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The megasporogenesis of Arceuthobium americanum Engelm

David Nathaniel Hudson
The University of Montana

THE MEGASPOROGENESIS OF ARCEUTHOBIUM AMERICANUM ENGELM

By

David N. Hudson

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INTRODUCTION

Many developemental studies have been done on Arceuthobium and related genera of the Loranthaceae. A majority of these investigations have been restricted to the endophytic system and various aspects of embryo developement. Several papers have been published on megasporogenesis, embryogeny, endosperm formation and endosperm haustorium developement but there is little agreement among these investigations. Johnson (1888) gives one of the earliest accounts of embryo sac developement in A. oxycedri. Later investigations also describe embryo sac developement and embryogeny (Theday and Johnson, 1930; Dowding, 1931; Gill, 1935; Kuijt, 1955; Cohen, 1963; and Maheswhari and Bhandari, 1965). Jones and Gordon (1965) give one of the most detailed accounts of embryo sac developement, endosperm formation, and haustorial developement in A. douglasii.

Investigations on embryo sac developement of related genera of the Loranthaceae are more detailed than those on the genus Arceuthobium. Some of these investigations find the embryo sac in certain species to be of a bisporic "Allium type" (Steindl, 1935; Rutishauser, 1937; and Schaeppi, 1945) while others find embryo sacs of a monosporic "Polygonum type" (Maheshwari and Singh, 1952; Jorhi, Agrawal and Garg, 1957; and Narayana, 1954, 1958). Due to the lack of detail and agreement in these studies on the developement of the embryo sac and endosperm of Arceuthobium there is a need for additional morphological study of the species of this genus. This paper will be limited to the study of one species

(Arceuthobium americanum) and will be confined to the development of megasporocytes, embryo sac, endosperm, endosperm haustorium, and early embryogeny.

MATERIALS AND METHODS

Specimens of staminate and pistillate flowers of Arceuthobium americanum were collected weekly from August 1964 to August 1965 with the exceptions of the fourth week of August 1964; third and fourth week of November 1964; third and fourth week of December 1964; third week of April 1965; and the first and fourth week of July 1965. Collections were made in various localities with the bulk of the collecting being made in Lolo National Forest, Lolo Hot Springs area, 35 miles west of Missoula on the Lewis and Clark Highway. A few collections were made in the Carlton Creek area 15 miles south of Missoula on Highway 93 and one collection from Glacier National Park. Although the materials were collected in various localities the internal and external anatomy of the staminate and pistillate flowers was similar regardless of the area collected in. Collections from the above localities show very little time lag in developmental stages.

Collected material was immediately fixed in Formalin-Acetic-Alcohol, brought into the laboratory, and aspirated under gentle vacuum for twelve hours. Individual flowers and clusters of flowers were processed through the Tertiary Butyl Alcohol dehydrating series and imbedded in 56°-57° Paraplast (Johansen, 1940). Serial sections were cut on the rotary microtome at 10, 12, and 15 microms, and stained by the Feulgens nuclear reaction and counterstained with Fast Green FCF (Johansen, 1940). Cover slips were mounted over the stained sections with Harleco Synthetic Resin. All morphological studies and photomicrographs were con-

ducted under phase contrast microscopy.

Line drawings have been inserted at the end of the text. Many of these schematic drawings will not be directly referred to in the text of the paper. However, the reader is referred to them in order to facilitate interpretation of the embryo sac, endosperm, and haustorium developement.

RESULTS

Development of the Ovarian Papilla. -- A. americanum, like other members of the genus, developes an ovarian papilla. This dome-shaped structure consisting of loosely arranged isodiametric parenchyma cells arises at the base of the ovarian cavity. A distinct epidermis delimits the papilla from the surrounding carpel walls and at no time does the papilla adhere to the carpel walls. New floral inceptions can be observed in March and sections reveal that the papilla has not differentiated at this time. By June the papilla is differentiating and in August megasporocytes are differentiating. By September the two megasporocytes have elongated to their maximum size but megasporogenesis does not occur until the following spring.

Development of the Megasporocytes. -- Two megasporocytes differentiate in the hypodermal layer of each papilla by August. The archesporial cell developes directly into the megasporocyte with no evidence of a prior division into a primary parietal cell and a primary sporogenous cell. The megasporocytes enlarge at the expense of the surrounding parenchyma cells although no crushed cells were noted in the region of their enlargement. Each megasporocyte can easily be distinguished from the surrounding parenchyma cells by its large size, dense cytoplasm and large nucleus (Fig. 1-2). The ovarian papilla cannot be considered a true ovule because the embryo sacs imbedded in it do not develope integuments and the papilla itself is forced aside during embryogenesis and forms no part of the mature fruit. Because of the lack of integuments it is difficult to access anatomical relationships to the

embryo sac. For the sake of discussion the terms "lower" and "upper" will be employed in order to establish the polarity of the embryo sac. The term "lower" will be used to denote that portion of the sac which contains the egg apparatus and the term "upper" to indicate the portion of the sac containing the antipodals.

Meiosis. -- Megasporogenesis occurs in the spring around the first week in May. Prior to this the resting megasporocyte nucleus lies in the "lower" portion of the cell. Before nuclear division, the nucleus migrates to the central portion of the cell. Meiosis occurs in both megasporocytes.

The first nuclear division is perpendicular to the long axis of the megasporocyte and results in two nuclei which, for a short period of time, are of equal size (Fig. 4). One nucleus migrates to the "lower" end of the megasporocyte and becomes smaller than its sister nucleus. A cell wall forms immediately above the lower nucleus resulting in a small, lower cell and a much larger upper cell (Fig. 5-7). After the formation of the cell wall the lower cell separates from the upper cell (Fig. 7). The second nuclear division does not occur simultaneously in the two daughter cells. The nucleus of the lower cell divides first and no cell wall forms between the two nuclei. This binucleate, nonfunctional dyad can be seen for approximately two weeks during the initial stages of embryo sac developement. After nuclear division in the lower cell is completed the nucleus in the upper cell divides. No cell wall forms between the nuclei and this

binucleate cell is the functional dyad or 2-nucleate embryo sac (Fig. 8-12).

Development of the Embryo Sac. -- Embryo sac developement in A. americanum is of the bisporic "Allium type". The nuclei of the functional dyad undergo two nuclear divisions. The first division results in a 4-nucleate embryo sac with the nuclei resting along the periphery of the sac (Fig. 13). Up to this point nuclear division has occurred simultaneously in both embryo sacs. Further developement of the two embryo sacs is asynchronous; however, in a few instances both developed in unison. Usually, the embryo sac, which will be the non-functional sac, is arrested in the 4-nucleate condition while the other proceeds to the 8-nucleate stage (Fig. 17). Sections of material collected in the third week of May reveal 8-nucleate embryo sacs in which two groups of four nuclei lie along the margins of the poles (Fig. 14). One nucleus from each group migrates to a central position in the embryo sac and are the polar nuclei (Fig. 42). The mature embryo sac consists of three antipodals (Fig. 15-16), two polar nuclei and an egg apparatus in which the egg lies off to one side and above the two synergids rather than between them. Cell walls form around five of the eight nuclei leaving the egg and two polar nuclei within the same cell.

