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Morphology and Cytology of Vallisneria spiralis L.

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I.—Introduction

The order Helobiae, to which the species under discussion belongs, has been an especially inviting field for morphological study. Many of the species have been investigated. Specialized features have been found which appear particularly advantageous for plants that live under aquatic or partially aquatic conditions. On the other hand, some unspecialized features have also been observed, and of which it may be a question whether they really represent a fundamental simplicity in the plant structure or whether they are generalized features allowed or necessitated by the comparative evenness of aquatic conditions.

The method of pollination in *Vallisneria spiralis* L.* has long been known and has been reinvestigated more recently by Wylie (1917) who also describes the occurrence and behavior of sperm cells in this species (Wylie, 1923). This plant, as Arber (1920) suggests, is a form intermediate between those that are water-pollinated and those that are pollinated in the air. It is thus one of the most specialized plants in the order. Furthermore, the plant is essentially dioecious. In view of the studies and conditions just mentioned, one is induced to think that a further investigation of the species may bring out new facts which should be valuable in the understanding of structure and relationship in the monocotylous plants.

II.—Materials and Methods

The first collection of material for this investigation was made in the summer of 1923. Staminate and pistillate flowers in various stages of development were collected from Lake Mendota, Wisconsin, and placed into fixing solutions on dates from June 29 to August 3 inclusive. Some of the material was also sectioned and stained during this period and the study of it was begun. My acknowledgments are due to Dr. C. E. Allen of the department of Botany, University of Wisconsin, for suggesting this plant and encouraging this investigation in its initial stages.

* Nomenclature is that of Gray's New Manual of Botany (7th ed. 1908) though the name *V. americana* Michx. is often used for plants from America.

The sectioning and staining of material already collected was resumed in the autumn of 1933. Mature plants were also obtained from a lake near Bloomington, Indiana, on November 6, 1933 and cultured in the greenhouse of the department of Botany at Indiana University. These cultured plants produced pistils, some of which were fixed and embedded. A new supply of material was collected from Lake Mendota on dates from August 13 to August 25 inclusive, 1934. On the last date of collection, maturing fruits were also taken, transported to Bloomington, Indiana, and kept alive in jars containing water. From these fruits, seeds were fixed the following September and October. Mature and unpollinated pistils were collected and preserved from a hydraulic canal at Goshen, Indiana on August 24, 1926 and again on August 29, 1934.

Of the several fixing solutions used, a Flemming's medium solution yielded the best results. The strong solution sometimes gave just as good results as did the medium solution. Chromo-acetic and formalin-acetic-alcohol solutions were satisfactory for some phases of the study. Sections were cut to a thickness of from 5 to 25 microns depending on the organ sectioned and the purpose. Some sections were stained by Heidenhain's iron-alum haematoxylin method. Most of the results of the study are based on sections stained by Flemming's triple (safranin-gentian violet-orange) method.

III.—Investigation and Discussion

A. The Staminate Flower

1. *Floral Development*

Several staminate inflorescences in different stages may be found on the same individual plant. These inflorescences are attached in the axils of the leaves and remain submerged during development although the individual flowers rise to the surface of the water when detached at maturity. The inflorescences consist of a spadix, on which the large number of male flowers are attached, surrounded by a spathe (Fig. 1).

The individual flower consists of three sepals, one rudimentary petal, two stamens and one staminodium. Figure 2 represents a cross section of a nearly mature and still closed flower. The staminodium is not present in this section. The zygomorphic nature of the flower seems to be associated largely with the fact that only two stamens are formed. The small sepal which is the first to open when the flower floats on the water following its abscission from the spadix, occurs on the upper, adaxial side of the flower (Fig. 2,*a*).

The youngest male flowers that were observed are rounded primordia pushing outward on the surface of the spadix as shown in figure 1. Each primordium consists of an epidermal layer enclosing a group of cells (Fig. 3). As the flower primordium elongates, the terminal part thickens (Fig. 4). From this thickened end the various parts of the flower will form. The narrow basal part forms the pedicel of the flower. Figure 5 shows a cross section of the terminal part at a little later stage when the three sepals have begun to develop, leaving the remaining cells as an undifferentiated group in the center.

