this; he found that its development to the eight-nucleate stage indicated Drusa type. Eunus (1950) worked out the later stages and confirmed it as Drusa type.

McAllister (1914) studied two other species of Smilacina. He stated that in S. sessifolia (S. sessilifolia) the embryo sac developed like that of S. stellata. The other species S. amplexicaulis, as far as he followed it to the four celled stage, developed in a manner similar to S. racemosa. Stenar (1934) suggested that S. sessifolia, if reinvestigated, would be classed as a Drusa type. McAllister's figures (1914) of S. sessifolia showed an eight nucleate sac with six chalazal and two micropylar nuclei, which is typical of the Drusa type. He did not refer to the organization of the mature sac.

It is interesting to observe that the latest taxonomic revision of the genus Smilacina (Galway, 1945) considers S. amplexicaulis Nutt. as a variety of S. racemosa, and S. sessilifolia Nutt. as a synonym for S. stellata.

McAllister (1914) also described Maianthemum canadense, which he reported as belonging to the Adoxa type. As cited by Stenar (1934) the European species M. bifolium was first described by Jonsson, 1879-1880. Jonsson's description was somewhat ambiguous, being interpreted later by Chiarugi and Schurhoff as Allium type, while Schnarf (1929) classed it as a doubtful normal type. Stenar's reinvestigation (1934) established it finally as a Drusa type. At the same time Stenar suggested that M. canadense might have the same type of development. This was confirmed by Swamy (1949b).

Convallaria majalis was studied first by Wiegand (1900), followed a year later by Schniewind - Thies. They gave conflicting reports, as noted by McAllister (1914). Maheshwari (1937) listed for C. majalis "Allium or Adoxa type". Stenar (1941) established it as Allium type.

Review of Adventive Embryony[1]

Although the embryo sac of Smilacina racemosa develops to the mature stage, the embryo is formed from enlarged nucellar cells, not from a fertilized egg. This type of apomictic development is adventive or adventitious embryony. It means that the new sporophyte plant is formed directly from the female parent sporophyte tissue, without fusion of gametes. The gametophyte generation is present but does not function directly in the development of the embryo.

The first instance of seed production without fertilization was described by J. Smith in 1841, in female plants of Alchornea ilicifolia. Strasburger later found that the embryos in these seeds were formed by adventitious nucellar budding. Discovery of other apomictic forms followed. The modern study of apomixis began with the work of Juel and Murbeck about 1900. The present accepted terminology was established by Winkler, modified by Gustafsson (1946).

In adventive embryony, the embryos arise within the ovules but outside the embryo sac. They develop from nucellar cells, or from cells of the integuments. Often more than one embryo is formed in an ovule, - thus polyembryony frequently accompanies adventive embryony. Pollination is usually necessary. The egg cell may be fertilized and the zygote undergo a few divisions before stopping its development or else, as in Citrus

[1] Except where the source is cited, information in this section has been obtained from a secondary source, Gustafsson's treatise "Apomixis in Higher Plants" (1946, 1947a, 1947b) and references to the original articles may be found in his bibliography (1947b).

(Swingle, 1927) normal embryos develop with the adventive ones. Even when pollination does not take place, endosperm is formed autonomously. As examples, Gustafsson (1946) mentions Alchornea ilicifolia, in which endosperm is formed and seeds are produced in female plants when there are no male plants growing in the vicinity. Endosperm is formed in Euphorbia dulcis, although the egg apparatus degenerates and the pollen is mostly inactive. Sarcocca pruniformis also has endosperm and numerous embryos in the vicinity of the sac, in spite of the early degeneration of the egg cell and pollen which does not function.

Here it may be pointed out that although Winkler regards adventive embryony as a form of vegetative reproduction since the embryos are sporophytic buds, there is a very important difference. Vegetative buds, bulbils or cuttings grow in the normal vegetative way, producing shoots, leaves or roots in the normal way. However, the adventive buds in the ovules develop like normal embryos, forming cotyledons, plumule and radicle in the same fashion. Most authors consider that the influence of the embryo sac is the factor causing adventive embryos to develop thus. The part of the embryo sac most important in this respect is the endosperm which is almost always formed, even without triple fusion. Haberlandt postulated a special "embryo sac regulating substance" which, in adventive embryony, causes undifferentiated cells to form true embryos and not callus tissue. Cook pointed out that "all of the differentiation evolved in the growth to maturity or even senescence is cancelled, and the bud begins over again and repeats its previous development as an embryo, with the specialized tissues and organs of an embryo" (Gustafsson, 1947a). Evidence of the difference

between vegetative reproduction and adventive embryony is shown by Citrus, in which cuttings eventually senilize a clone, while adventive embryo seedlings rejuvenate it (Maheshwari, 1950).

In 1940, Swingle suggested that a series of hormones is secreted in the embryo sac apparatus; these hormones act on both sexual and nucellar embryos and are responsible for embryos being formed at all. He said that the embryo sac represents a powerful morphogenic field of force - a "magic bath".

Haberlandt developed a "Necrohormone Theory" (Gustafsson 1947a) which aroused great interest for a while. The theory was that dying cells in the nucellus secrete a hormone which is responsible for the development of embryo sacs; and he claimed that he could induce adventive embryony in Oenothera by squeezing the ovaries. His method was repeated by other workers, Beth in particular, and their findings discredited the theory. However, in many cases of spontaneous adventive embryony, necrotic cells are present near the embryos. Furthermore, Gustafsson observes that since a wound-hormone which induces mitotic division has been discovered, there may be some value in Haberlandt's theory.

Finally in this connection, there is the work of van Overbeek, Conklin and Blakeslee who obtained "pseudoembryos" in Datura by auxin treatment. The "pseudoembryos" are growths or proliferations from the inner layer of the integument. They frequently consist of several hundred cells and lack the differentiation of an embryo. Gustafsson believes that possibly they need the "magic bath" of the fertilized embryo sac, and further work would show whether they could be likened to adventive embryos. If this is so, then the experimental control of adventive embryony is not

is not far off (Gustafsson, 1947a).

Very little actual knowledge has been gained about the genetic aspects of adventive embryony. Strasburger and Rosenberg were among the earliest to claim that polyploidy is common in apomictic plants. It is now known that polyploidy is often found in other types of apomixis, but species with adventive embryony are usually diploid. Plants with adventive embryony do not have disturbed meiosis, unless the disturbance is connected with hybridity, as is suspected for Alnus rugosa (Gustafsson, 1947a). Failure of the chromosomes to pair at synapsis does not occur in adventive embryony.

Whether there is any connection between adventive embryony and hybridity is not really known, except in the Citrus group, which has been studied very thoroughly, chiefly by Swingle, Frost and Webber. Swingle separated three genera, Fortunella, Poncirus and Citrus, which are closely allied (Webber and Batchelor, 1943). The original wild forms are unknown, and there is much hybridity among the many species and among the genera. There is no evidence that the heterozygous condition has induced adventive embryony. Instead, Frost points out that adventive embryony has made it possible for a very complex condition of heterozygosis to exist. Possibly the original wild forms were self-sterile and had a weak tendency to produce seeds asexually. The strength of this apomictic tendency increased through crossing, since the progeny were chosen for constancy. Selection would be for the nucellar seedlings which were all like the female parent. At the same time self-sterility would disappear. Thus in Citrus as often elsewhere, apomixis and self-fertility would have been acquired together. The hybrid vigour is maintained subsequently due to apomixis (Gustafsson, 1947a).

Gustafsson considers that heterozygosity attributes to apomixis by indirect methods, such as by recombination of special factors for apomixis. This does not exclude the possibility that specific apomixis-genes are present in many cases, especially in instances of adventive embryony in which the change from sexuality to apomixis only concerns the sporophyte. For Citrus "it is evident that the adventitious embryony is genetically determined and in certain cases is recessive to sexuality" (Gustafsson, 1947a). Gustafsson believes that the Citrus study may indicate that adventitious embryony is also inherited according to a simple factorial scheme.

In his paper on Biotype and Species Formation (1947b) Gustafsson points out that many diploid sexual species possess a high rate of vegetative reproduction. In his list of such plants he included Convallaria and Polygonatum species. Previously he had said that many groups producing seeds asexually were characterized by a high capacity for vegetative spread; and that agamospermous seed production means a vegetativization to some degree of the plant. He pointed out that plants reproducing by seed only are rare in northern regions. According to him, there is an obvious relation between chromosome number and vegetative development: annuals are diploid, while perennials with much vegetative reproduction are predominantly polyploid or have high basic numbers.

Species of the Liliaceae which have adventive embryony are (Gustafsson, 1947b):

Allium odorum

Hosta caerulea

Nothoscordum fragrans

Smilacina racemosa

Tulipa Gesneriana

It is apparent that none of these species is closely related to Smilacina racemosa.

Germination Studies

Seed germination studies have been reported for Smilacina racemosa. McAllister (1913) attempted to germinate seeds without success. Adams, in 1927, got 49% germination. Barton and Schroeder (1942) reported that S. racemosa seeds showed a new type of epicotyl dormancy. The seeds require cold treatment for root development; this is followed by a warm period, and then a longer cold period to after-ripen the epicotyl so that the first green leaf appears above the surface. Seedlings can be obtained in fourteen months with controlled temperatures. When planted out of doors, green seedlings do not show above the ground until after the second winter. The seeds germinated best when planted out of doors immediately after being harvested. The authors suggested that the stored seeds are short-lived, losing their vitality after one winter. This is typical of fleshy fruits.

MATERIALS AND METHODS

Collections of Smilacina racemosa inflorescences were made in late May and early June, 1950 by R.B. Roper at Ile Perrot, Lachine Woods and Morgan's Woods, near Montreal. This material was fixed in equal parts of absolute alcohol and glacial acetic acid, and stored in 70 per cent alcohol.

In 1951, collections were made from early May to late June, once a week and more often around the time of pollination. The plants were collected at Ile Perrot, Mount Royal and Val Morin. The first collection, on May 9, did not appear to contain the earliest stage of ovule development, so a trip was made north to Val Morin in the Laurentian Mountains, where growth in general was two weeks later than at Montreal. On May 9 at Ile Perrot the S. racemosa plants were over a foot high, with an elongated stem and expanding leaves. At Val Morin on May 16, most of the shoots were about eight inches high, the stem had not elongated, and the leaves had not uncurled. This collection contained a younger stage than was found in the first collection.