Pollination and Fertilization. -- Pollen sacs of A. americanum were observed to be open in early March and pollen was found on the stigmas later in that month. The pollen was held to the stigma by secretions from the pistil. Pollen tubes grow down

through the style during April preceding megasporogenesis. Double fertilization was not observed in this investigation; however, it is assumed that two sperm are released from the pollen tube and that syngamy and tripple fusion are effected in the usual manner.

Fertilization of the egg by one of the male gametes and fusion of the second gamete with the polar nuclei initiates embryo and endosperm developement. Prior to endosperm and haustorium growth the embryo sac segregates into two parts by the formation of a diagonal cell wall. The "upper" chamber of the embryo sac contains the primary endosperm nucleus, fusion product of the polar nuclei and sperm, and the antipodals. The "lower" chamber contains the zygote and synergids (Fig. 43).

Development of the endosperm, haustorium and embryo. — The endosperm and haustorium develope in two phases. The endosperm haustorium initiates as a protuberance from the "upper" end of the sac (Fig. 44, 45). It protrudes initially at a 90° angle to the long axis of the embryo sac and then curves downward and elongates toward the base of the papilla (Fig. 22, 25). At the onset of haustorial extension the primary endosperm nucleus divides once. One of the resulting nuclei remains adjacent to the zygote and the other migrates into the protruding haustorium (Fig. 18-21). A cell wall forms between the two nuclei and in this manner delimits the endosperm initial adjacent to the zygote and the primary haustorium (Fig. 45). The basal portion of the haustorium enlarges and becomes bulb-like and always remains larger in diameter than the rest of the haustorium.

Initial endosperm development begins in June. The endosperm developes after preliminary haustorial extension to the base of the papilla. The endosperm initial undergoes repeated divisions, giving rise to a series of cells which completely surround the zygote in one plane (Fig. 25, 26). At this time the endosperm cells are oriented around a central axis and appear plate-like with the zygote in the center (Fig. 46,47). In this phase the endosperm is composed of large triangular to rectangular cells. Either during or immediately after primary endosperm formation, and while the endosperm is in one plane, the haustorium nucleus divides repeatedly and a free nuclear condition exists for a short period of time before cytokenesis (Fig. 21, 46). Eventually, tiers of cells form within the tubular haustorium (Fig. 29, 30, 47). The haustorium is persistant through embryogenesis. It lies imbedded in the endosperm and portions of it can be seen extending beyond the base of the endosperm (Fig. 36). After the tiers of cells have formed in the haustorium the large triangular endosperm cells undergo anticlinal and periclinal divisions and eventually completely surround the zygote. The zygote divides once after being surrounded by the endosperm and remains quiescent for three to six weeks as the endosperm continues to develope. Rapid cellular division in the basal portion of the endosperm passively conveys the embryo upward and by August it lies imbedded in the endosperm near the apex of the ovarian cavity (Fig. 31-34). During its growth the endosperm forces its way through the papilla and in the immature

seed the crushed remnants of the papilla can be observed off to one side of the endosperm (Fig. 31).

The quiescent stage of the embryo ends and by August it has become multicellular. Growth of the embryo and endosperm ceases in September or early October and remains in this resting state through the winter. Growth resumes in the spring and the embryo is mature and ready for dissemination by late August, approximately 28 months after floral inception.

In one instance two embryos were observed in the same endosperm indicating that both embryo sacs were fertilized. One embryo was found imbedded in the apex of the endosperm - its normal position; the other was imbedded in the base of the endosperm (Fig. 34, 35). This was the only observed instance of two embryos in one endosperm.

When sections of one female inflorescence collected in March was analyzed, three stages of development were observed - floral initials, flowers containing mature megasporocytes, and immature seeds with well developed endosperm and embryos. Sections of one female inflorescence collected in late July revealed flowers with maturing megasporocytes, mature embryos, and embryos and endosperm in various stages of development. From the available evidence it was concluded that A. americanum has a two year reproductive life cycle.

DISCUSSION

The ovarian papilla, present in many genera of the Loranthaceae, has been the subject of much discussion. In A. americanum, its development agrees with observations made on other species of the genus (Johnson, 1888; Thoday and Johnson, 1930; Dowding, 1931; Gill, 1935; Kuijt, 1955; and Jones and Gordon, 1965). Initial developement appears to be similar in most species of Arceuthobium but as developement proceeds the compactness of the cells in the papilla changes. The papilla of A. douglasii remains compact throughout embryo sac developement. The papilla of A. americanum begins to degenerate rapidly and by the time the embryo sacs are mature the cells of the papilla have become very loosely arranged.

The megasporocytes of A. americanum originate from one of the parenchyma cells in the hypodermal layer of the papilla. This parenchyma cell differentiates directly into the megasporocyte. The development of the megasporocytes of A. americanum agrees with the observations made by Jones and Gordon (1965) on A. douglasii. These investigators reported that two parenchyma cells located in the hypodermal layer formed the megasporocytes. At no time did they observe a division of the archesporium. Thoday and Johnson (1930) also failed to observe any division in the archesporium. This direct differentiation of the archesporium into the megasporocyte is in disagreement with the findings of Johnson (1888) and Dowding (1931). Johnson reported that the archesporium of A. oxycedri divided twice. The first division

resulted in a small, upper primary tapetal cell (which divided again into two smaller cells) and a larger, basal "mother-cell of the embryo sac". A second division of the mother cell resulted in two smaller cells at its lower end. The mother cell enlarged into the embryo sac. Dowding (1931) also reported a division of the archesporium in which the upper cell became the embryo sac. Some of these discrepencies may be explained if one considers that these investigators may have been observing stages of megasporogenesis rather than maturation of the archesporium into the megasporocyte.

Embryo sac developement in A. americanum differs from all reported cases in the literature. Johnson (1888) inferred that the embryo sac of A. oxycedri followed the monosporic "Polygonum type" developement. Dowding (1931) inferred that A. americanum also followed the "Polygonum type" development. The monosporic "Polygonum type" has been reported for other genera of the Loranthaceae (Maheswari and Singh, 1952; Johri et. al., 1957; and Narayana, 1958). Jones and Gordon (1965) indicated that the embryo sac of A. douglasii followed the tetrasporic "Adoxa type" developement. Careful study of the prepared material of A. americanum shows the embryo sac to be of a bisporic "Allium type". Personal correspondence of Maheshwari and Bhandari (1965) with Gordon stated that A. minutissimum also developed a bisporic type embryo sac. They questioned Jones and Gordon's (1965) report of a tetrasporic type embryo sac in A. douglasii and suggested further study of that species. The bisporic type embryo sac has been

reported for other genera of the Loranthaceae (Steindl, 1935; Rutishauser, 1937; and Schaeppi and Steindl, 1945).

Several stages of the developement of the embryo sac in A. americanum were observed beginning with the resting megasporocyte. A definite cell wall is formed between and immediately above the lower nucleus after the first nuclear division. The immediate division of the nucleus in the lower non-functional dyad and the delayed division of the nucleus in the future embryo sac may account for the variation in interpretation of embryo sac developement in other species of the genus. Reports of a preliminary division of the archesporium (Johnson, 1888; and Dowding, 1931) may in fact be the initial phases of megasporogenesis. Johnson's (1888) report of the mother-cell of the embryo sac cutting off two small cells from the lower end of the sac may in fact be the separation of the non-functional dyad as found in A. americanum. Dowding's (1931) observation of the disintegration of the small lower daughter cell of the embryo sac mother-cell corresponds to our interpretation of the disintegration of the non-functional dyad. The mature embryo sac of A. americanum contains eight nuclei formed from two nuclear divisions of the two nuclei in the functional dyad. Dowding's (1931) report of a 7-nucleate embryo sac may be due to the fusion of the polar nuclei prior to fertilization.

