EARLY ENDOSPERM DEVELOPMENT AND EMBRYOGENY IN *CASSIOPE HYPNOIDES**

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One of several trips made to collect material for study of the several aspects of floral morphology in the Ericales was up Mt. Washington, N. H. This trip was during the latter part of June 1949, in the company of Dr. Stuart K. Harris of Boston University, who is well acquainted with the flora of the Presidential Range. Here, at the head of Oakes Gulf, a single patch of *Cassiope hypnoides* (L.) D. Don was found. The flowering material was carefully removed and placed in Conant's modification of Navashin's killing and fixing solution. The material was dehydrated in an ethyl alcohol-tertiary butyl alcohol series and embedded in paraffin. Both cross and longitudinal sections were cut at 10 μ. Three different stains were used: Kraus' modification of Flemming's triple, Foster's tannic acid-ferric chloride-safranin, and Heidenhain's iron alum hematoxylin with Orange G. The latter two were more satisfactory, but of these it was easier to prevent overstaining of embryo and endosperm by using the last. The flowers of this one collection proved on study to show the developmental stages immediately following fertilization, and it is these which are reported here. All drawings were done with a camera lucida at the same magnification, each from a single section if possible, but occasionally two adjacent sections were combined into a composite figure.

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Observations

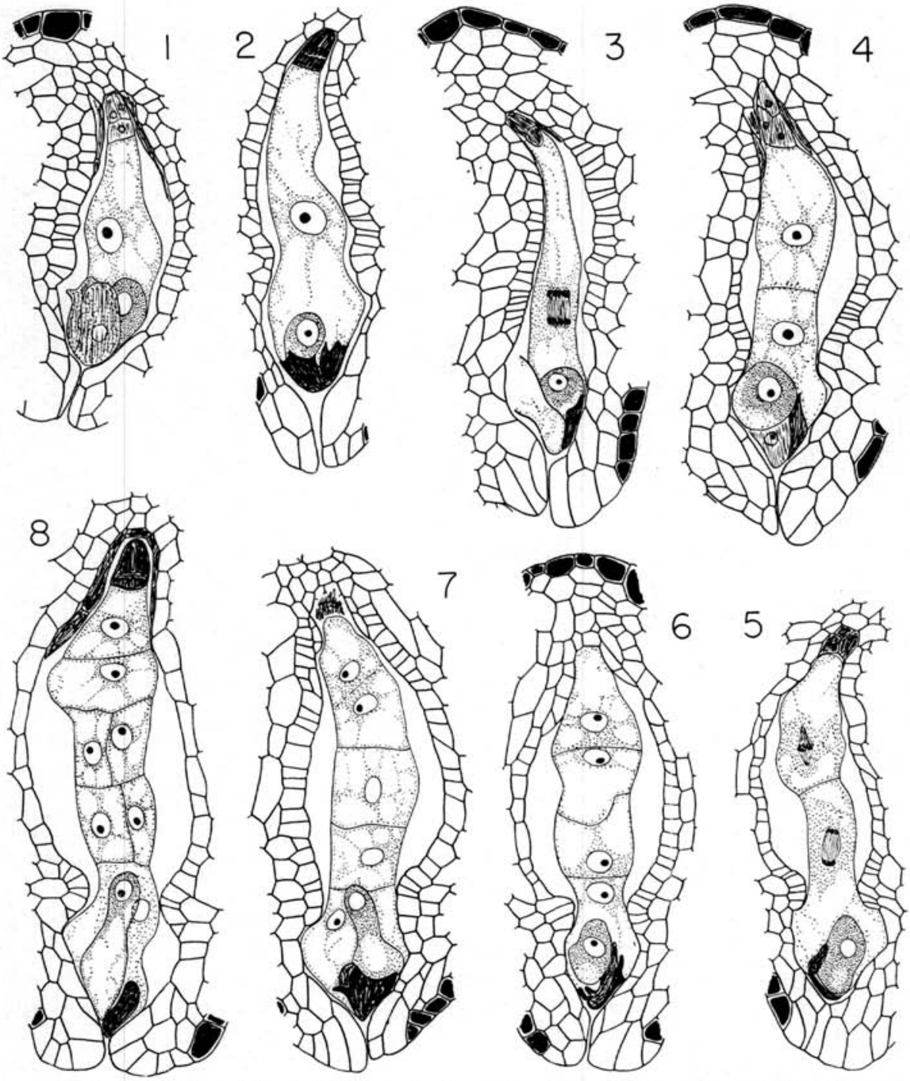
The pistil in *Cassiope hypnoides* is five-carpellate. A large downward projecting placenta is axile in position in each of the five locules at the base of the ovary. In the upper portions a split in the central column, continuous with the stylar canal, extends radially and divides each placenta into two portions so that the placentation becomes parietal with the placentae extending very deeply into the single locule. The ovules are small and very numerous —seventy to eighty per locule. They have been called anatropous but are are not strictly so, as there is a definite bend in the chalazal end of the gametophyte. Their orientation on the placentae is somewhat varied, so that sections suitable for study of developmental stages can be obtained from a relatively small proportion of the ovules in either cross or longitudinal sections of the flower, though the percentage is somewhat greater in the former.