The 1951 collections were fixed in 3:1 absolute alcohol-acetic acid, Craf III, and Craf IV (Sass, 1940). The absolute-acetic fixative was more convenient for field use, due to its rapid penetration. Craf, being an aqueous solution, required the prolonged use of a vacuum pump to aid its penetration of the tissues. However, since absolute-acetic produced some shrinkage of the cytoplasm, especially in the early stages, Craf was considered to be the better fixing fluid. There was no difference in effect between Craf III and Craf IV.

Material containing the four-nucleate and later stages (collected after May 20) was softened in hydrofluoric acid before proceeding toward embedding. Some of the 1950 material was embedded in celloidin, but as the need for serial sections became apparent, all further embedding was done by the paraffin method from the butyl alcohol series (Johansen, 1940). Sections were cut at ten microns for the youngest flowers, eighteen microns for mature flowers, and twenty-two microns for young fruits. All sections were mordanted with three per cent iron alum and stained regressively with 0.5 per cent haematoxylin.

In addition to the permanent slides, temporary preparations of fresh material were used to obtain additional information. To find the number of nuclei in the pollen grains, acetocarmine smears were made of ripe unopened anthers. Pollen grains were germinated on agar coated slides and stained to show male cells in the pollen tubes (Bishop, 1949). To determine the presence of pollen tubes in the stylar canal, pistils were dissected and crushed. The condition of the stigmatic papillae served as an indication of pollination. Attempts were made to dissect embryo sacs out of the ovules. This was not successful, as the embryo sac could not be separated from the nucellus. Lactic acid was found to be a good mounting medium for crushed pistils or ovules, since it also cleared and softened the material.

Young seeds from preserved material were dissected and stained in acetocarmine. Although the micropylar region of the sac could not be separated from the nucellus, the nuclear endosperm was clearly visible.

OBSERVATIONS

Ovule Development

Megasporogenesis

The youngest stage in my preparations shows the young ovules extending horizontally from the placenta. The position of the inner integument is already indicated by slight distensions of the nucellus near the apex of the ovule. There is no sign of the outer integument. One hypodermal layer separates the archesporial cell from the nucellar epidermis. The archesporial cell is nearly isodiametric, and measures 19 microns in length (Fig. 1). At this stage cell division is going on in all parts of the young ovule. Divisions in the hypodermal nucellar layer give rise to two parietal nucellar layers. Depending on the time of this division, one or two parietal layers may be seen at the megaspore mother cell stage, while by the end of the first meiotic division two parietal layers are generally present.

The archesporial cell enlarges to form directly the megaspore mother cell. The chromosomes very soon appear in the thread-like form of early prophase, and gather into a knot at one side of the large nucleus (Fig. 2). During the ensuing stages of prophase, the megaspore mother cell and its nucleus elongate considerably, so that the cell attains a length of 60 microns (Fig. 3). At the same time, the ovule has bent downward in its growth, the inner integument is about four cells long, and the outer integument can be seen near the base of the inner integument.

The megaspore mother cell divides transversely (Fig. 4). At telophase (Fig. 5), the cell plate extends completely across the cytoplasm, so that in later stages a complete separation between the dyads is seen; however, no visible cell wall is formed. Although the ovules in a flower are at approximately the same stage of development, cell divisions are not quite synchronized. Thus prophase may be seen in one ovule, while another in the same ovary shows metaphase. In the second meiotic division, both spindles may lie parallel to the length of the sac, or the chalazal spindle may be at right angles to it (Fig. 6). Consequently, either a linear tetrad (Fig. 7a, Fig. 8) or a $\perp$ shaped tetrad may be formed (Fig. 7b). The cell plates formed after the second meiotic division usually disappear before being completed but occasionally they can be seen extending all the way across the cytoplasm (Fig. 7a). The result is always two binucleate dyad cells. The chalazal dyad is usually smaller than the micropylar one (Fig. 8); occasionally they are almost equal in size (Fig. 7a).

Elongation of the sac apparently is arrested during meiosis, as the length of the tetrad is 65 to 70 microns, nearly the same length as a megaspore mother cell in late prophase. By the end of meiosis, the outer integument extends to the apex of the nucellus, while the inner integument is longer (Fig. 8). The thick walled hypostase which is prominent in the mature ovule can first be recognized at this time.

Megagametogenesis

The micropylar dyad forms the mature embryo sac. The chalazal dyad is non-functional; by the four-nucleate stage its nuclei sometimes have fused (Fig. 11), but the fused nuclei persist at least until the eight nucleate stage and occasionally until after the initiation of endosperm. The nuclei of the functional dyad move apart and a vacuole forms between them, while the sac enlarges (Fig. 9). The first mitotic division (Fig. 10) results in a four-nucleate sac with two micropylar and two chalazal nuclei, separated by a large vacuole (Fig. 11). The four nuclei are not all in the same plane, as the spindles are at an angle to each other. In the micropylar region the inner parietal layer of the nucellus shows signs of being absorbed; the nuclei are small, distorted, and deeply stained. Occasionally a nucellar cell of the outer parietal layer is noticeably larger than its neighbouring cells, and periclinal divisions in the nucellar epidermis are sometimes observed. The nucellar cells at the sides of the sac elongate as the sac grows larger (Fig. 11). The inner integument encloses the nucellus. The outer integument is as long as the inner one, and grows over the tip of it on the side away from the placenta.

In the second mitotic division the two spindles at the micropylar end are perpendicular to each other as are the spindles at the chalazal end (Fig. 12). The nuclei resulting from this division move apart almost immediately. Although cell walls are not always visible there is a definite separation between the cytoplasm of one cell and the next (Fig. 13). At this stage the sac is 116 microns long.

Organization of the mature gametophyte occurs rapidly (Fig. 14). The upper polar nucleus moves to the lower part of the primary endosperm

cell, until the two polar nuclei are adjacent. They gradually become appressed. The antipodal cells are small, but may persist as irregular, dark-stained masses during the first few endosperm divisions. The synergids are somewhat triangular, and in some cases have a well-marked filiform apparatus. A vacuole has been observed occasionally in the lower part of a synergid. Normally, the egg is pear-shaped, with a large vacuole in the upper part of the cell, while the nucleus occupies most of the lower part (Fig. 16a). The embryo sac is constricted below the middle so that the polars, and especially the antipodals with the non-functional dyad, lie in an elongated narrow region. There is a thick-walled hypostase surrounding the chalazal region. The mature gametophyte together with the non-functional dyad, measures 180 microns in length.

The egg apparatus frequently shows signs of degeneration. In such cases the synergids are more or less shrunken and deeper staining (Fig. 19b). The vacuole in the egg cell may be slightly or almost entirely collapsed. The synergids usually remain at the micropylar end, while the egg may be found, detached from the synergids, in a lower position. Occasionally the egg cell appears to be attached to the upper wall of the primary endosperm cell, both being withdrawn from their original positions in the shrinkage accompanying fixation.

Embryogeny

The inner parietal layer of the nucellus becomes absorbed between the two-nucleate and the four-nucleate stage. Absorption of most of the outer parietal layer is completed by the eight-nucleate stage. However, as early as the two-nucleate stage in some ovules, one or rarely

two cells of the outer parietal layer can be distinguished from the adjacent cells by their larger size. By the eight-nucleate stage more than half of the ovules contain one or more such enlarged nucellar cells. These cells are actively metabolic in appearance, with a large round nucleus and dense granular cytoplasm. A vacuole may be present, in which case the nucellar cell resembles an egg cell (Fig. 16c).

Divisions of the enlarged nucellar cells produce the embryos. The time of the first division is variable, but usually occurs during the elongation of the mature embryo sac. The "pro-embryos"[1] come to occupy the same region as the egg apparatus.

Various stages in embryo formation are seen in Figs. 15-19. The nucellar cell may be in prophase while the egg apparatus is still intact (Fig. 15). At other times the egg apparatus shows degeneration while the nucellar nucleus is still in a metabolic condition (Figs. 16, 19). Frequently, in one ovule there are two nucellar cells in different stages of embryo formation (Figs. 16, 19). The nucellar nature of the embryos is indicated by the fact that the cell walls enclosing them come from the nucellar layer next to the epidermis.

The "pro-embryos" are variable in form, but usually their structure indicates origin from a single parietal nucellar cell (Fig. 20, a-i). In ovules where the polar nuclei are still appressed, frequently there are "pro-embryos" of two to four cells, rarely eight cells. In other ovules, there is not even a recognizable enlarged nucellar cell.

[1] These are not actually pro-embryos, since Johansen (1950) says "in all instances the pro-embryo originates from the zygote". The embryo proper starts with the fifth cell generation.

In some ovules at a later stage when a hundred or more endosperm nuclei are present, small embryonic masses, different from those already described, may be seen in the micropylar region. These embryos are several cells wide, and by the arrangement of their cells appear to have arisen from proliferations of the nucellar epidermis (Fig. 20, j,k). Occasionally, ovules containing between 100 and 200 endosperm nuclei are without any indication of an embryo.

Endosperm Formation

During the elongation period of the mature gametophyte the two polar nuclei remain pressed together. The nuclear membranes between them become faint and difficult to distinguish, especially when the section being examined has been cut parallel to the plane of the appressed surfaces. Careful observation, however, shows the membrane between them even after the time of pollination.

Only rarely has the secondary nucleus been seen. It is very large, containing four or five nucleoli, while the unfused polars each contain two or three nucleoli. The secondary nucleus functions as the primary endosperm nucleus. After it divides, one of the nuclei moves toward the micropylar end of the sac (Fig. 19, b). The first two or four endosperm nuclei are large (Fig. 19, b), but after a few more divisions the nuclei appear small and less sharply stained, embedded in dense cytoplasm (Fig. 18). Divisions of the endosperm nuclei are simultaneous at first. Two-, four- and eight-nucleate stages have been seen, as well as later stages estimated at 50, 100, 200 and 300 nuclei. The endosperm is free nuclear, forming a peripheral layer around the greatly enlarged,

nearly spherical vacuole. The size of the ovule increases proportionately.

In ovules with over 200 endosperm nuclei, small "vesicles" can be seen extending inward from the band of endosperm near the chalazal end. This seems to indicate the beginning of wall formation in the endosperm.

The size of the ovules does not increase in proportion to the amount of endosperm. When there are about 300 endosperm nuclei, the embryos usually contain three to eight cells although one embryo (Fig. 20, k) contained at least twenty cells in a broad low mass. At this stage there are frequently two or three embryos in the ovule, all situated near the micropyle.