The staminodium and the rudimentary petal are cut off from opposite sides of the remaining central and terminal mass of cells (Fig. 6,*a*). What still remains in the center goes to form the two stamens.

Most of the flowers in the upper part of the spadix exhibit more advanced stages in development. In one inflorescence, for example, the flowers in the apical region have sepals long enough to partly enclose the flower across the top. In the middle region of the inflorescence the flowers have sepals which only begin to curve over the top of the flower. Flowers in the basal part have not yet begun to form sepals. These, of course, are average conditions. Some flowers may be advanced beyond or lagging behind the stage prevailing among their neighbors. Perhaps the succession of development from apex to base is to be correlated with the fact that when mature, the flowers become functional in this successive order. Further on, other successions in development will be noted.

2. *Microsporogenesis*

A layer of hypodermal cells appears in the central mass of tissue before it becomes divided into the two stamens. The layer extends at right angles to the long axis of the flower (Fig. 7). At first the cells of this layer differ from the surrounding cells chiefly in the fact that they form a definite hypodermal layer. Later, it is evident that they have denser protoplasmic contents, larger nuclei and the cells themselves are elongated in a direction parallel with the long axis of the flower.

The two stamens come into existence by the depression of the epidermal layer in the center and a sterilization of the hypodermal archesporial cells in that region (Fig. 8). This separates the hypodermal cells into two groups. By this time these sporogenous cells have increased in number, have lost the palisade form, but are still distinguished from surrounding cells by denser protoplasm.

A second sterilization process divides each of the two groups of sporogenous cells resulting in four groups,—two to each stamen (Fig. 9). These groups represent the two loculi in each anther. The sporogenous cells in the loculus divide and form a parietal layer on the side of the epidermis. The larger cells remaining in a more interior location are the microspore mother cells. The cells of the parietal layer in turn divide forming an outer, wall layer, which lines the epidermis and an inner, tapetal layer (Fig. 11,*t*), which surrounds the spore mother cells. Figure 10 shows the parietal layer between the epidermis and the spore mother cells. Two of the parietal cells shown have recently undergone the division that has just been described. In some cases tapetal cells may be supplied by the spore mother cells. In other cases tapetal cells divide, thus forming a layer outside of the spore mother cells with a thickness of four cells instead of the usual three. In still other cases the layer of cells next outside of the tapetum also takes on the appearance of a tapetum, thus making a two-layered tapetum.

The sporogenous cells which have not undergone sterilization in the subdivision of the original group and in forming the layers described above, are

the microspore mother cells. There are most commonly five, six or seven of these cells in each of the two loculi belonging to one stamen. The orientation of the loculi in this plant is an unusual one. The long axis of the loculus is at right angles to the longitudinal axis of the stamen (Fig. 11). The mother cells form a single row except at the ends of the loculus where there is a tendency to form more than one row. It is readily seen that such an arrangement of loculi allows a transverse section through the flower to be longitudinal through the loculi as is the case represented in figure 2. In this figure the loculi are shown filled with immature pollen spores.

One of the problems is to homologize the two loculi found in one stamen of this plant with the four-sporangiate condition so generally found in the stamens of angiosperms. Campbell (1897) found that the condition of a single loculus to a stamen is representative for the genus *Naias*. In *Naias flexilis,* however, some stamens were found that had a partition dividing the sporogenous mass into two loculi. Conditions intermediate between the one loculus and the two-loculate type were found. In *Naias major* four loculi to the anther occur. Campbell thinks that the single loculus represents the primitive condition and that a plural number are formed by the dividing of the original one.

The findings in this study of *Vallisneria* are in some respects similar to those just cited for *Naias*. In *Vallisneria,* however, it appears that we have the types from the single loculus through intermediate stages to the four-loculate condition, all represented in one species. It has already been shown above, that, typically, the sterilization of some of the sporogenous cells results in two loculi to the anther. In several instances observed the process of sterilization failed to go far enough to form a complete partition between the two loculi. This results in allowing the two loculi to remain connected at one end, in which case one continuous horseshoe-shaped row of spore mother cells is formed (Fig. 12). It may also be noted here that all such connections observed occur toward the adaxial side of the flower.