There is a delay of some two months between pollination and fertilization in A. americanum. Pollen tubes were observed to grow down through the style and penetrate the papilla prior to

megasporogenesis. This differs from most reports on other species of the genus. Jones and Gordon (1965) observed in A. douglasii that the period between pollination and fertilization was a matter of three or four days. Johnson (1888) reported a delay in fertilization after pollination in A. oxycedri. In A. oxycedri, pollination occurs in September and pollen tubes grew down into the papilla and remained in close contact with the embryo sac until the next spring at which time two sperm were released. A. oxycedri is a fall flowering species.

Developement of the haustorium and endosperm in A. americanum agrees with the findings of Jones and Gordon (1965) in A. douglasii. After formation of the primary endosperm nucleus, by the fusion of the polar nuclei and male gamete, a diadonal cell wall divides the embryo sac into two chambers. The "lower" chamber contains the zygote and synergids; the "upper" chamber contains the primary endosperm nucleus and antipodals. The primary haustorial protuberance originates from the "upper" chamber. Division of the primary endosperm nucleus and the formation of a cell wall between the two nuclei divides the "upper" chamber into an endosperm initial and the haustorium. Thoday and Johnson (1930) observed haustorial extension and endosperm developement in A. pusillum. According to their findings, the embryo sac had "thrust a haustorial extension down into the base" of the papilla. They did not observe segmentation of the embryo sac into chambers nor division of the "upper" chamber into an endosperm initial and haustorium. Although they found the haus-

torium to be persistent until at least August, they did not observe the formation of tiers of cells. They found occasional degenerate nuclei which they assumed were the remnants of the antipodals or endosperm cells. Their data on endosperm formation is similar to ours. They found a single layer of endosperm cells surrounding the zygote and separating it from the wall of the embryo sac.

Endosperm development in Arceuthobium differs from that reported for other genera in the family. Narayana's (1958) study on Lysiana exocarpi indicated that the primary endosperm nucleus migrated into the lower portion of the embryo sac and divided repeatedly to form a cellular endosperm. Division and elongation of the suspensor pushed the proembryo downward into the endosperm. This condition is found also in the genus Dendrophthoe (Narayana, 1954) and Helicanthes (Johri et. al., 1957). In Maheshwari's textbook, An Introduction to the Embryology of Angiosperms, there is a discussion of endosperm formation similar to that of Arceuthobium in the genus Balanaphora. This unusual method of endosperm formation in Arceuthobium may be due to the lack of an embryonic suspensor.

One interesting aspect of endosperm and haustorium developement is the intricate manner in which they avoid interaction. The haustorium, by protruding at a 90° angle to the long axis of the embryo sac and then elongating downward, avoids interfering with the encirclement of the zygote by the endosperm.

Some stages of embryogeny were followed and are in agreement

with the findings of Cohen (1963). The rapid growth of the endosperm ruptures the papilla and the crushed remnants of the papilla can be seen at the base of the ovarian cavity and off to one side of the mature endosperm. In the process of growth, the endosperm carries the embryo along with it out of the papilla. The embryo does not mature until the next summer at which time it completes developement and is ready for dissemination, approximately 28 months after floral inception.

SUMMARY

Arceuthobium americanum developes an ovarian papilla in the usual manner described for the genus. It cannot be considered a true ovule since no integuments are formed and the papilla does not form part of the mature fruit. Two megasporocytes differentiate in the summer but meiosis does not occur until the following spring. Embryo sac developement is of the bisporic "Allium type". The non-functional dyad degenerates within two weeks after its formation. Pollination occurs in March but fertilization is delayed until May. Meiosis proceeds simultaneously in both megasporocytes. However, division of the functional dyads is asynchronous and one of the embryo sacs is often arrested in the 4-nucleate state. If both embryo sacs develope to the 8-nucleate state, usually only one developes an embryo although two embryos were observed in the same endosperm in one instance. A diagonal cell wall forms after fertilization segregating the embryo sac into two chambers. The "lower" chamber contains the zygote and synergids, the "upper" chamber contains the primary endosperm nucleus and antipodals. The haustorium protrudes at a 90° angle to the long axis of the sac and then elongates downward to the base of the papilla. Division of the primary endosperm nucleus and the subsequent formation of a wall between the two nuclei delimits the endosperm initial adjacent to the zygote and the haustorium. The endosperm initial divides only in one plane and surrounds the embryo. After encirclement of the embryo by the

endosperm, the free nuclear haustorium forms tiers of cells. The haustorium is persistent throughout embryogenesis. Further division of the endosperm increases its volume and conveys the embryo out of the papilla. Developement of the embryo is arrested in the autumn and the immature embryo completes its developement the following year and is mature and ready for dissemination approximately 28 months after floral inception.

PLATE I

Figs. 1-6. -- Longitudinal sections of the ovule of A. americanum showing the first nuclear division of the megasporocyte. Fig. 1-2. Sections of the papilla (p) with the megasporocyte (m) imbedded in it. The papilla has a distinct epidermis (ep) which is not fused with the carpel wall (cw). Fig. 3. An ovarian papilla containing two megasporocytes in metaphase. The first nuclear division of the megasporocyte is perpendicular to the long axis of the megasporocyte. Fig. 4. Megasporocyte in telophase. Fig. 5. Cell wall (w) formation between the two nuclei of the first nuclear division. The upper cell (u) will give rise to the embryo sac. The lower cell (l) will divide again to form the non-functional dyad which degenerates. Fig. 6. Cytokenesis has been completed and the lower cell is separating from the upper cell.