Pollen Development

The youngest stage collected, containing archesporial cells in the ovules, shows in the anthers microspore mother cells in early prophase. They are densely cytoplasmic and packed together so that they take a hexagonal shape. The nuclei are large, the chromosomes appearing first as very delicate threads before they gather into a knot at one side of the nucleus. The wall of the anther consists of one tapetal layer, one middle layer already flattened, endothecium and epidermis. Pollen development is successive, the cells in the centre of a pollen sac being slightly more advanced than those next to the tapetum. Although there is some variation, the anthers of one flower are usually at the same stage of development.

When the ovules contain elongated megaspore mother cells, the microspore mother cells have separated from one another. Their nuclei are in early to late prophase. By this time mitotic nuclear division has taken place in the tapetal cells, so that each tapetal cell contains two to four nuclei.

During meiosis in the ovules, meiosis also takes place in the anthers. The process occurs rapidly, as late prophase and metaphase are found in one anther, or metaphase and telophase in another. Separation of the chromosomes at anaphase is regular. The second meiotic division follows immediately after the first telophase, and is completed very quickly. The spindles of second division are at right angles to each other so that the tetrads are tetrahedral, the microspores appearing first as small flattened cells inside the large pollen mother cell wall.

When the tetrad stage has been reached in the ovules, the microspores frequently have separated from each other, or they may still be in tetrads. While the embryo sac is in the two-nucleate stage, the microspores enlarge and become round. They have a large vacuole in the cytoplasm and a heavy wall. Division of the microspore nucleus usually occurs just before division of the two nuclei in the embryo sac. The tapetal layer in the anther breaks down, and scattered droplets are seen among the pollen grains. The epidermis of the anther at this stage is flattened and irregular.

By the four-nucleate stage in the embryo sac, the pollen grains are two-celled. The lens-shaped generative cell is cut off against the wall of the pollen grain, and a vacuole is apparent. At this stage an estimated ten percent of the pollen grains are flattened and empty. Fibrous bands are seen in the endothecium, although nucleus and ctyoplasm

are still present in the cells.

The pollen grains are ripe when the ovules contain eight-nucleate embryo sacs. The exine coat of the pollen grain contains thickenings, and the germinal furrow is visible. The cytoplasm is dense and uniform; there are two nuclei. The wall between the pollen sacs breaks down at this stage. In some sections the anthers are split open on the line of dehiscence, but the pollen grains have not escaped.

At the mature gametophyte stage in the ovules, the anthers are shedding pollen. The mature pollen grain does not contain a vacuole; the generative nucleus is smaller and more deeply-staining than the vegetative nucleus. Frequently the vegetative nucleus is irregular in outline, indicating the start of degeneration. The number of empty pollen grains is higher, about one-quarter to one-third of the total amount. Rarely, three-celled pollen grains have been seen but normally division of the generative cell takes place in the pollen tube soon after germination.

When pollen grains land on the stigma, the stigmatic papillae collapse. Pollen tubes are numerous in the stylar canal. They have been observed occasionally near the micropyle of the ovules, but for the most part they are not present in the locules. No instances have been found where a pollen tube has penetrated the ovule.

The appearance of pollen tubes in the locules is unlike that of the pollen tubes observed in the stylar canal. Those in the stylar canal contain male cells and granular cytoplasm; occasionally a burst pollen tube has been seen, with its discharged contents beside it. The pollen tubes in the locules are pale staining, without any visible contents. No trace of discharged nuclei or cytoplasm has been found near these pollen tubes.

Irregularities in Development

Although the young inflorescence of Smilacina racemosa contains a large number of flowers, relatively few of them develop into mature berries. Furthermore, although the young ovaries contain six ovules, there are only one or two seeds in the berries. In the sections it was noted that some ovules are degenerated within two weeks after pollination. To seek some explanation of these occurrences, all slides from the youngest stages onward were re-examined in an effort to discover when and how the abnormalities developed.

At the megaspore mother cell stage, occasionally there are two megaspore mother cells in one ovule, either side by side or one below the other. In a sample of 120 ovules counted, four of these twin megaspore mother cells were seen. Ovules containing a degenerated megaspore mother cell were extremely rare. One ovary in this group contained ovules in one carpel only, the other two carpels having no trace of ovules. In seventy ovules examined at the end of meiosis, two twin tetrads were present and three ovules showed degeneration of the group of megaspores.

Twin sacs are occasionally found in ovules at the mature gametophyte stage. The number of degenerated sacs is greater than at the tetrad stage, but varies from flower to flower. In one flower there may be six good ovules; in another, all may be degenerated. Most frequently, there is an intermediate number. Exact counts were not made, because it became obvious that these ovaries could be separated into two groups, one of which showed normal growth while the other appeared abnormal.

At the time of pollination sections of the flowers in the group designated "abnormal" show a constricted area at the base of the ovary, between the attachment of the perianth and the base of the locules (Fig. 21). The ovaries of the normal flowers are convex in this region (Fig. 23). Later collections show that the ovaries displaying this constriction are no larger than pre-pollination ovaries, and their walls are wrinkled (Fig. 22). The other ovaries in the same collection have grown into young fruits (Fig. 24). The two groups of ovaries contain similar numbers of degenerated ovules, although early degeneration of the egg apparatus appears to be more frequent in the "abnormal" group. When endosperm is present in ovules of the "abnormal" flowers, the sac and outer layers of the ovules are not enlarged. In the normal group, on the other hand, when endosperm is present the whole ovule is much enlarged, and the sac is nearly spherical (Fig. 24).

Examination of a flower cluster at the pollination stage shows that the flowers at the top of the inflorescence and those at the tips of the secondary peduncles have upright stamens and smaller ovaries than the ones near the base of the inflorescence, which have spreading stamens. Where stamens are present in sections of "abnormal" flowers, they are upright, (Figs. 21 and 23), while those of the normal group are spreading. An abscission layer is present in all the flowers of both groups. As recorded in the field notes for the 1951 collection, most of the flowers near the top of the inflorescence absciss about three weeks after pollination without forming fruit. In sections of "abnormal" flowers collected about this time the ovary wall shows signs of degeneration, and all the ovules are degenerated.

From the above description it is apparent that the factors resulting in abscission of flowers are not the same as those resulting in one- or two-seeded berries.

Before the pollen is shed, the ovaries usually contain six ovules at the mature gametophyte stage. This stage is of long duration, during which time the sac elongates and the polar nuclei become appressed. The egg apparatus starts to degenerate, and nucellar "proembryos" are formed in some ovules, while in others not even an enlarged nucellar cell is visible. The mature gametophyte stage terminates when the polar nuclei fuse. Divisions of the fusion nucleus follow very quickly, forming the endosperm. This takes place about the time of pollination.

Although endosperm is formed in one or two ovules of the ovary, occasionally even in three, the remaining ovules are in various stages of degeneration. In sections of ovaries at pollination time or afterward, the size of degenerated ovules gives an indication of the stage at which their growth ceased. Most of them are the same length as normal ovules of the mature gametophyte stage. Usually the degenerated ovules are withered, and the embryo sacs are collapsed, or full of diffusely stained material. When it is possible to distinguish their contents, appressed polar nuclei can be seen near the chalazal end of the sac. These observations show that development is arrested at the mature gametophyte stage in most degenerating ovules.

In addition, the small size of some degenerated ovules and the very large size of a few others, shows that degeneracy may set in before the mature gametophyte stage or even after endosperm has become established.

DISCUSSION

General Development

The archesporial cell of the Smilacina racemosa ovule is found in shoots which have emerged a few inches above the ground. It passes into the megaspore mother cell stage long before the leaves unfold. This may explain why the archesporial cell has not been described before in S. racemosa or the related species.

Reports on the position of the megaspore mother cell show much variability. McAllister (1913) found that in S. racemosa, by the time of synapsis the megaspore mother cell was usually separated from the nucellar epidermis by one layer, occasionally by more than one, and rarely by none. In S. stellata (McAllister, 1909) usually there were two layers, sometimes one or even none, between the megaspore mother cell and the nucellar epidermis. Stenar (1934) described the megaspore mother cell of Maianthemum bifolium as separated from the nucellar epidermis by one cell division. He could not agree with Jonsson's earlier statement that there were two parietal rows of cells. One or a pair of cell divisions separate the megaspore mother cell from the nucellar epidermis in Convallaria majalis (Stenar, 1941).

The position of the archesporial cell as seen in Smilacina racemosa helps to explain these differences. It is situated underneath one parietal nucellar layer, which at this time is two cells wide. As the archesporial cell enlarges to form the megaspore mother cell, the parietal layer divides, one cell frequently dividing before the other (Figs. 5 and 6). Stenar's figures for Convallaria show a similar arrangement of nucellar cells.

The occasional occurrence of two megaspore mother cells in an ovule has been reported for Smilacina racemosa (McAllister, 1913), Maianthemum bifolium (Stenar, 1934), M. canadense (Swamy, 1949b), and Convallaria majalis (Stenar, 1941). In Smilacina racemosa both may develop into mature gametophytes. Those in Convallaria develop equally to the two-nucleate stage, after which one sac always degenerates. Swamy reported finding several ovules with two mature sacs in Maianthemum canadense. In one case the separating wall had disappeared and two pollen tubes had effected the fertilization of both sacs.

The present observations on the embryo sac development of S. racemosa are in agreement with McAllister's description up to the eight-nucleate stage. However, from the eight-nucleate sac onward my observations are at variance with his.

McAllister described the eight-nucleate sac of Smilacina racemosa as having "three plump normal nuclei" at the micropylar end, three distorted ones at the chalazal end, and two polar nuclei approaching each other. In his words, "at a somewhat later stage membranes are formed around the micropylar nuclei forming an irregular group of cells ... apparently they very rarely become organized to form the typical egg apparatus"; and again "the cells of the micropylar end were irregular in size, number and arrangement, bearing no resemblance whatever to an egg apparatus and lacking any cell which might be regarded as an egg cell" (McAllister 1913, p. 620-621).

My description shows that cell formation follows immediately after the second mitotic division. The three antipodal cells and the three cells of the egg apparatus are separate from the sac-like primary

endosperm cell containing the two polar nuclei. Whether cell walls are always formed around the synergids is not clear but a thin wall is often visible. The wall of the egg cell is definite. The egg apparatus does not appear to touch the nucellus. The present observations show that the enlarged nucellar cells are joined to the nucellar epidermis, although McAllister considered that they tended to become separated from it. The egg apparatus and the enlarged nucellars together constitute McAllister's "irregular group of cells".