The ovules are unitegmic and tenuinucellate. The single integument is from four to six cell layers in thickness and broader in the region of the raphe. The nucellus has disappeared by the time the megagametophyte is mature except for occasional disintegrating cells seen at the chalazal end. The inner layer of the integument, surrounding the region of the nucellus, differentiates as an epithelial layer with cells elongated perpendicularly to the long axis of the ovule. The cells of this layer usually contain more cytoplasm than other integumentary cells. The outer layer of the integument, except for a few cells around the micropyle, are filled with an opaque, highly stainable material, thought to probably be tanniniferous in nature.

An occasional ovule showed a mature megagametophyte. In every case where one was observed, it seemed apparent that no pollen tube had reached that ovule and that the thus unfertilized gametophyte was beginning to disintegrate (fig. 1). The micropylar region of the gametophyte, containing two rather large synergids and the egg, is slightly expanded anterior to the epithelial layer of the integument. The secondary nucleus (fused polars) is located just back of the beginning of the epithelial layer, while the narrower slightly bent chalazal region is occupied by three small antipodal cells. These normally occur in the form of a T rather than linearly, though the latter condition is found on rare occasions. The two cells located side by side usually occur at the very end, but occasionally the single cell occupies this position. Because the antipodals are located in the curved end of the gametophyte it is necessary to have a longitudinal section of the ovule cut through the raphe to see them. Sections cut at right angles, or almost right angles, to this plane will show most of the gametophyte (or endosperm), but not the antipodals.

The penetration of the pollen tube destroys one, and usually both, of the synergids. Occasionally one synergid will persist for a short time after fertilization (fig. 4). Apparently the fusion of egg and sperm and secondary nucleus and sperm occurs rather rapidly, the former somewhat more rapidly than the latter. In all instances where a pollen tube had penetrated the micropyle, a zygote with a single nucleus was observed, but in at least two cases the fusion of the sperm with the secondary nucleus was not complete. The antipodals may start to disintegrate even prior to fertilization, but the process is rather slow and their remains can be distinguished for a considerable period subsequent to fertilization (fig. 8). Immediately after fertilization (fig. 2), therefore, an embryo sac shows a zygote and the remains of pollen tube and synergids at the micropylar end, the primary endosperm nucleus in the center, and disintegrating antipodals at the chalazal end. The remains of the pollen tube and synergids may also persist for a considerable period of time.