In attempting to determine how many microsporangia are represented in an anther of *Vallisneria,* attention was drawn to the ends of the loculi. The fact, already mentioned above, is, that a greater amount of sporogenous tissue occurs in the ends. This suggests that the four ends of the two loculi may be the true microsporangia. These four sporangia develop little in the direction of the longitudinal axis of the stamen and their transverse connections uniting them in pairs form two loculi situated transversely in the stamen.

As the examination of material continued, staminate flowers were found in which sterilization has proceeded to the point of separating the two ends of loculi and in this manner forming four microsporangia similar to those regularly found in angiosperms. All gradations were found from four loculi to the flower to the condition of eight loculi. Figure 13 illustrates an anther in which sterilization proceeded to the point of separating the sporogenous tissue on one side into two sporangia, although on the other side the sporogenous tissue remains connected in the usual manner in the form of a transverse loculus. Finally, it was observed that when the ends of the usual type of loc-

ulus were separated as distinct sporangia, these sporangia developed in a direction parallel with, instead of transverse to the longitudinal axis of the stamen. In this point *Vallisneria,* when the microsporangia are separated, still further simulates the usual angiospermous condition.

From the description already given, it is seen that the spore mother cells of both anthers of a flower arise from a common archesporium instead of from eight independent, sporangial groups of archesporial cells as is common for angiosperms. In this respect *Vallisneria* recalls the condition described by Caldwell (1899) for *Lemna minor.* The question of the identity of the microsporangium is discussed by Coulter and Chamberlain (1903). If the archesporium is to be considered the sporangium, then in *Vallisneria* the two stamens together have only one sporangium. May it be that there is only one stamen in the male flower of *Vallisneria?* The union of the basal portions of the two filaments would favor this view. On the other hand, it would seem that the relative positions of the floral parts and the occasional presence of eight microsporangia, indicates that there are here two stamens.

After the microspore mother cells have enlarged, they undergo the meiotic divisions. The spindle figures of the first division lie at right angles to the longitudinal axis of the loculus. The figures of the second division lie in the same plane as those of the first. This results in the formation of a flat (bilateral) tetrad, the four members of which are most frequently visible in a transverse section of the loculus (Fig. 14). A longitudinal section of a loculus shows most or all of the tetrads in the loculus; but, as the tetrads are viewed edgewise only two members of each may be visible.

The cells of the tetrad separate as shown in the outer loculus of the two represented in figure 14. A little later the tapetum which has up to this point formed a jacket of definite cells around the young microspores, breaks open toward the interior of the loculus. In this manner the cytoplasm is released. Part of it flows into the cavity of the loculus among the microspores, forming a periplasmodium (Fig. 15). Some of the tapetal nuclei also move interiorly in the periplasmodium which evidently nourishes the developing microspores. At this stage the exine, a heavy spiny wall of the pollen spore is evident for the first time.

Just as the floral parts in the upper portion of the inflorescence develop in advance of those in the lower portion, so microsporogenesis in the upper flowers runs ahead of the same process in the lower flowers. In one inflorescence, for example, there were various stages ranging from the cutting off of the tapetal cells in the lowermost flowers to the first contractions of the nucleus of the spore mother cell in the uppermost flowers. Advanced development is also noted in the outer loculi as it has already been pointed out in figure 14.

3. *The Development of the Pollen Spore*

The microspores, likely to be irregular in shape (Fig. 15), possess an exine; but an intine does not appear until later. A denser mass of cytoplasm usually occurs in the central part of the cell and the primary microspore

nucleus occupies a position a little to one side. In this position the nucleus undergoes mitotic division and gives off a smaller, antheridial nucleus to one side or corner of the microspore. A cell plate is well developed and forms, what is especiallv noteworthy, a definite cell wall (Fig. 16) which cuts off an antheridial cell. The larger, tube cell occupies the main part of the microspore and it retains the denser mass of cytoplasm in its central portion.