Even when the egg apparatus exhibits marked degeneration, its cells can be recognized by their typical shape. They can always be distinguished from nucellar cells. However, it is easy to understand McAllister's misinterpretation. Since the mature gametophyte is so large as to extend through several sections, the whole micropylar group of cells can be visualized only after a study of several planes. Thus in a single section the cells seen at one focus may be completely different from those seen at another focus. Sometimes five or six serial drawings through three sections are necessary to co-ordinate the arrangement of the cells.

The appearance of the egg apparatus in several related species resembles that of S. racemosa. The synergids in S. stellata sometimes have a filiform apparatus (Eunus, 1950). In Swamy's figures of Maianthemum canadense, the synergids resemble those of Smilacina racemosa, being densely cytoplasmic and rather triangular in outline. In Maianthemum bifolium also the synergids sometimes display a filiform apparatus; the egg is not quite apical in position, and broadly inserted. The parietal layers of the nucellus degenerate so that the mature embryo sac lies adjacent to the nucellar epidermis, due to the degeneration of the intervening nucellar layers (Stenar, 1934).

McAllister reported that in Smilacina racemosa some of the nucellar cells became rounded and "considerably vacuolated". They proceeded to form adventitious embryos and could sometimes be seen dividing before the polar nuclei had fused. At other times they remained undivided until after the first division of the endosperm nucleus. He found that frequently the embryo appeared to develop from a single nucellar cell, but occasionally there was a mass of cells whose origin might be multicellular.

That the embryos arise from nucellar cells and not from the egg is clearly illustrated by ovules containing both a nucellar embryo and the complete egg apparatus (Figs. 15 to 19). The embryos are variable in structure (Fig. 20) but most of them appear to have a unicellular origin. None of them has a suspensor cell. Occasionally the arrangement of the cells indicates a multicellular origin. This is in accordance with McAllister's findings. The multicellular embryos appear to rise from the nucellar epidermis. Since they have been found only in young seeds, it seems that they start to develop only after the endosperm begins to multiply, whereas the embryos coming from single nucellar cells often start to develop before the polars have fused.

According to Johansen (1950), few of the Liliaceae for which the embryogeny is known conform to any of the well-known embryo types. Allium ursinum (which he placed in the Amaryllidaceae) is one carefully investigated species of the Liliaceae, considered by Johansen to exemplify those monocotyledonous embryos which do not have a suspensor. The genus Lilium he considers to be "characterized by an excessive degree of irregularity in the location and disposition of the cell walls during both the early and later proembryonic stages"(Johansen, 1950, p. 243). However, Smilacina

racemosa should not be compared too closely to these, since the relationship between adventive and zygotic embryos is not known for the early stages; older embryos are similar, whether nucellar or zygotic.

Measurements of the length of the sac, interestingly enough, showed that elongation of the sac is not a continuous process, but progresses by "pulsations". The megaspore mother cell elongates until the end of prophase, but growth practically ceases during meiosis. Some enlargement follows between the tetrad- and the eight-nucleate stage. Another period of elongation takes place during maturation of the gametophyte. Once endosperm formation begins, the sac grows rapidly as indicated by the scarcity of ovules containing less than fifty endosperm nuclei.

McAllister considered there was strong evidence of fertilization as well as nucellar embryony because of the abundance of pollen tubes in the styles, which were "not infrequently found in the vicinity of the micropyle and in several cases were seen entering the micropyle". He stated that "the confused collection of nuclei ... at the outer end of the nucellar cavity makes it very difficult to recognize a male nucleus"; and he found the lack of an easily recognizable egg cell an obstacle to determining whether any nuclear fusion occurred (McAllister, 1913, p. 623). From the results of bagging experiments, he concluded that "although an embryo is probably rarely developed from an egg, still pollination is necessary to initiate the adventive budding of the nucellar cells". However, he did not examine the contents of the flowers which withered in the absence of pollination. Since embryos may form before pollination they may have been present in those flowers; McAllister's experiments did not prove that pollination is necessary for the initiation of adventive embryos.

In the present study, no evidence of fertilization has been found. Pollen tubes occasionally seen near the micropyle of ovules are abnormal in appearance and most of the pollen tubes do not reach the locules. The occurrence of burst pollen tubes in the stylar canal is too infrequent to have significance. Pollen tubes contain nuclei in their early growth but do not have them near the tip of such tubes as elongate to reach the ovules. These circumstances indicate that there may be a sterility factor associated with the pollen. At the present time, the pollen has no function in embryo and endosperm development, but it is possible that pollen sterility may have been a factor in inducing the apomictic condition.

The observation that pollen tubes do not penetrate the ovule and the accompanying lack of fusion of a male cell with the secondary nucleus leads to the conclusion that endosperm is formed autonomously. McAllister did not refer to the possibility of fertilization of the polar nuclei in S. racemosa. He described the polars as remaining in contact, but distinct from each other, until about the time of endosperm formation, when they were seen to be fused; again, he referred to "the fusion of the polar nuclei and the first division of the resulting endosperm nucleus".

My observations, like McAllister's, show that in Smilacina racemosa the secondary nucleus is also the primary endosperm nucleus. The polar nuclei, situated in the lower part of the sac, remain pressed together for a long time while the membranes between them grow thinner. Because most ovules show either this stage or many endosperm nuclei, in different flowers of the same collection or even in different ovules of one flower, it is apparent that fusion of the polar nuclei is followed very

quickly by the first few endosperm divisions. Although over fifty ovules containing endosperm and at least 200 ovules with appressed polar nuclei have been examined, only rarely has the secondary nucleus been found, and division of the primary endosperm nucleus has been observed only twice. Great enlargement of the ovule normally accompanies the rapid multiplication of the endosperm nuclei.

In S. stellata, according to Eunus (1950) the polar nuclei meet in the centre of the sac. He did not indicate when they fuse, nor did he refer to fertilization. Swamy described the secondary nucleus of Maianthemum canadense as being situated nearer the antipodals than the egg apparatus. This means that the polars fuse before fertilization. He stated that fertilization occurs, and free nuclear endosperm is formed (Swamy, 1949b). The polar nuclei in Maianthemum bifolium usually lie in the lower part of the sac, and do not fuse until the time of fertilization (Stenar, 1934). One synergid is usually damaged by the entrance of the pollen tube, whose refractive point continues to be visible for some time after fertilization. The endosperm divides before the zygote, developing far more rapidly so that there are 120 to 130 free nuclei before the embryo reaches the four-celled stage.

In the opinion of Gustafsson (1946) and Maheshwari (1950), endosperm formation without triple fusion is extremely rare even when the embryos are not fertilized. Maheshwari considers most of the reports he cites to be of doubtful validity. In those reports which he considers valid, the phenomenon is described as an occasional occurrence in species which usually have double fertilization. These species are Vincetoxicum nigrum, Mitella pentandrum, Zostera marina, Taraxia ovata and Zauchneria latifolia. Maheshwari states: "sooner or later . . . such embryos are

likely to stop growth so that no viable seeds are produced" (Maheshwari, 1950, p. 209). Gustafsson, however, cited a few species where autonomous endosperm formation occurs regularly (see p. 9). These cases are less open to doubt than those listed above, since most of his examples are species with non-functional pollen, or ones in which seeds are formed without the presence of pollen. Smilacina racemosa differs from them since pollination takes place although the pollen tubes do not penetrate the ovules.

A full discussion on the nature of endosperm with its relation to fertilization phenomena and especially to the embryo, has been given by Brink and Cooper (1947). The function of the endosperm is to mediate certain growth processes of the young embryo in the early stages before it is able to perform these functions for itself. The double fertilization of angiosperms is considered to be a mechanism whereby the advantages of hybridity become available to the endosperm, enabling it to become aggressive in the ovule.

These investigators point out that the conditions permitting seeds to mature without fertilization of the endosperm have not been discovered. They suggest that many apomictic species should be reinvestigated to determine whether triple fusion occurs, and when it does not, whether the polar nuclei fuse before endosperm formation. Further investigation of autonomous endosperm formation might "shed light on the unique secondary fertilization in which the endosperm of angiosperms takes its origin" (Brink and Cooper, 1947, p. 518).

Whether pollination is necessary for seed formation in Smilacina racemosa is not known. McAllister's experiments, designed to show that

pollination was necessary for adventive embryo formation, were inconclusive, since he did not determine the contents of ovules of the unpollinated flowers.

Recent studies on fruit development show that one of the functions of pollen is to stimulate the production of growth hormones in the ovary, leading to development of the fruit. The phenomenon of induced parthenocarpy shows that this role of the pollen can be separated from its function in seed formation, since fruits can be produced without pollination by the application of synthetic growth substances (Gustafson, 1942a).

Seeds containing embryos or endosperm are not formed in parthenocarpic fruit production. However, the outer layers of the ovule may be affected. In parthenocarpic Ilex fruits, the integuments of the seeds grow as rapidly as those of pollinated fruits, although endosperm and embryo are lacking (Gardner and Kraus, 1937). The formation of "pseudoembryos" in parthenocarpic Datura fruits has been described (see p. 10). Gustafsson considered that further investigation of this phenomenon would lead to the experimental control of adventive embryony.

Since Smilacina racemosa does not require fertilization for endosperm or embryo formation, experiments with the application of growth substances would conceivably result in the formation of viable seeds without pollination, and thus could contribute to knowledge of the nature of adventive embryony.

Adventive Embryony

Although the occurrence of adventive embryony has been reported in several species, this form of apomictic development has been studied very little. Its history and mechanisms have been reviewed by Gustafsson (1946, 1947a, 1947b) in his series of papers on apomixis in higher plants. He also discussed what is known of the causal aspects including genetic and physiological factors, the relation of adventive embryony to ecological distribution, and to biotype formation.

Smilacina racemosa is like most species with adventive embryony in that the embryos arise from the nucellus, there are no disturbances of meiosis, there is a well-marked condition of polyembryony, and pollination takes place. It differs from most of them in having endosperm formed in the absence of fertilization.

Except for the Orchidaceae and Podostemonaceae, where endosperm formation is suppressed (Maheshwari, 1950), endosperm is necessary for the development of seeds. In consideration of the postulated "magic bath" influence of the embryo sac apparatus which has been suggested as governing the growth and differentiation of embryos (see p. 10), the presence of endosperm in S. racemosa is important, even though it lacks the genome supplied by a male nucleus. Although the Necrohormone Theory (see p. 10) as an explanation of adventive embryony has been discredited, it is worth noting the presence of necrotic nucellar cells at the micropylar end of the embryo sac in S. racemosa.

The connection between adventive embryony and hybridity has been studied in Citrus, which is the only intensively investigated example of adventive embryony. The Citrus group displays much hybridity among the

many cultivated varieties. Fertilization takes place, and both zygotic and nucellar embryos grow into seedlings. Frost's hypothesis of self-sterile and weakly apomictic original forms has been described (see p. 11). It is believed that the wide variety of forms resulting from hybridizing is maintained by the nucellar seedlings.