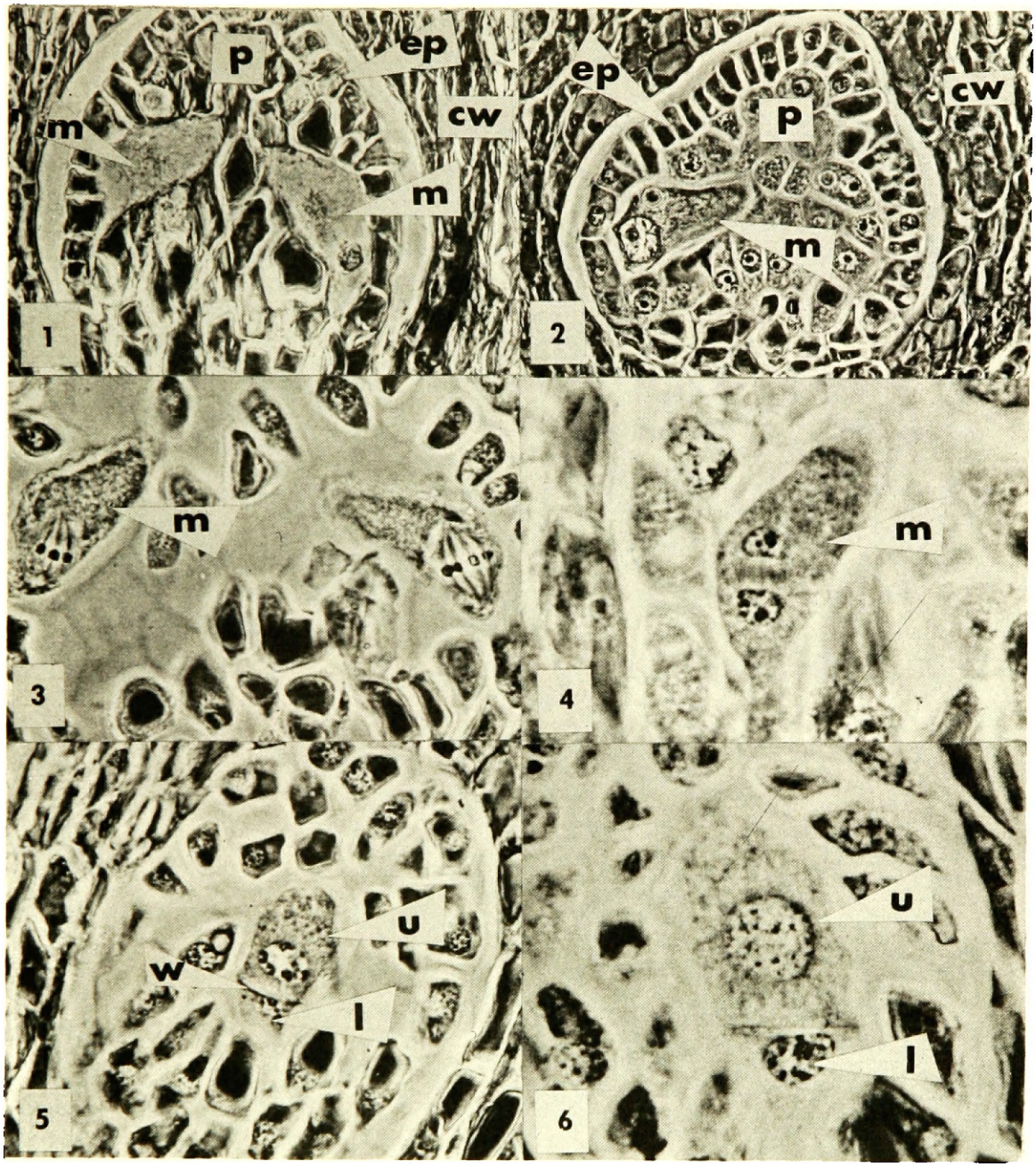


PLATE I

m, megasporocyte; p, papilla; ep, epidermis of papilla; c.w., carpel wall; w, cell wall; u, "upper" cell of megasporocyte; l, "lower" cell of megasporocyte.

PLATE II

Figs. 7-12. -- Sections of the ovule of A. americanum showing the formation of two dyads. Fig. 7. This section shows the completed separation of the two cells formed from the first nuclear division. Fig. 8-12. A series of sections showing the second nuclear division. This division results in an upper functional dyad (fd) and a lower non-functional dyad (nd). The non-functional dyad degenerates rapidly and remains visible for approximately two weeks during the development of the embryo sac. The functional dyad may be considered to be the 2-nucleate embryo sac.

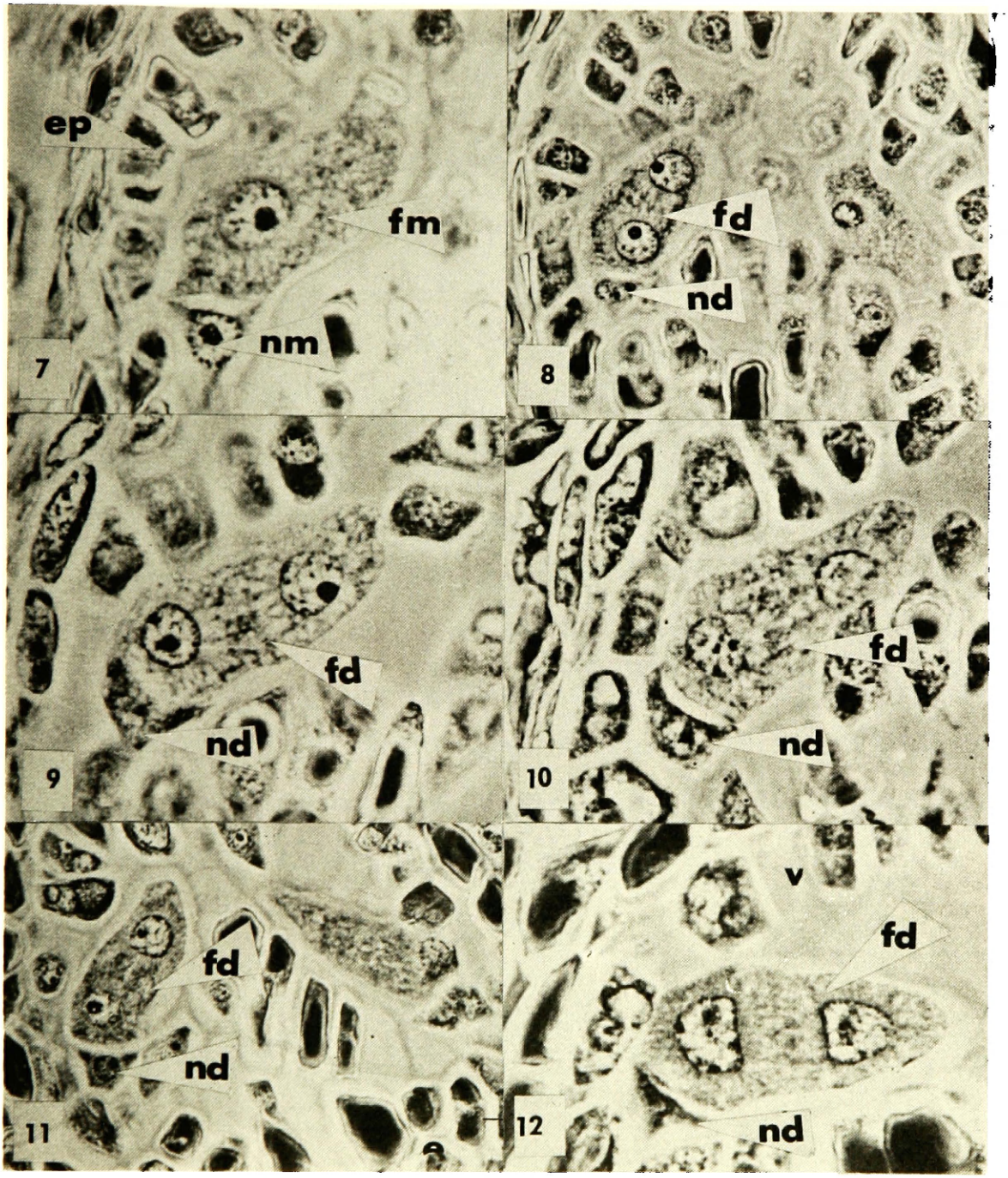


PLATE II

ep, epidermis of papilla; f.n., "upper" cell of the megasporocyte; n.m., "lower" cell of the megasporocyte; f.d., functional dyad; n.d., non-functional dyad.