A much discussed question is whether the aquatic monocotyledonous plants possess primitive or degenerative characters. A definite cell wall formed within the microspore recalls similar conditions found in lower groups. This one feature alone would indicate a primitive rather than a degenerative nature.

A rather complete series of stages in the development of the pollen spore was obtained. It is, therefore, possible to follow the history of the antheridial cell and the development of the sperms in some detail.

The definite cell wall separating the antheridial cell from the tube cell disappears (Fig. 17) leaving a narrow clear space between the plasmic membranes which remain on either side. The antheridial cell becomes rounded and, to an increasing extent, it projects into the interior of the pollen spore. Contact with the outer wall, is at the same time lessening (Fig. 18) and eventually lost, as the cell migrates in a more rounded form into an interior location. By this time the intine has appeared. The denser central mass of cytoplasm has been replaced by vacuoles which reach their maximum in size and form in a single, large, central vacuole (Fig. 19).

Located in the interior, the antheridial cell gradually assumes an elongated form (Fig. 19) and eventually it becomes spindle-shaped with pointed ends (Fig. 20). The cell is also likely to be somewhat curved and frequently the ends lie in contact with the opposite walls of the spore. The large vacuole which occupied the center of the spore has by this time disappeared. A comparatively continuous and even mass of cytoplasm again fills the spore.

The division of the antheridial cell and the organization of the two sperm cells resulting from this division is of special interest. It involves the apportioning of cytoplasm as well as of nuclear material. The cytoplasm of the sperm, Wylie (1923) has pointed out in *Vallisneria,* probably has a part in the fusion of the sperm with the egg. The sperm cells formed in the division become spindle-shaped and finally lie in contact with one another only by their pointed ends. This raises a question concerning the method by which the cytoplasm may be divided so as to result in this condition.

In describing the division of the generative cell in *Elodea canadensis,* Wylie (1904) states that a partition is formed between the daughter nuclei. He observes also that the adjoined ends of the male cells become long drawn out and when fully formed are connected by their tips.

Herrig (1919) observed in *Echeveria Desmetiana* that a spindle-shaped generative cell divides soon after it has passed into the pollen tube. In the binucleate condition the generative cells were sometimes found somewhat constricted in the middle. No sign of a separation membrane between the two nuclei was observed. Later, the two cells are distinctly separated and of an

elongated, oval form. But a clearly defined intermediate stage was not observed.

Piech (1924) finds in *Scirpus lacustris* L. that the anaphase brings about a characteristic elongation of the spindle-shaped generative cell, which becomes much narrowed in the middle and finally the two sperms, also spindle-shaped, are formed by the tearing apart of the equatorial part of the phragmoplast.

Finn (1925) observed in *Asclepias Cornuti* a prominent cell plate taking part in the separation of the protoplasts of the two newly formed male cells. This author also states that Murbeck and Graves have shown that in *Ruppia* the division of the generative nucleus is followed by a laying down of a rather fine cell plate.

Poddubnaja (1927) working on *Echinops sphaerocephalus* refers to the division of the spindle-shaped generative cell. He states that at the equator of the spindle a thin delicate wall is laid down. In this manner the two nuclei are separated and each has its own cytoplasmic envelope.

In the present study of *Vallisneria spiralis,* two factors are observed to effect the division of the cytoplasm of the antheridial cell and to separate the two resulting sperm cells. These two factors are the cell plate and the constriction of the antheridial cell in the region between the two daughter nuclei. These two factors vary in their importance. Either may be almost entirely excluded from the process of division itself.

In the earlier telophases of the division of the antheridial nucleus a definite cell plate occurs (Fig. 21). Generally, this cell plate fades to a considerable extent, leaving the actual separation of the sperm cells to a constriction which soon develops (Fig. 22). In some cases (Fig. 23), no cell plate is any longer discernible while a broad constriction is in process of dividing the cytoplasm. The weak cell plate may, during the constriction, be drawn into an oblique position, or the deepest part of the constriction may not coincide with the position of the cell plate.