Smilacina racemosa is not known to be a hybrid. However, Galway's description of it as a species with variable morphological characters suggests a heterozygous condition. This is at variance with the fact that only adventive embryony is known to occur, since every new plant should be similar to its female parent. As in the hypothetical original stock of Citrus, there is a possibility of a self-sterility factor, in the pollen. But adventive embryony is the only means of seed formation known in S. racemosa, while the original Citrus forms were only weakly apomictic, and the present Citrus varieties are self-fertile.

Nigritella is another example of a heterozygous condition combined with adventive embryo formation (Gustafsson, 1947a). Nigritella rubra and N. nigra are both polymorphic species. They reproduce sexually, and hybrids between them are formed in certain geographic areas. In other regions they reproduce by adventive embryony. N. nigra reproduces sexually and hybridizes with N. rubra in the Alps, while in Scandinavia N. nigra is apomictic and uniform.

Smilacina racemosa has two forms: the species and one variety, var. amplexicaulis. Galway's distribution map (Galway, 1945) shows the following range: the species extends from the Atlantic Coast to the Rocky Mountains, while var. amplexicaulis extends from the Pacific Coast to the Rockies. The ranges of the two overlap, and in this region a group of

intergrading "forms" is found. If S. racemosa and its variety can be considered comparable to Nigritella nigra and N. rubra, then it is possible that sexual reproduction in Smilacina racemosa may occur in the region where the intergrading forms are found.

McAllister's material, collected near Beloit, Wisconsin, as well as the collections for the present study, were obtained from regions well to the east of the region where, on the above basis, sexual reproduction might be expected to occur.

Gustafsson (1947b) considered that there is an obvious relationship between chromosome number and vegetative development; and that perennials with much vegetative reproduction are either polyploid or have high basic numbers. S. racemosa is an herbaceous perennial with a creeping rhizome which permits some vegetative reproduction. Its chromosome number, as reported by McAllister (1913), is twenty-four. Woolery (1915) obtained a haploid number between twenty and twenty-four. S. stellata and Maianthemum bifolium have a haploid number of eighteen (Stenar, 1934), while Convallaria majalis has a haploid number of nineteen (Löve, 1948). Thus it appears that Smilacina racemosa is probably not a polyploid, although it has a high basic chromosome number.

Irregularities in Development

Irregularities in the development of S. racemosa seeds and fruits have been described. There are two separate trends, one toward the degeneration of ovules, the other toward the abscission of young fruits. Both are initiated at the pollination stage, irregularities earlier in development being insignificant in amount.

The abscission of young fruits is related, for the most part, to the position of the flowers in the panicle. There is very little difference from apex to base of the immature inflorescence, in either the size of the flowers or their stage of development. However, when the panicle is fully extended and the lowest flowers are mature, it is seen that the upper flowers are retarded in their development. Differences are noticeable both microscopically and macroscopically. Comparison of these flowers with the normal mature ones shows that growth of the retarded flowers becomes arrested approximately at the pollination stage; development in the embryo sac may continue for a short time without, however, the usual accompanying increase in the size of the sac.

The cause of arrested development cannot be attributed to lack of pollination, since pollen tubes are frequently found in the stylar canal of these flowers. It cannot be attributed to lack of endosperm formation, since endosperm may be found in the ovules. The fact that the ovary wall ceases growth first rather than the ovules, indicates that the ovary is unable to take advantage of pollination and the initiation of endosperm.

In normal flowers, pollination stimulates the production of auxins (growth hormones) in the ovary wall, so that it forms a fruit. Auxin produced in the ovary diffuses down to the pedicel, preventing abscission of the young fruit. Abscission usually takes place by alterations in the tissues at a special region in the pedicel, the abscission layer.

The presence of an abscission layer in both the normal and retarded flowers of S. racemosa makes it obvious that this layer is only the means, and not the cause of abscission in the retarded flowers.

Abscission of flowers within a few weeks after pollination occurs in many plants. Usually plants produce an enormous number of flowers of which only a fraction form seeds (Murneek, 1951). The abscission of young fruits has been investigated in detail for tree fruits, where methods have been devised to delay fruit drop by the application of synthetic growth substances. Usually, there are at least two waves of abscission of young fruits, one shortly after pollination and another just before the fruits reach maturity. Abscission may also take place between these two periods.

Until recently, it has generally been assumed that application of synthetic growth substances shortly after pollination causes abscission (Gardner, 1951). However, recent work indicates that the function of growth substances at early, as well as late drop, is to delay abscission and that the heavier fruit set resulting from the application of chemicals is followed by a heavy nutritional dropping (Struckmeyer, 1951). That is, abscission results from starvation through competition for nutrients.

Gardner (1951) in discussing the effect of NAA, which results in a heavy drop in apples when applied just after pollination, suggested that the young fruits affected were "less advanced" than those which did not abscis. He believed that the position of the flowers on the spur, by appecting their food supply, might be a factor in causing abscission. Murneek has noted Gardner's statement that the young fruit is sensitive for two weeks after fertilization, and may abort easily (Murneek, 1951).

A histological study of aborting fruits of Prunus cerasus was made by Bradbury (1929). She discussed the possibilities of attributing their drop to self- or cross-sterility, lack of pollination, lack of fertilization after pollination, improper nutrition of the tree, or competition of individual blossoms for nutritive material. She concluded that in Prunus cerasus the cause of fruit drop was nutritional deficiency. It could not be attributed to lack of pollination. She reported that the ovules did not increase in size after pollination, in the aborted fruits.

Considering the "arrested" flowers of Smilacina racemosa in relation to the studies discussed above, it becomes apparent that the most probable reason for the arrested development and abscission of flowers at the pollination stage is a deficient supply of nutrients.

In. S. racemosa some of the young fruits which survive the early drop period absciss later. Although this was not studied closely, it appeared to be a gradual dropping.

Phenomena occurring in S. racemosa should not be compared too closely with those in the tree fruits. Prunus is a stone fruit and apple a pome; both are dicotyledonous woody perennials. S. racemosa, on the other hand, is a monocotyledonous herbaceous perennial whose fruit is a berry.

Degeneration of a portion of the ovules also occurs in many plants. In Prunus and in the Compositae, for example, regularly one ovule degenerates while the other grows into a seed. In others including Smilacina racemosa, the nuber of degenerating ovules is variable.

The non-functional ovule in Prunus cerasus begins to degenerate before the mature gametophyte stage (Bradbury, 1929). In Smilacina racemosa, degeneration commences most frequently during the mature gametophyte stage, after the pollen has been shed.

Cooper, Brink and Albrecht (1937) reported that some ovules of Medicago sativa (alfalfa) abort after fertilization. The first indication of degeneration is a constriction of the embryo sac near the endosperm mother cell, or a constriction of the endosperm. Cooper et al suggested that in alfalfa nutritional deficiency is a result, rather than a cause, of the ovule's arrested development. Brink and Cooper (1940) stated that the ovules collapse within forty-eight hours after fertilization when the embryo-endosperm relationship is disturbed. Collapse may be induced by self-pollination.

Brink and Cooper believe that hybridizing gives a stimulus to growth, and double fertilization is a mechanism whereby the advantages of hybridity become available to the endosperm. There is no fertilization in Smilacina racemosa. However, the polar nuclei may be of different genic constitution, since in plants with bisporic development the two polar nuclei are descended from different megaspores (Brink and Cooper, 1947). This may give a stimulus to the endosperm.

The collapse of ovules in alfalfa is attributed to a disturbed endosperm-embryo relationship. However, in S. racemosa, where the embryos

arise from parent sporophyte tissue and the endosperm from fused polar nuclei, the endosperm-embryo relation is always the same and thus cannot explain ovule degeneration.

The large number of ovules whose development becomes arrested at the mature gametophyte stage, while the polars are appressed, suggests that failure of the polar nuclei to fuse is associated with ovule degeneration. The cause of their failure to fuse is unknown. It does not appear to be correlated with the absence of an enlarged nucellar cell or embryo since in degenerated ovules these may be present or absent, and also, in good ovules embryo formation may be delayed until after endosperm has become established.

In some species related to S. racemosa (see p. 34) the polars do not fuse until the time of fertilization. Possibly the lack of a fertilization stimulus in S. racemosa may help to explain why the polars frequently fail to fuse. If and when they do fuse, belatedly, then endosperm divisions follow rapidly and development of the seed takes place.

Nutritional supply is a possible factor in ovule degeneration which may be influenced by fusion of the polar nuclei or may act independently. It has already been mentioned that the embryo sac enlarges greatly within a short time after the initiation of endosperm. If endosperm were formed in one ovule first, it could take all or most of the nutrient supply to the ovules, and retard the development of the other ovules in the ovary. However, two or even three developing seeds are often found in one ovary soon after pollination, and they are not all the same size, as would be expected if endosperm formation were initiated in all three at the same time.

The possibility of a nutritional factor is sustained by the observation that when endosperm formation occurs it proceeds very rapidly, and a large number of endosperm nuclei is soon established. However, in ovaries with arrested development, a condition believed to be due to nutritional deficiencies, the sac does not enlarge with endosperm initiation.

Some ovules which have produced endosperm begin to degenerate when they contain over a hundred endosperm nuclei. This ovule degeneration is not necessarily due to competition for the nutritional supply, since the ovule which collapses may be the largest in the ovary. If an unbalanced embryo-endosperm relation were the cause of degeneration, it would be expected to take effect within a few days, as in alfalfa. The large degenerated ovules, however, were from young fruits collected three weeks after pollination.

The occurrence of irregularities in development has been reported for Maianthemum bifolium also (Stenar, 1934). Stenar pointed out that in this species only a few flowers of the cluster form berries, and each berry has only one or two seeds, occasionally three. The number of ovules in the young ovary is four. Stenar found that normal mature embryo sacs were formed in all ovules of most flowers. Moreover, embryos and endosperm could be seen in at least three, often all four, of the sacs in an ovary. Sometimes one ovule of the four ceased development at the eight-nucleate stage, and did not become organized into a mature gametophyte. In one ovary which Stenar described, three ovules contained embryos and endosperm, while the fourth was very big and degenerated - he believed it had been fertilized. This indicates that degeneration of ovules in M. bifolium begins sometimes after endosperm formation is established.