PLATE III

Figs. 13-17. -- Sections of A. americanum showing the 4 and 8-nucleate stages of the embryo sac. Fig. 13. Ovarian papilla (p) containing a 4-nucleate embryo sac (es). The nuclei lie along the periphery of the sac. Note that the non-functional dyad is no longer visible. Fig. 14. An immature 8-nucleate embryo sac with two groups of four nuclei (arrows) at the poles of the sac. Note the synchronous division (telophase) of the nuclei. Fig. 15-16. Serial sections of an 8-nucleate embryo sac. Cell walls form around the antipodals (a) and the synergids (s) but not around the egg (eg) and polar nuclei (p.n.). The egg lies off to one side and above the synergids. Fig. 17. An ovarian papilla with an 8-nucleate embryo sac with the antipodals (a) visible and a 4-nucleate embryo sac. The development of the second embryo sac is delayed and in some instances remains arrested in the 4-nucleate condition.

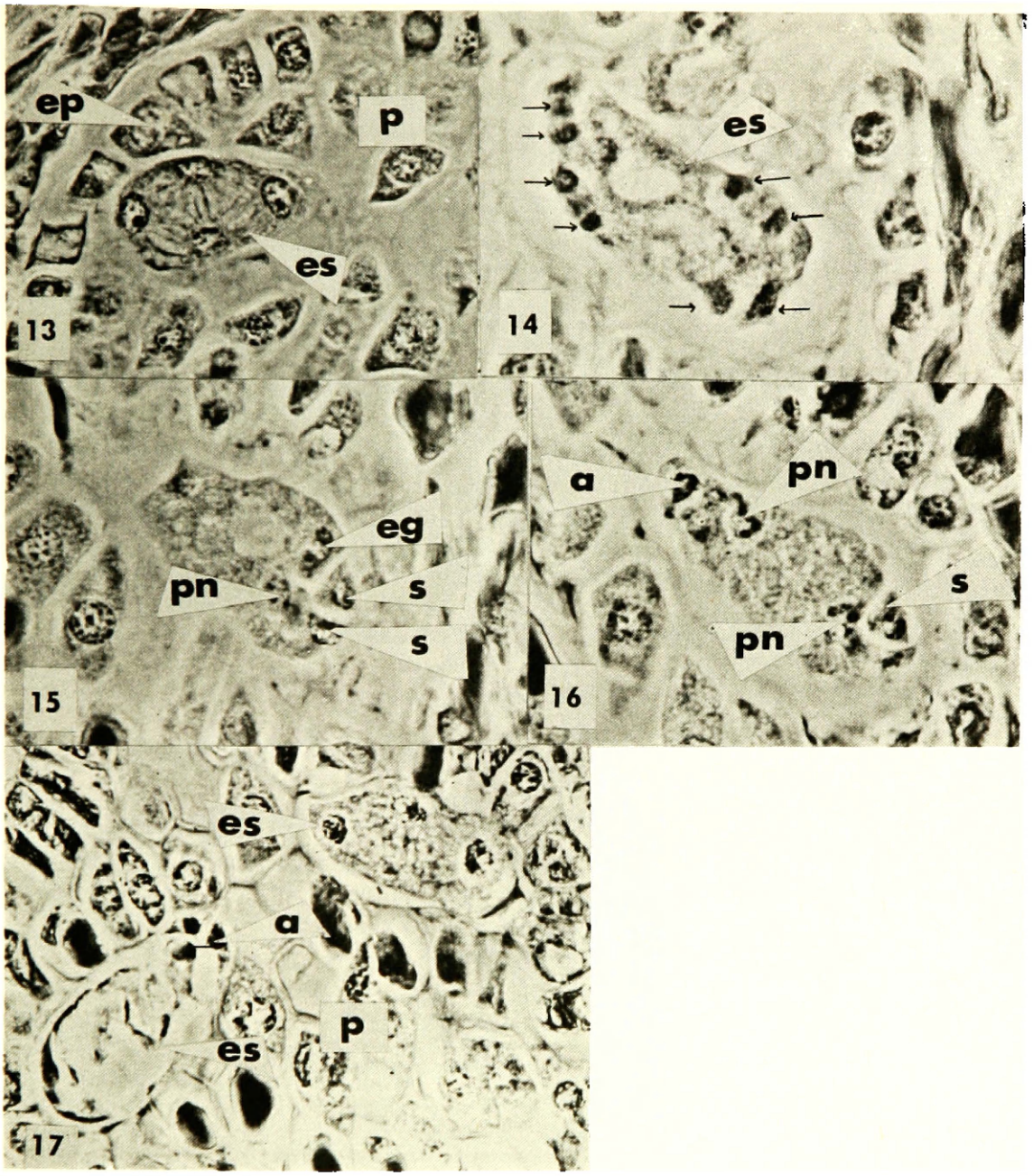


PLATE III

p, papilla; e.s., embryo sac; p.n., polar nucleus; eg, egg; s, synergid; a, antipodal; ep, epidermis.

PLATE IV

Figs. 18-24. -- Fig. 18-20 are serial sections of the same embryo sac showing the zygote (z), the endosperm nucleus (en) and haustorium (h), and the haustorial nucleus (hn) prior to its migration into the haustorial protrusion. Fig. 21. This section shows the haustorial nucleus (hn) after its migration into the haustorium (h). Figs. 22-24. Serial sections of an embryo sac cut at approximately 60° to the long axis of the sac. Fig. 22. The endosperm (e) has begun to surround the zygote (z) and the haustorial protrusion (h) is forming. Fig. 23-24. Sections showing the endosperm-surrounded zygote.