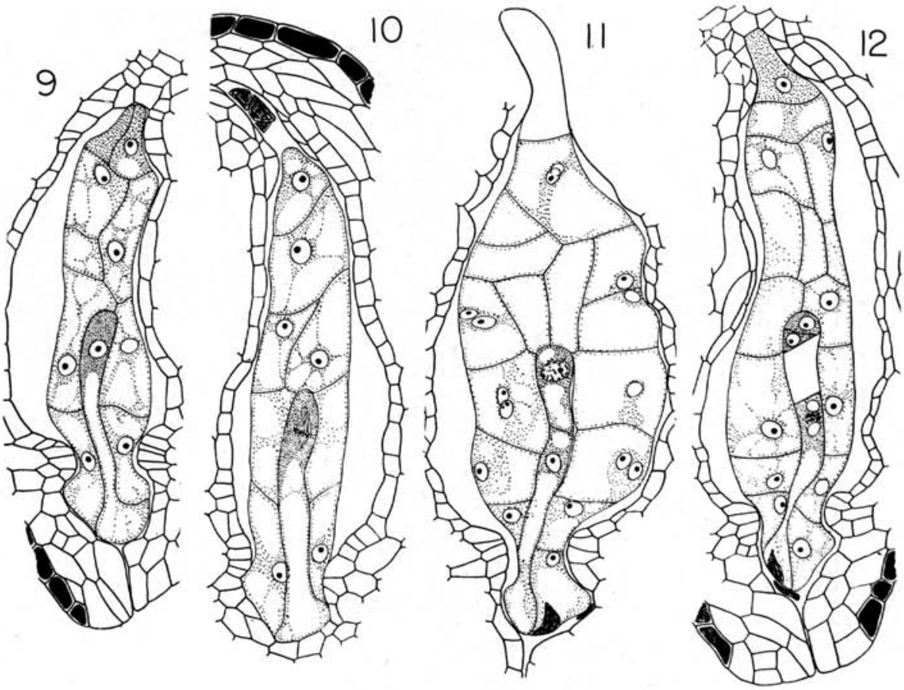
During subsequent development, the most anterior cells of the epithelial layer of the integument remain elongated in the usual fashion and, with the expansion of the embryo sac both in front of and behind this, form a narrow constricted area near the micropylar end of the embryo sac. The region anterior to the constriction forms the micropylar haustorium, while the main bulk of the endosperm and the embryo proper develop posterior to the constriction. The epithelial cells of the integument posterior to the constric-



FIGS. 1-8.—*Cassiope hypnoides*, about x365. Fig. 1, unfertilized megagametophyte beginning to disintegrate. Fig. 2, zygote and primary endosperm nucleus. Figs. 3-8, stages in the development of endosperm and zygote.

tion become considerably flattened in the direction of the long axis of the ovule during the expansion of the endosperm (fig. 12).

The initial stages of endosperm development p r e c e d e those of the zygote and embryo. The first division of the primary endosperm nucleus (fig. 3) is oriented parallel to the long axis of the embryo sac, and is followed by cell wall formation thus dividing the embryo sac into two approximately equal chambers (fig. 4). These two cells divide



FIGS. 9-12.—*Cassiope hypnoides*, x365. Later stages in development of endosperm and early stages in development of embryo.

again, the micropylar one slightly in advance of the chalazal (fig. 5), and again nuclear division is followed by cell division (fig. 6). As shown in figure 6, the nuclei lie close to each other and to the separating wall immediately following a division, but soon move to occupy a central position in the cell as seen in figure 4. The second division of the endosperm results in a linear tier of four cells; the wall of the micropylar cell is located just posterior to the constriction and the chalazal cell extends back into the narrow region containing the antipodals.