Stenar could not explain the reason for the abscission of flowers in M. bifolium. He decided that it probably was not due to poor pollination or to anomalies in the male apparatus, although he pointed out that he had not studied pollen development beyond the two-celled stage. He did not consider that dropping was due to anomalies in embryo sac development either, since they were too infrequent.

SUMMARY

1. This study is a reinvestigation of embryo sac development in Smilacina racemosa.

2. The observations in the present study agree with those of McAllister (1913) from the megaspore mother cell to the eight-nucleate stage.

3. The ovules are bitegmic and crassinucellate.

4. The archesporial cell is separated from the nucellar epidermis by one hypodermal layer.

5. Division in the hypodermal layer gives rise to two parietal nucellar layers.

6. The archesporial cell functions directly as the megaspore mother cell.

7. Two binucleate dyad cells result from reduction division. The chalazal dyad is non-functional, but persists to the early endosperm stage.

8. Development is bisporic: the embryo sac is formed by two divisions of the micropylar dyad.

9. Contrary to McAllister's observations, cell formation takes place immediately after the eight nuclei are formed.

10. A pear-shaped egg cell, two triangular synergids, three antipodals and two polar nuclei are formed.

11. The egg apparatus degenerates during the mature gametophyte stage.

12. The embryos are adventitious, arising by divisions in single enlarged parietal nucellar cells, or by proliferations from the nucellar epidermis.

13. The enlarged nucellar cells adjoin the nucellar epidermis. The egg apparatus appears not to be in contact with the nucellus.

14. Contrary to McAllister's report, the egg cell is always recognizable, even when degenerate, and nucellar cells can always be distinguished from the egg apparatus.

15. Nuclear endosperm is formed autonomously, immediately after fusion of the polar nuclei.

16. Pollen formation is successive. The tapetum is glandular. The pollen grains are two-celled.

17. Pollen germinates readily on the style; division of the generative nucleus is completed very soon after germination.

18. Pollen tubes sometimes reach the locules, but do not penetrate the ovules.

19. The long pollen tubes do not show male cells or cytoplasm near the tip.

20. Embryos may become multicellular before endosperm formation begins.

21. Polyembryony is frequent.

22. Contrary to McAllister's opinion, there is no evidence of fertilization.

23. The development of Smilacina racemosa is compared with other species in the same taxonomic group.

24. The conditions accompanying adventive embryony and autonomous endosperm formation are compared with other species.

25. Possible causal aspects of adventive embryony in this species are discussed.

26. The possibility of fertilization and hybridity between Smilacina racemosa and var. amplexicaulis is suggested.

27. Irregularities in development are described. These cause
a) a reduction in the number of flowers developing into berries;
b) a reduction in the number of seeds per berry.

28. Reduction in the number of berries is attributed to nutritional deficiency causing arrested development of distal flowers at the time of pollination; abscission of the flowers follows.

29. The effects of deficiency are shown first in the ovary wall, later in the ovules.

30. The small number of seeds results from ovule degeneration, chiefly at the mature gametophyte stage. This is considered to be related to failure of fusion of the polar nuclei.

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Explanation of Plates

All figures were drawn with the aid of an Abbe camera lucida and a Reichert research microscope.

Plate I

Fig. 1. Young ovule; archesporial cell separated from nucellar epidermis by one hypodermal layer. x 350.

Fig. 2. Megaspore mother cell in early prophase; two parietal nucellar layers present. Start of inner integument. x 350.

Fig. 3. Megaspore mother cell, late prophase. One parietal nucellar layer. Outer integument present. x 350.

Fig. 4. Megaspore mother cell in metaphase. x 350.

Fig. 5. Early telophase of Meiosis I. Cell plate not completed. x 350.

Fig. 6. Metaphase of Meiosis II. The two dyads are separated. x 350.

Fig. 7. Tetrad of megaspores following second meiotic division.
a) Four separate cells, nearly equal in size; linear arrangement.
b) Cell division not complete, giving two binucleate dyad cells in ⊥ form; the chalazal dyad is smaller. x 500.

Fig. 8. Linear tetrad of megaspores. Functional dyad enlarged, non-functional chalazal dyad very small. Inner integument extends beyond nucellus. x 350.

Plate II

Fig. 9. Two-nucleate sac with central vacuole. Cells of inner parietal nucellar layer partially absorbed. x 450.

Fig. 10. Metaphase of first mitotic division, spindles at right angles to each other. Non-functional dyad at chalazal end. Absorbed parietal cells at micropylar end. x 450.

Fig. 11. Four-nucleate sac. Fused nuclei of non-functional dyad at lower end of sac. Nucellar cells at sides of sac elongated. x 450.

Fig. 12. Metaphase of second mitotic division. Spindles oriented in different planes. x 450.

Fig. 13. Seven-celled gametophyte, just after the division in Fig. 12. Lowest cell contains the fused nuclei of the non-functional dyad. x 450.

Fig. 14. Typical mature gametophyte; at micropylar end, two triangular synergids with vacuolate egg cell between them; polar nuclei in lower part of primary endosperm cell; three small antipodals, and non-functional dyad in the lowest position. At the micropylar end, one cell of the parietal nucellar layer has not been absorbed. x 450.

Plate III

Fig. 15. Serial sections through micropylar end of mature sac. a) Enlarged parietal nucellar cell in prophase, surrounded by delicate walls of absorbed cells. b) Egg apparatus; egg (at right) withdrawn from the apex. x 450.

Fig. 16. Serial sections through micropylar end of mature sac.

a) Egg, with one synergid at right, somewhat degenerated. Parietal nucellar cells above partly absorbed.

b) Second synergid at right, enlarged nucellar cell at centre, in metabolic state.

c) Another enlarged nucellar with large vacuole in upper part of the cell. x 450.

Fig. 17. Egg apparatus and dividing nucellar cell. Synergids at left, one below the other; the egg in atypical form, at right below the nucellar cell. x 450.

Fig. 18. Two celled "pro-embryo" from outer parietal nucellar layer, surrounded by walls of absorbed cells. Endosperm nuclei below the embryo. x 450.

Plate IV

Fig. 19. Early endosperm stage. Serial sections.

a) Micropylar end only; one synergid at left, telophase of first division in an enlarged nucellar to the right.

b) Entire length of sac. Remainder of synergid shown in first section, with the other synergid below it and egg at centre. The egg apparatus is in a late stage of degeneration. Upper right, enlarged nucellar cell in metabolic state. Two large endosperm nuclei from division of the primary endosperm nucleus in lower part of sac. At the chalazal end, three small antipodals, with the non-functional dyad below them. Thick walled hypostase surrounds the chalazal region. x 450.

Plate V

Fig. 20. Variability of embryos. Diagrams. x 150.

a-i: 2- to 8-celled embryos, unicellular origin from parietal nucellar layer.

j, k: embryos with multicellular origin from nucellar epidermis. j) 4-celled, and k) ca. 24-celled embryo.

a, b: ca. 8 days after pollination.

c-i: three weeks after pollination.

j,k: shortly after pollination.

c and h: 2 embryos in one ovule.

d and e: 2 embryos in one ovule.

Fig. 21. "Arrested" flower at pollination time. Note long narrow region of ovary below the ovules, and upright stamen. x 24.

Fig. 22. "Arrested" flower just before abscission. Ovary wall has irregular outline, base of ovary constricted, filament of stamen upright. x 24.

Fig. 23. Normal flower at pollination time. Base of ovary is convex. x 24.

Fig. 24. Young fruit. Developing seed at left, with nearly spherical endosperm sac. Degenerated ovule at right. From same collection as Fig. 22. x 24.

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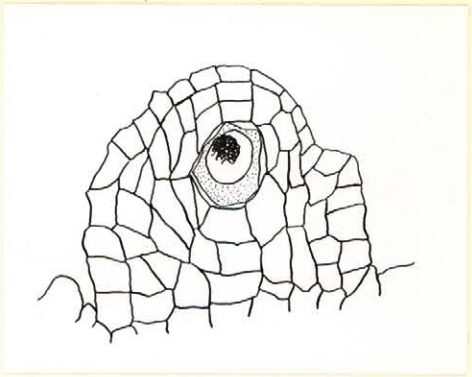

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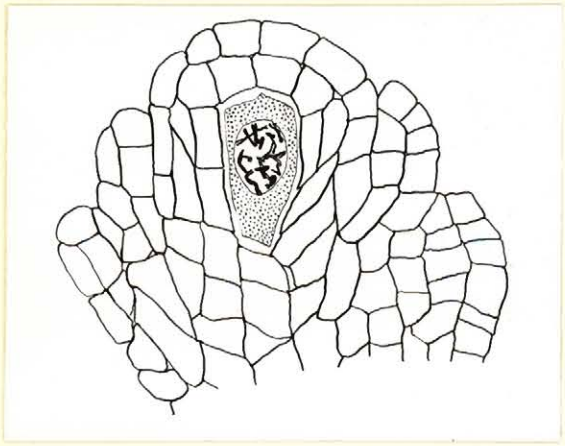

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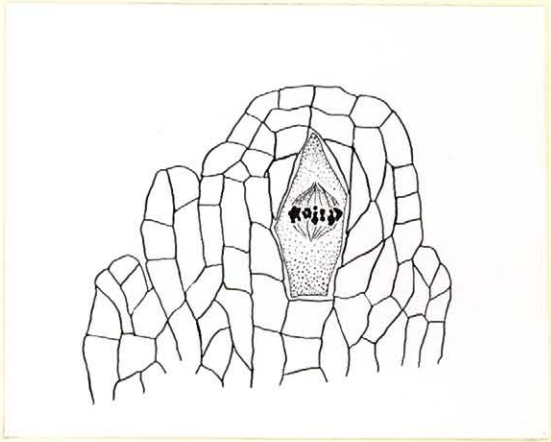

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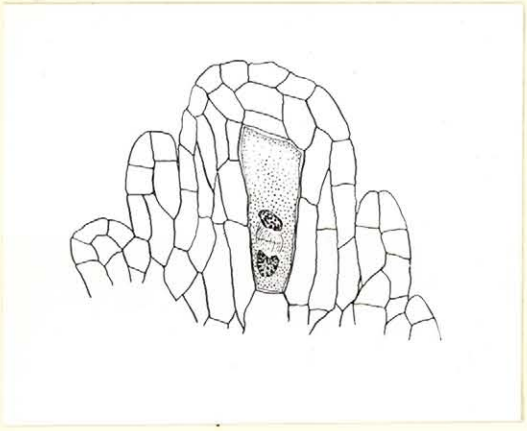