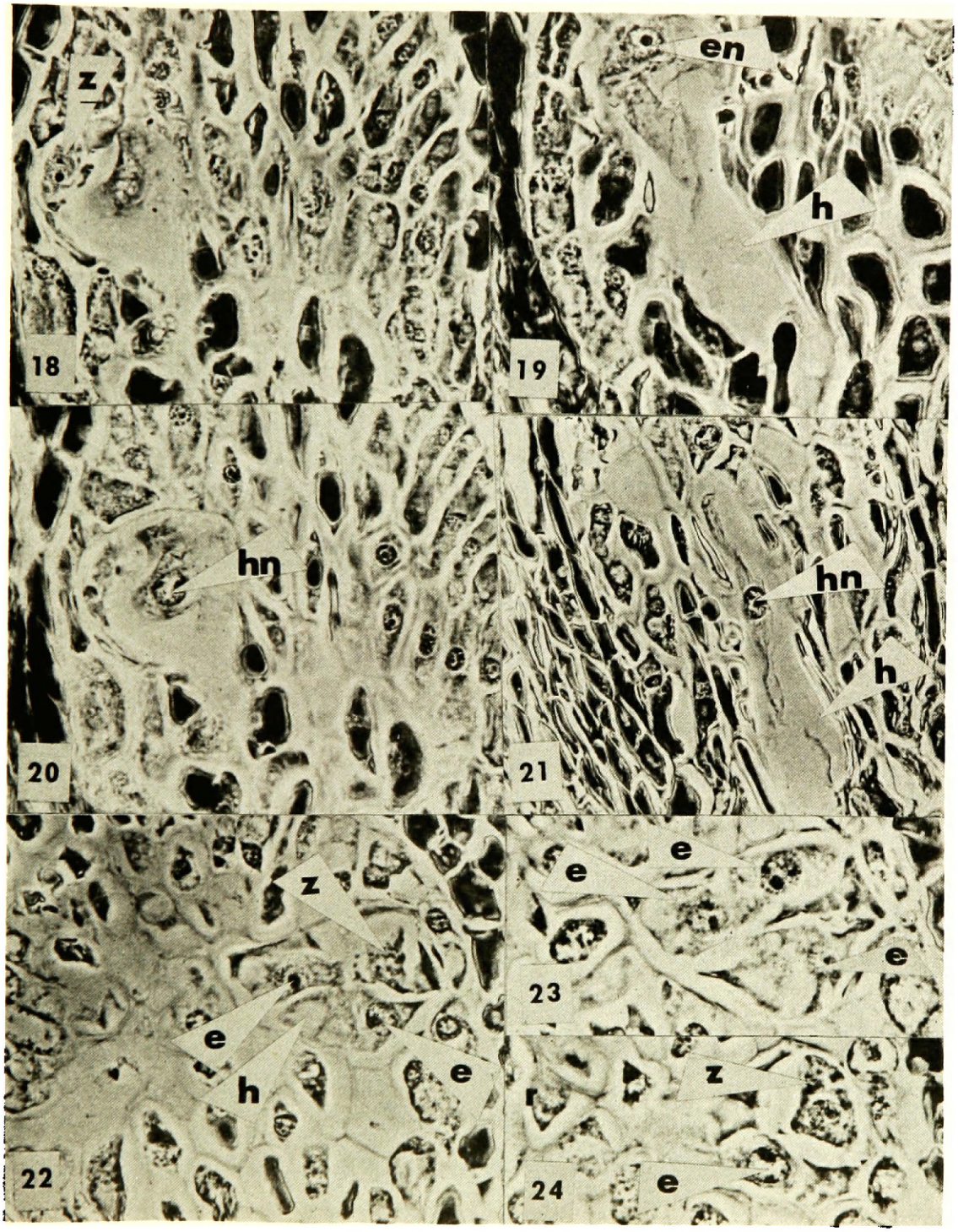


PLATE IV

z, zygote; e.n., endosperm nucleus; h, haustorium; h.n., haustorial nucleus; e, endosperm.

PLATE V

Figs. 25-30. -- Serial sections showing the zygote, endosperm and haustorium. Fig. 25. This section was cut at approximately 60° to the long axis of the embryo sac. It shows a centrally located zygote (z) surrounded by endosperm (e). The haustorium (h) is now in the multicellular state. Note that the haustorium does not interfere with the encirclement of the zygote by the endosperm. Fig. 26. A section of the zygote and endosperm. Fig. 27-28. Sections of the endosperm cut past the zygote indicating that the endosperm at this time has divided in more than one plane. Fig. 29-30. Longitudinal sections of a multicellular haustorium.

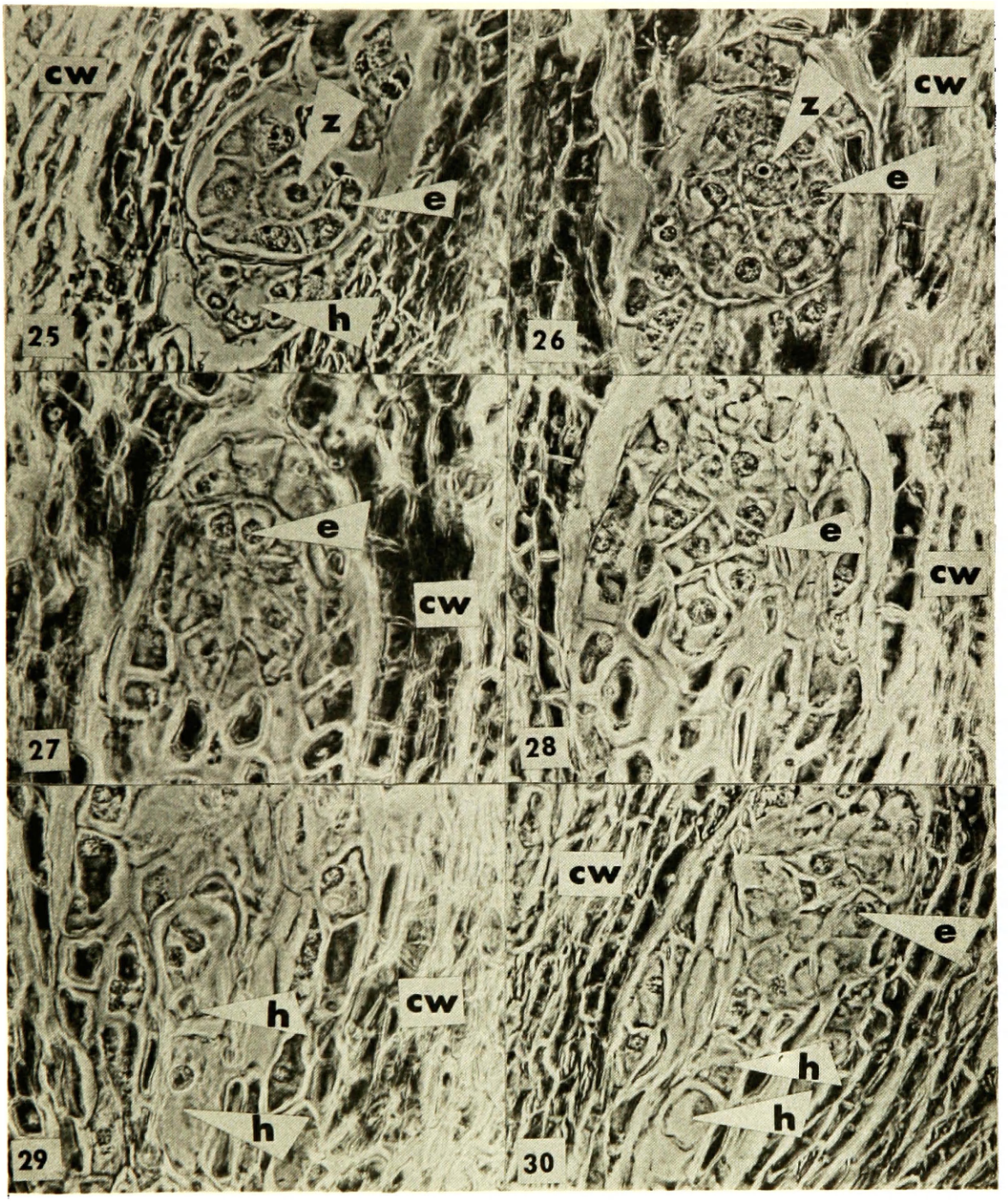


PLATE V

z, zygote; e, endosperm; h, haustorium; c.w., carpel wall.

PLATE VI

Figs. 31-36. — Longitudinal sections of the maturing endosperm and embryo. Fig. 31-32. Longitudinal sections of a four celled embryo (em) imbedded in the apex of the endosperm (e). Note the crushed remnants of the papilla (p) at the base of the endosperm (Fig. 31). Fig. 33. Section of the maturing seed. Viscum (v) and endocarp (en) are well developed. Fig. 34. Longitudinal section of a mature seed with one embryo in the normal apical position in the endosperm and one embryo imbedded in the base of the endosperm. Fig. 35. Enlarged view of the secondary embryo imbedded at the base of the endosperm in Fig. 34. Fig. 36. Section of the mature endosperm with a segment of the haustorium (h) still present at the base.