Subsequent divisions may vary a little in their order of occurrence. Frequently the chalazal endosperm cell divides transversely forming a linear sequence of five cells (fig. 7). Occasionally longitudinal divisions of one or both of the two central cells may precede this transverse division, but they more frequently follow it. Figure 8 shows both types of division completed. Rarely division of the chalazal cell may be longitudinal rather than transverse (fig. 9). Longitudinal division of the micropylar cell follows (figs. 9, 10). Subsequent divisions occur in various planes in the cells derived from the two central cells, and in the micropylar one of the two derived from the chalazal cell, of the endosperm quartet; transverse divisions may occur in the micropylar cells at about the level of the constriction (figs. 11, 12). The most anterior cells of the endosperm form the micropylar haustorium, and the most chalazal cell (or, rarely, cells), which usually has more cytoplasm than the cells just anterior to it (or them), can probably be considered as forming a little-developed (at least in this young material) chalazal haustorium (figs. 9, 12).

The development of the zygote and embryo lags behind that of the endosperm, but the earliest stages of embryogeny are accomplished during the endosperm divisions described above. At first the zygote is an essentially globular cell located anterior to the micropylar constriction (figs. 2, 3, 4). At about the four-celled stage of the endosperm the zygote starts to elongate in a chalazal direction (figs. 5, 6), passes the region of constriction (figs. 7, 8), and penetrates the main body of the endosperm to about the level of the third cell of the original endosperm quartet (fig. 9). Most of the cytoplasm of the zygote remains near the tip of the highly elongated cell and its nucleus is also located in this position (figs. 7, 8, 9). The first division of the zygote is transverse (fig. 10), cutting off a small terminal cell from a much elongated suspensor cell. The plane of the second division (fig. 11) was not determined. Figure 12, which is of a later stage in development, shows a somewhat longitudinal division of the terminal cell and an intercalary division in the lower suspensor cell, so it may be that the second division of the embryo proper approaches the longitudinal and that the suspensor is derived by intercalary divisions. The small size of the terminal cell in figure 12 as compared to figure 11, however, suggests that the second division may be transverse, cutting off a short, highly cytoplasmic terminal cell, and a longer cell which forms part of the suspensor. The intermediate stages necessary to determine the actual sequence of divisions here were not found in the available material.

Discussion

Several papers have been written which are concerned with megagametophyte development and/or certain phases of seed development in the Ericales. Artopoeus (1) and Peltrisot (10) described a few species in somewhat general terms without much detail. Samuelsson (11) has given a more thorough treatment and one of the species he considered was *Cassiope hypnoides* though this is not described in detail. Stevens (14) followed the development of the endosperm and embryo in *Epigaea repens*, and more recently Copeland (3-6) has described certain stages of development in some of the Pyroleae, Monotropoideae, and Rhododendroideae. Schnarf (12) has summarized the information on the Ericales up to 1930, and Johansen (7) has summarized the work on embryogeny.

The Ericales are apparently characterized throughout by unitegmic, tenuinucellate, almost anatropous ovules. The megagametophyte is of the Polygonum (Normal)-Type (8). The nucellus disintegrates and frequently the inner layer of the integument differentiates as an epithelium surrounding the megagametophyte. The polar nuclei may fuse prior to fertilization (the pollen tube penetrates porogamously) or occasionally fusion may be delayed so that a triple fusion leads to the formation of the primary endosperm nucleus. The antipodals in most cases are small and degenerate rather early. Except for Peltrisot, and Brough (2) on *Styphelia* (Epacridaceae), all the authors mentioned above have described endosperm development as cellular, with the first divisions resulting in a linear tier of four cells. Subsequent divisions are followed in less detail. All, except for the Diapensiaceae (11), appear to be characterized to a greater or lesser extent, by the development of micropylar and chalazal endosperm haustoria. These haustoria are frequently small in the Pyroleae, but may be quite large in the Vaccinioideae or Arbuteae. The micropylar haustorium is frequently larger and contains more cells than the chalazal which often is unicellular. The time of appearance of the haustoria is variable. The region of the micropylar one may become apparent even before fertilization and the chalazal shortly thereafter, as in *Cassiope hypnoides*, or neither may appear until the endosperm has undergone considerable development, as in *Epigaea repens* where there are approximately fifty cells in the endosperm before the haustoria develop (14).