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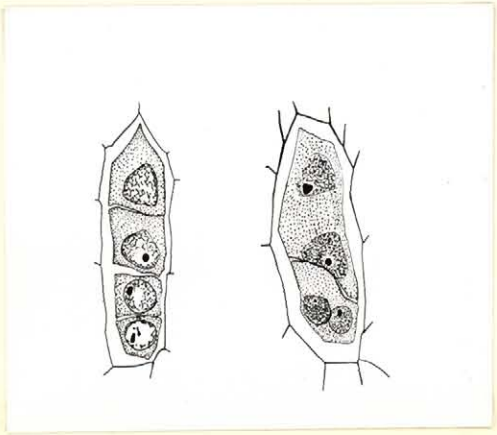

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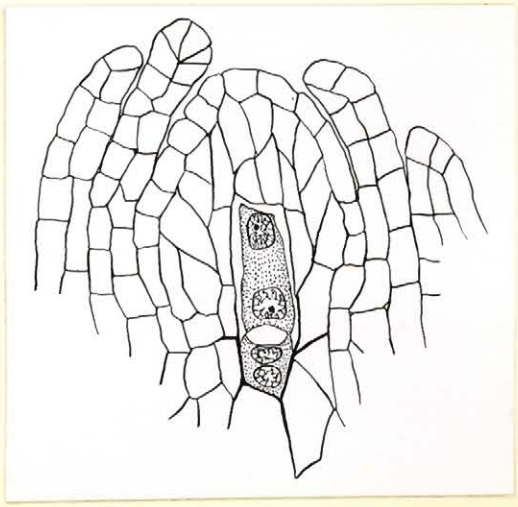

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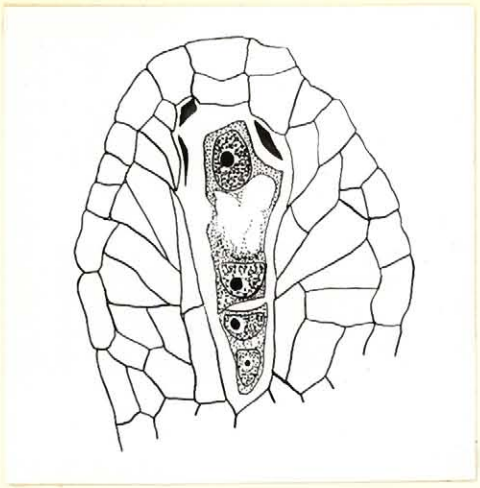

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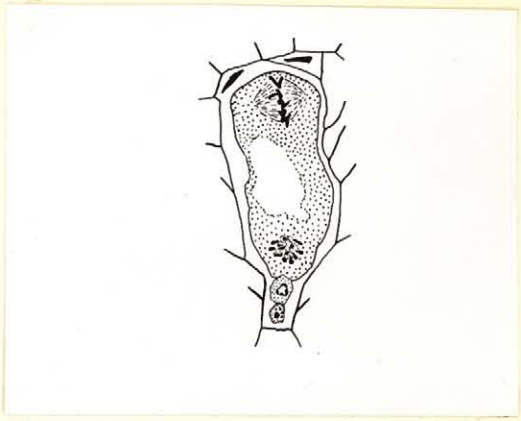

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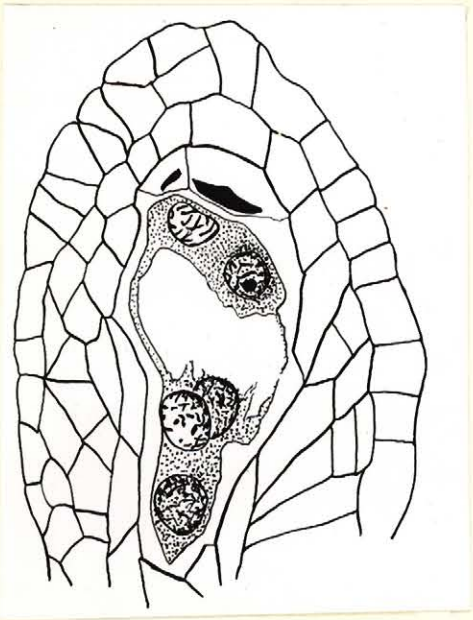

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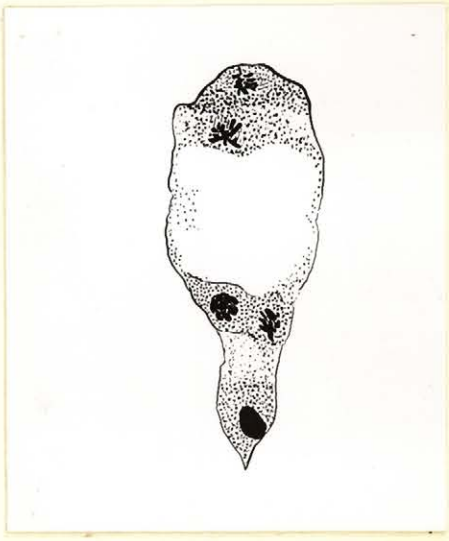

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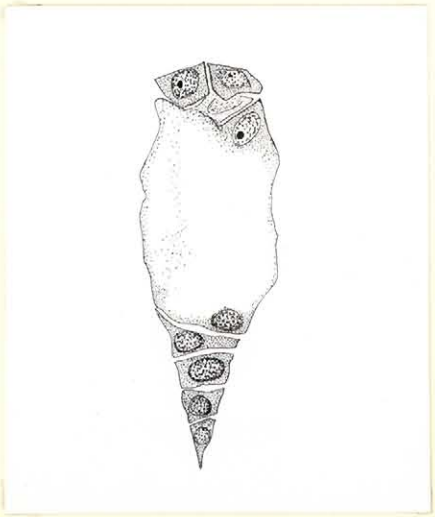

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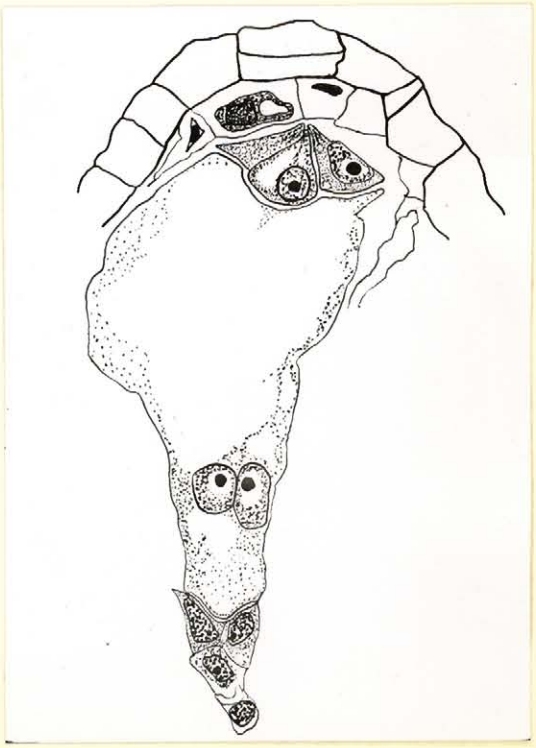

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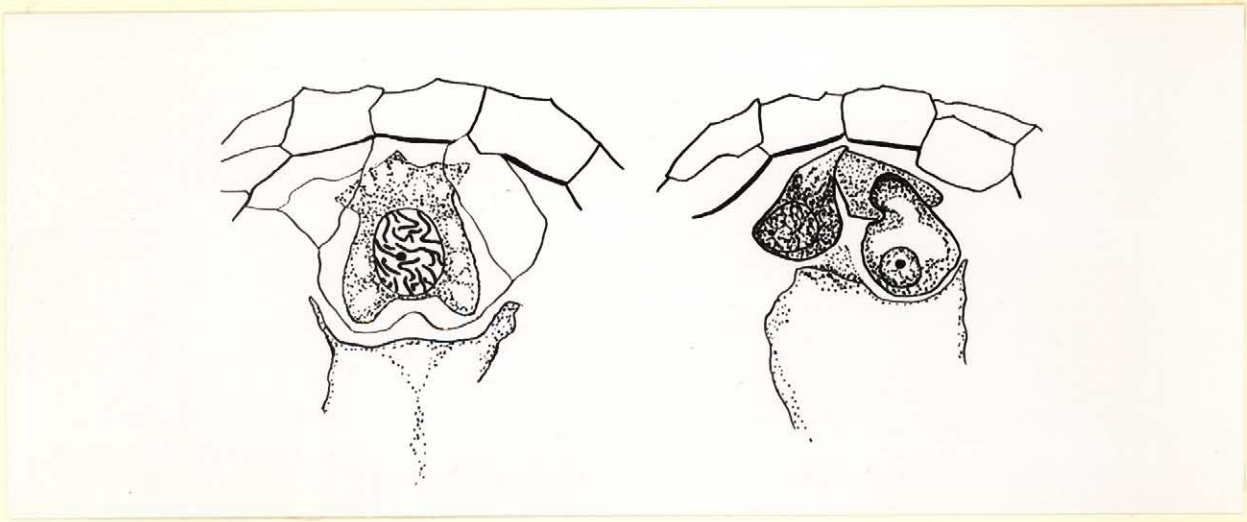

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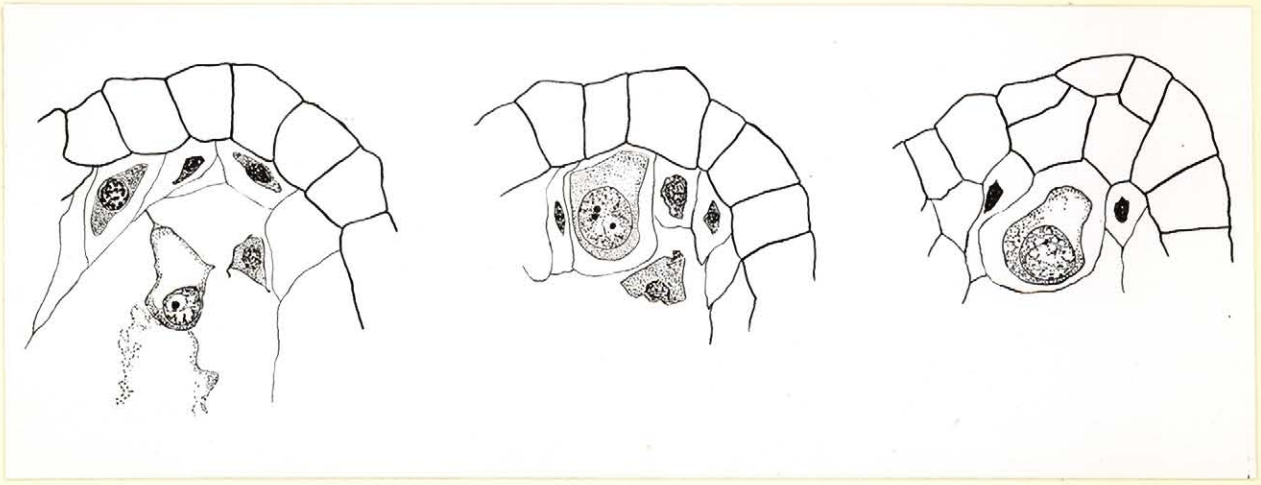