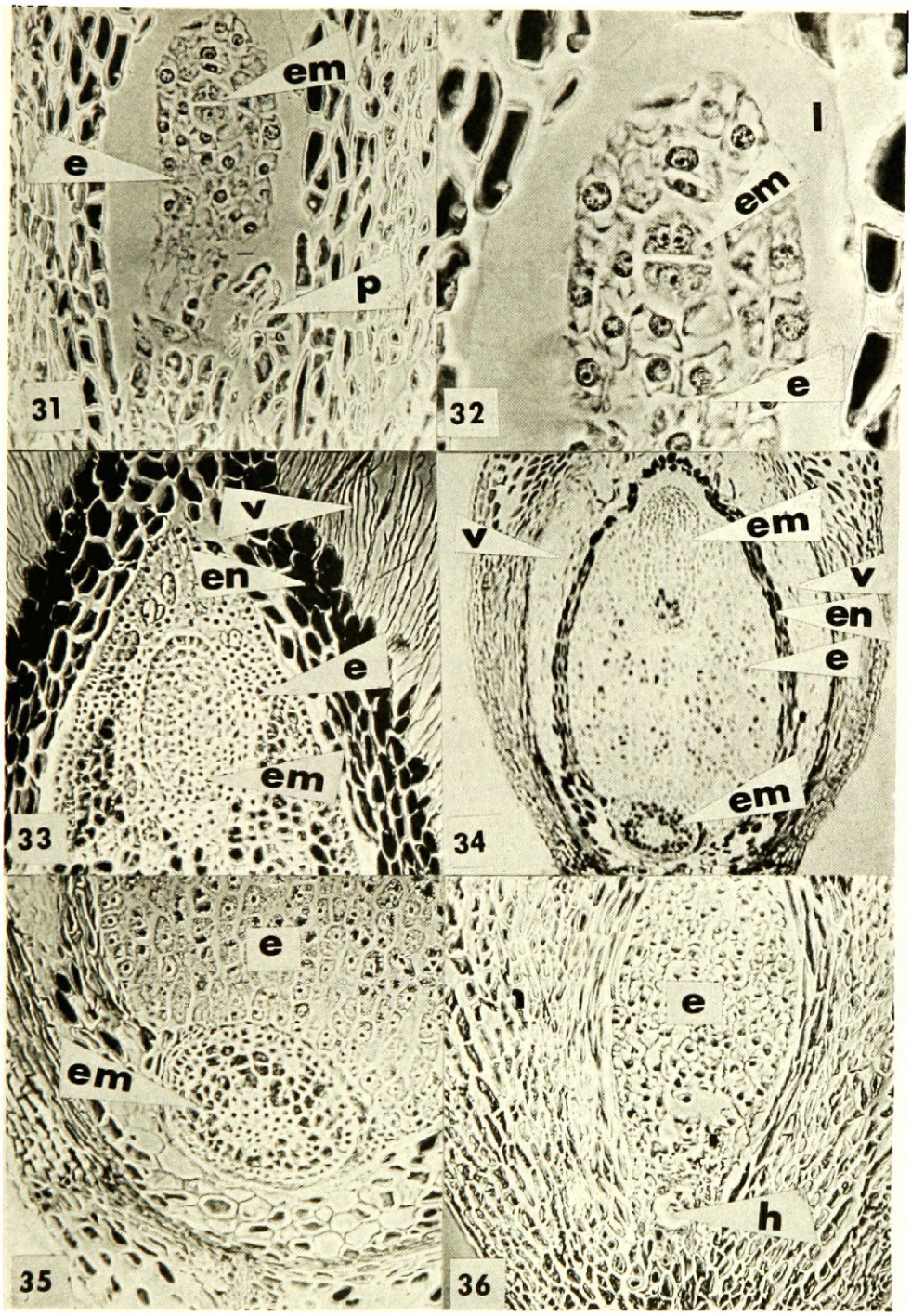


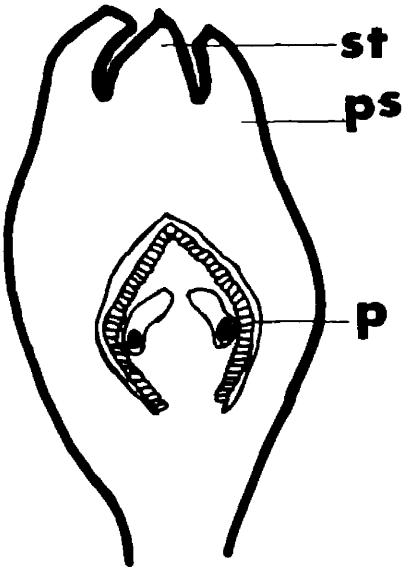
PLATE VI

em, embryo; e, endosperm; p, remnants of papilla; v, viscum; en, endocarp; h, haustorium.

PLATE VII

Figs. 37-42. — These are a series of line drawings showing the development of the megasporocytes, megasporogenesis, and embryo sac development. Fig. 37. This is a line drawing of a single ovule showing floral anatomy; style (st); perianth segments (p.s.); epidermis of the papilla (p); This figure represents an entire flower while the remainder of the figures shows only an enlarged view of the papilla with a few cells of the epidermis drawn in. Fig. 38a. Megasporocyte in the resting stage with the nucleus in the "lower" portion of the cell. Fig. 38b. Megasporocyte prior to megasporogenesis. The nucleus migrates to the central portion of the cell. Fig. 39c. Megasporocyte immediately after the first nuclear division. Fig. 39d. Megasporocyte after one of the nuclei has migrated to the "lower" pole and a cell wall has formed immediately above it. Fig. 40e. The "lower" cell has separated from the "upper" cell. A nuclear division has occurred in the "lower" cell. No cell wall forms between the nuclei and this cell is the non-functional dyad. Fig. 40f. Nuclear division in the "upper" cell has occurred giving rise to the functional dyad or 2-nucleate embryo sac. Fig. 41g. 4-nucleate embryo sac. Fig. 41h. 8-nucleate embryo sac. Fig. 42i. A mature 8-nucleate embryo sac

with antipodals (an); polar nuclei (p.n.); egg (eg); and synergids (s).



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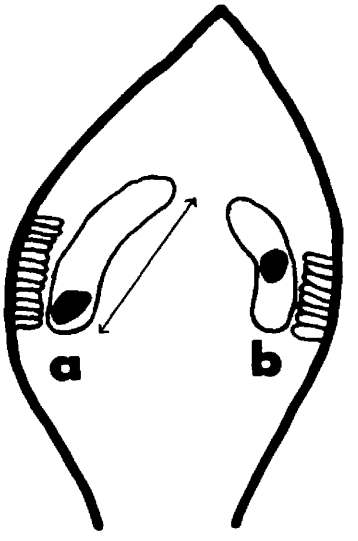

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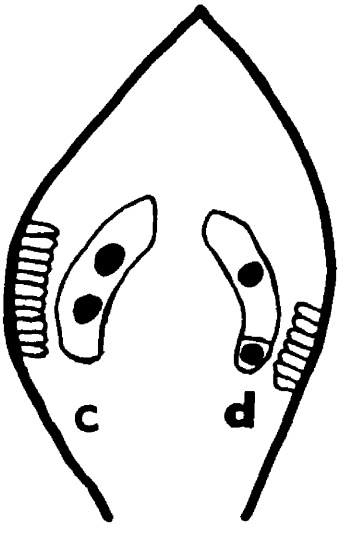