In other instances the cell plate is more persistent. The progress of the constriction may even be delayed in the region of the cell plate (Fig. 24) although evident on either side of it. The constriction may at first be narrow and involve the cell plate by serving to separate the two plasma membranes formed and pushing them back in the process of tapering the ends by which the sperm cells remain connected. Finally, there are instances in which the splitting of the cell plate separates the sperms before the constriction has progressed far (Fig. 25). In a few instances a slight shrinkage served to demonstrate that the sperm cells may easily break apart at the cell plate before there is any constriction.

The completely formed sperm cells are spindle-shaped and may have various positions within the pollen spore. Commonly they form a V-shaped figure about the tube nucleus (Fig. 26). In the mature pollen spore the cytoplasm of the sperm cells is readily distinguished from the starch-filled cytoplasm of the pollen spore proper. With Flemming's triple stain, the sperm cell may be made to appear orange-colored in contrast to the intense blue of the surrounding starch grains.

The exine of the pollen spore, it has been noted, appears soon after the tetrads have broken apart and the microspores are still in the one-nucleate stage. Wylie (1923) refers to the pollen grain of *Vallisneria spiralis* L. as having a nearly smooth surface. Figure 27 shows sections of the exine, at high magnification, as found in mature pollen spores. The figure shows the relative length and number of the spines which form part of the exine and project outwardly on the surface of the pollen spore.

Advanced development in the terminal part of the inflorescence was noted again in connection with the development of the pollen spore. In one example, the microspores in the basal part contained a large vacuole and the antheridial cells were only beginning to assume the shape of a spindle. In the middle region there were fewer vacuoles and many spindle-shaped antheridial cells. In the apical region of the inflorescence the antheridial cells were in process of division and forming the sperm cells.

B. The Pistillate Flower

1. *Structure of the Flower*

The pistillate flower develops under water. As development proceeds the scape of the flower elongates, slowly at first, more rapidly when the embryo sacs are practically mature. The scape usually continues to elongate even after the tip of the flower has reached the surface of the water and enough scape is formed to allow the pistil to lie horizontally on the water by the time the flower opens. It is in this horizontal position of the pistillate flower that pollination occurs. Subsequent to pollination the scape begins to coil and the pistil is tipped, base downward, again leaving only the apex of the flower at the surface.

The pistillate flower is epigynous. The three sepals are attached to the outer edge of the receptacle at the upper end of the ovulary. The three rudimentary petals, alternating with the sepals, are situated on the flattened space of the receptacle between the base of the calyx and the base of the style. The three staminodia are situated a short distance up on the style. They remain very small and entirely functionless as far as the production of pollen is concerned. The style is hollow in the mature flower. The ovularian cavity, therefore, is open to the exterior through this stylar canal (Fig. 28). This condition offers a contrast to that found by Wylie (1904) in *Elodea canadensis*. This investigator finds in *Elodea* a triradiate canal extending up through the center from the cavity of the young ovulary to the exterior at the stigmas. But as development progresses the parts above coalesce and the cavity of the ovulary is roofed over. In *Vallisneria spiralis* the cavity of the ovulary communicates with the exterior through the stylar canal at maturity as well as during the stages of development.

Figure 29 shows a cross section of the ovulary. This particular ovulary has four main conducting strands instead of the usual three found accompanying the paired projections. The orthotropous ovules are surrounded by a mucilage which the fixing solution did not dissolve in this case and which may frequently remain undissolved when certain fixing solutions are used.

The three pairs of projections shown in the figure extend only a short distance into the cavity of the ovulary.

Troll (1931) finds that each pair of these projections constitutes the margins of the two adjoining carpels. The three carpels, he explains, are really separate—an apocarpous condition. The tissue on the outer sides and between the carpels is axial tissue. Applying Troll's interpretation, we may add that the carpels are also connected in this manner in the lower part of the style. In the upper part of the style the three carpels are actually separated (Fig. 30), very much as Troll observed them at the level of the ovulary in other genera considered to be more primitive members of the order to which *Vallisneria* belongs. Each of the three carpels represented in figure 30 is an upward continuation of a carpel of the ovulary shown in figure 29. Each carpel, it is seen, is open throughout its entire length from the base of the ovulary to the stigma. The protruding margins of each carpel are continued upward through the stylar region and terminate in the two parts of one of the three stigma lobes.