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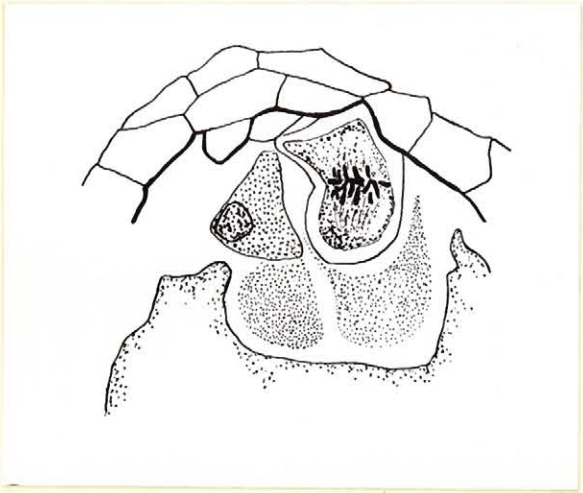

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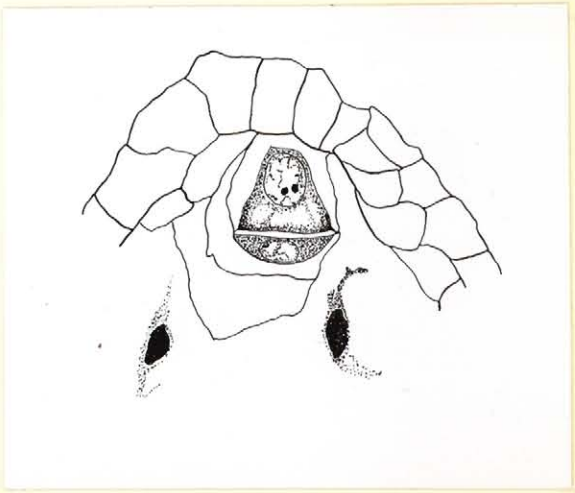

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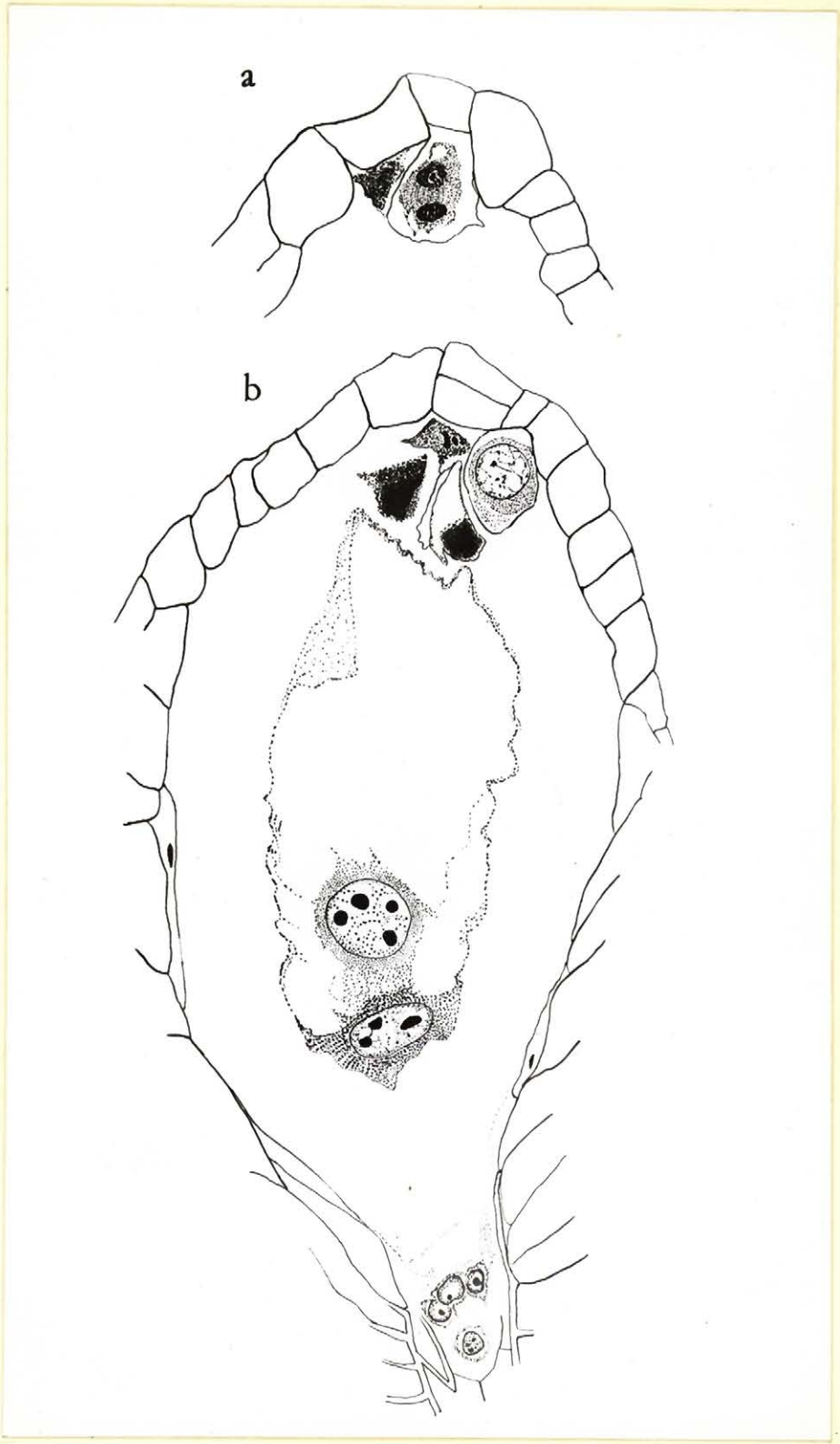
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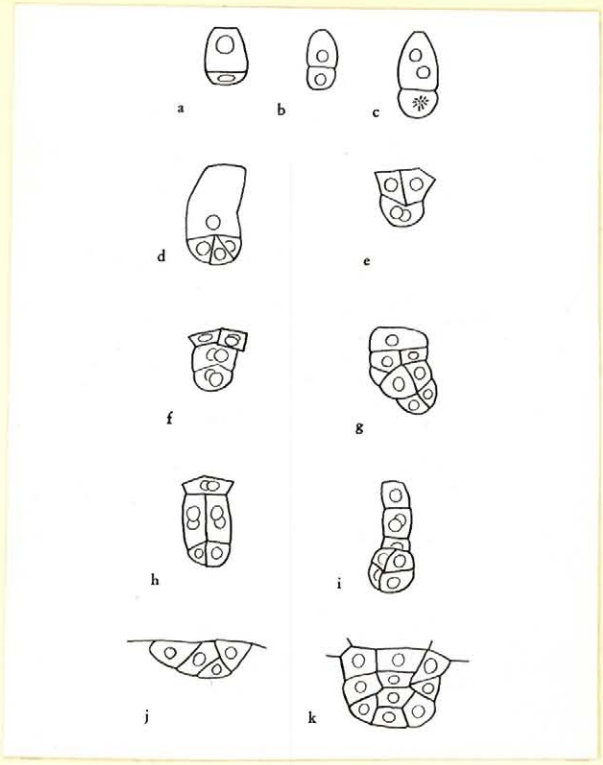

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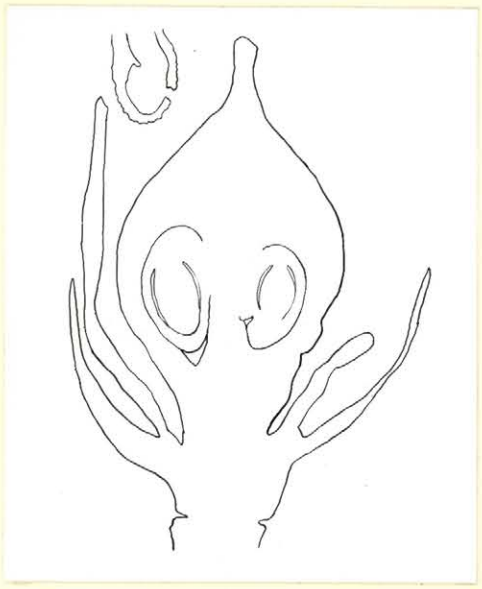

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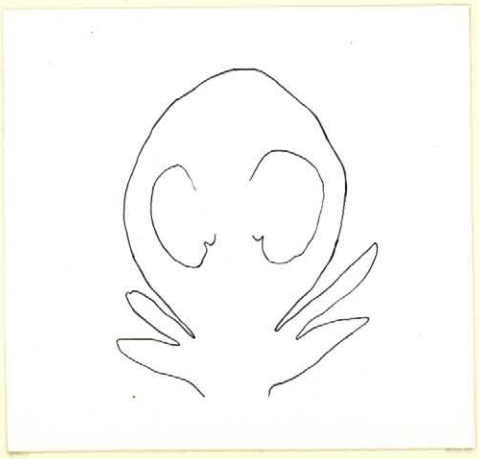

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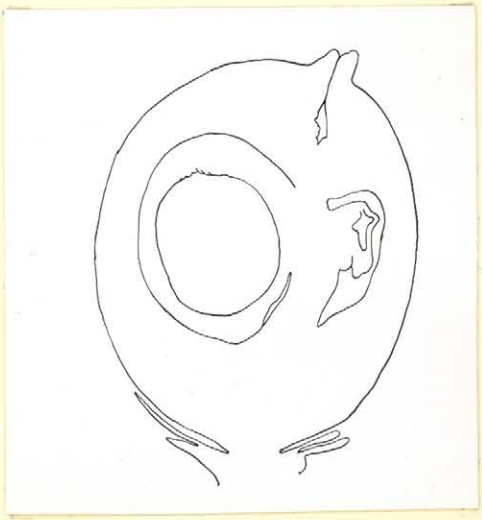

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