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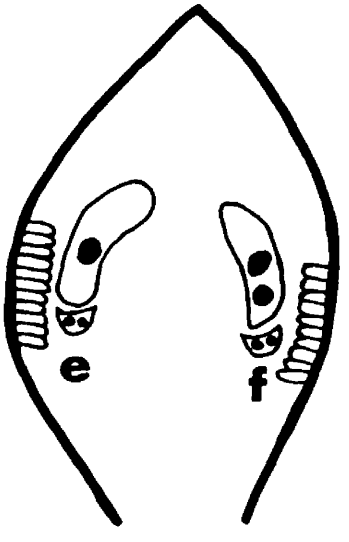

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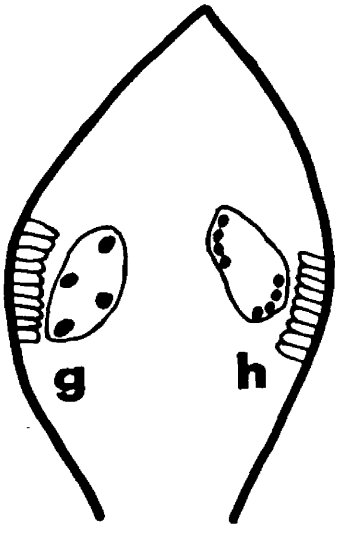

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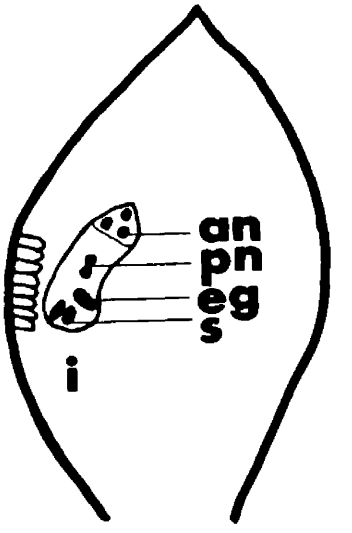

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PLATE VII

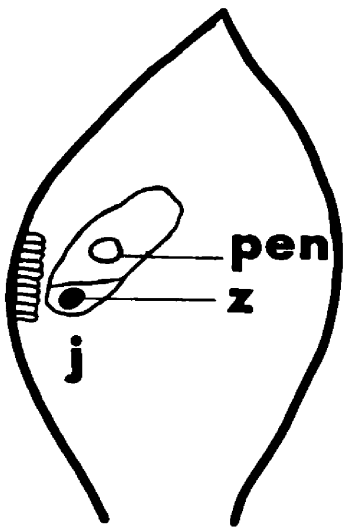
st, style; p.s., perianth segment; p, epidermis of papilla; an, antipodal; p.n., polar nuclei; eg, egg; s, synergids. Arrow in Fig. 38a shows the long axis of the megasporocyte.

PLATE VIII

Figs. 43-47. — A series of line drawings showing the organization of the fertilized embryo sac. Fig. 43j. An embryo sac after fertilization and triple fusion. The primary endosperm nucleus is a fusion product of the polar nuclei and second male gamete. A diagonal cell wall segregates the embryo sac into two chambers. The "lower" chamber contains the zygote (z); the "upper" chamber contains the primary endosperm nucleus (p.e.n.). Fig. 44k. An embryo sac with the haustorium (h) protruding. The dotted line indicates that the haustorium is not in the same plane as the embryo sac proper. The haustorium protrudes initially at a 90° angle to the long axis of the embryo sac and then elongates downward to the base of the papilla. The primary endosperm nucleus (p.e.n.) does not divide until the haustorium elongates to the base of the papilla. Fig. 45l. An embryo sac in which the primary endosperm nucleus has divided into the endosperm initial (e.i.) and the haustorial nucleus (h.n.). The endosperm initial remains adjacent to the zygote; the haustorial nucleus migrates into the haustorium. Fig. 46m. This figure shows an embryo sac in which the endosperm initial has divided several times in one plane. The arrows indicate the line of encirclement. In this stage of develop-

ment the haustorium is in a free nuclear state.

Fig. 47n. This figure shows an embryo sac in which the encirclement of the zygote (z) by the endosperm (e) is complete. The endosperm cells are in one plane. After initial encirclement of the zygote, tiers of cells form in the haustorium (h) ending the free nuclear state.



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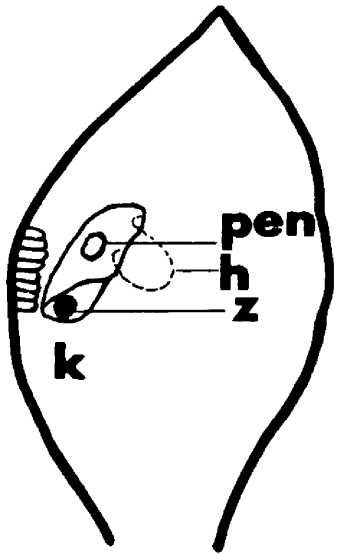

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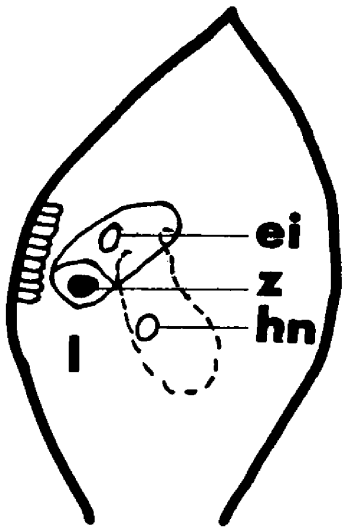

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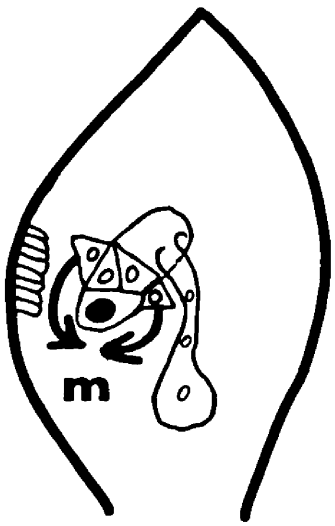

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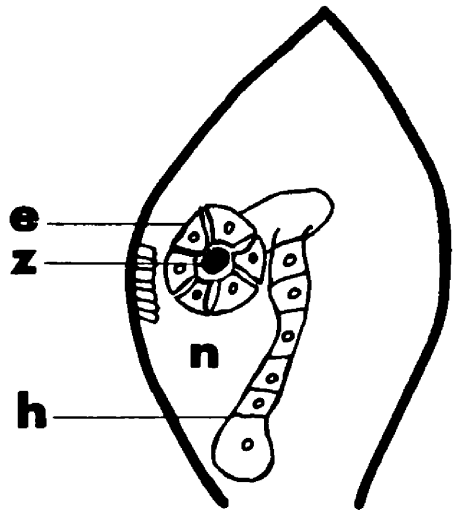

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PLATE VIII

p.e.n., primary endosperm nucleus; z, zygote; h, haustorium; e.i., endosperm initial; h.n., haustorial nucleus; e, endosperm. Arrows in Fig. 46 show the line of encirclement of the zygote by the endosperm.

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