Cassiope hypnoides corresponds closely to these characteristics. Samuelsson (11) has described the first division after the four-celled stage of the endosperm to be longitudinal or oblique. This type of division was seen in the present study, but perhaps more frequently the division was transverse in the chalazal cell of the quartet. He has also described and figured the older seeds of *Cassiope hypnoides* as having two distinct endosperm haustoria, the micropylar better developed than the chalazal. This would be expected on the basis of the present study.

The details of the sequence of divisions in the embryo are inadequately described for most of the Ericales and in most cases the pattern of the embryo development cannot be assigned to one or another of Johansen's types (7). The only clear case is Souèges' (13) study of *Pyrola rotundifolia* which Johansen classifies in the Myriophyllum Variation, Caryophyllad Type. Other possible assignations to types are to other variations of the Caryophyllad Type, to the Onagrad Type, and to the Solanad Type. None of these, however, are for plants in the Andromedeae, the tribe in which *Cassiope* is placed, or even in the Ericaceae proper. The critical early stages and necessary older materials are missing from the present study so that no definite assignation to type can be made for *Cassiope hypnoides.*

The first division of the zygote is transverse, thus the Piperad Type of embryo is eliminated. If the second division is longitudinal the embryo would probably belong to the Onagrad Type. If, on the other hand, it is transverse, as may be the case, it is more probably of the Solanad Type; the basal cell becomes so elongated and crushed that it would not be expected to take much part in the formation of the embryo, and there appear to be intercalary divisions in the suspensor, though only one embryo old enough to show this was observed.

The general characters of the early seed development are apparently so similar within the order that they cannot serve as characters of taxonomic importance for distinguishing between tribes or genera. It is possible, however, that a thorough study of the sequence of divisions in the endosperm and embryo of a number of species might show slight variations which would be of taxonomic use at these lower levels. These could then add to the usual morphological characters, studies of floral anatomy (such as the author's recent paper on Andromedeae (9)), and other morphological features, in determining relationships within the various families, subfamilies, tribes, and genera.

REFERENCES

1. Artopoeus, A., Uber den Bau und die Offnungsweise der Antheren und die Entwicklung der Samen der Erikaceen. Flora 92:309-345. 1903.
2. Brough, P., Studies in the Epacridaceae. I. The life history of *Styphelia longifolia* R. Br. Proc. Linn. Soc. N. S. Wales 49:162-178. 1924.
3. Copeland, H. F., The development of seeds in certain Ericales. Amer. Jour. Bot. 20:513-517. 1933.
4. ———, Further studies on Monotropoideae. Madroño 6:97-119. 1941.
5. ———, A study, anatomical and taxonomic, of the genera of Rhododendroideae. Amer. Midl. Nat. 30:533-625. 1944.
6. ———, Observations on the structure and classification of the Pyroleae. Madroño 9:65-102. 1947.
7. Johansen, D. A., Plant embryology. Chronica Botanica Co., Waltham, Mass., 1950.
8. Maheshwari, P., An introduction to the embryology of angiosperms. McGraw-Hill Book Co., New York, 1950.
9. Palser, B. F., Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. Bot. Gaz. 112: (in press). 1951.
10. Peltrisot, C. N., Developpement et structure de la graine chez les Éricacées. Jour. de Bot. 18:309-367, 386-402. 1904.
11. Samuelsson, G., Studien uber die Entwicklungsgeschichte der Blute einiger Bicornes-Typen. Svensk. Bot. Tidskr. 7:97-188. 1913.
12. Schnarf, K., Vergleichende Embryologie der Angiospermen. Borntraeger, Berlin, 1931.
13. Souèges, R., Embryogénie des Éricacées. Developpement de l'embryon chez le *Pyrola rotundifolia* L. Compt. Rend. Acad. Sci. Paris 209:635-637. 1939.
14. Stevens, N. E., Dioecism in trailing arbutus, with notes on the morphology of the seed. Bull. Torr. Bot. Club 38: 531-543. 1911.