A more extensive study of the anatomy of the female flower does not fall within the scope of the present paper. Otherwise, what is presented here should be supplemented by an approach to the subject in the light of the solid carpel theory developed by Edith R. Saunders.

2. *Development of the Ovule and Megasporogenesis*

In the youngest pistils sectioned, the ovules had already begun to develop as rounded knobs consisting of an epidermal layer covering about three layers of cells lying in the interior (Fig. 31). A hypodermal archesporial cell then appears (Fig. 32). It may be distinguished from surrounding cells by its larger size, its larger nucleus, the more even distribution of material on the nuclear reticulum and by the different staining reactions of this reticulum. It stains somewhat red as compared with the blue in the nuclear reticula of surrounding cells when Flemming's triple stain is used.

The division of the archesporial cell results in the megaspore mother cell and a primary parietal cell which lies between the mother cell and the epidermis. The parietal cell undergoes division and thus two or more cells eventually come to lie between the mother cell and the epidermis.

The megaspore mother cell frequently is of such form and position that a pointed end is directed toward the base of the ovule (Fig. 33). By the time the mother cell has been formed, the outer and inner integuments of the ovule are indicated (Fig. 33) in the epidermal layer. In the main, the development of the integuments and the development of the megaspore mother cell run parallel,—a feature utilized by Mottier (1909) in a cytological investigation of *Lilium*. In *Vallisneria spiralis*, the inner integument has risen to the level of the base of the mother cell when the latter is mature and its nucleus still in the resting stage (Fig. 34). At this stage the two cell-layers of the inner integument have split apart and they occur slightly separated throughout most of the length of the integument. During the stages of the first contraction of the nucleus of the mother cell, the inner integument continues to rise. At

the stage of the hollow spirem it reaches the level of the top of the mother cell. By this time the outer integument also has split into two slightly separated layers. It, however, continues to lag behind the inner integument in the whole development of the ovule.

From the stage of the hollow spirem on, the mother cell elongates very noticeably in the direction of the longitudinal axis of the ovule. When the bivalent chromosomes have formed and before a spindle of achromatic fibers has been organized, the cytoplasm exhibits masses of fibers (Fig. 35) similar to those observed by Mottier (1897) in Podophyllum and in other plants. In the first meiotic division, the upper half of the spindle was found to be distinctly curved (Fig. 36).

The two cells resulting from the first division are slightly separated along part of the interlying boundary. The lower cell is the larger of the two. The outer integument at this stage has risen to the level of the base of the lower cell and the inner integument exceeds the nucellus in height.

The second meiotic division occurs with the two division figures not always in the same relation to each other. This results in three different types of tetrad arrangements of the megaspores. Figure 37 represents one of the linear tetrads. The second cell from the top is somewhat back of the others. In none of the linear tetrads observed are all of the four cells actually in a straight line or on the same plane. The second type of tetrad encountered in this plant is the socalled T-type. The two lower megaspores lie in the longitudinal axis of the ovule, while the outer two lie transversely (Fig. 38). Such an arrangement of the megaspores has been described by Rosendahl (1909) for *Symplocarpus foetidus*. In *Vallisneria spiralis* the wall separating the upper two cells is sometimes so delicate that it could easily be overlooked, and, since the nuclei degenerate early, the group of cells could be mistaken for three instead of four megaspores.

The third type of tetrad is more nearly a true tetrad in the form of a tetrahedron. Figure 39 represents a second division forming this type of tetrad. The lower division figure lies obliquely in the ovule and shows that the process has run ahead of that represented in the upper division figure. The lower cell is in telophase and the upper cell is in a late anaphase which has been sectioned transversely through the spindle fibers. The chromosome groups which belong to the upper division figure (Fig. 39,*a* and *b*) are found in the adjacent sections.

The meiotic divisions take place in such a manner that the lowermost, functional megaspore is at once the largest of the four. The three megaspores which do not function, disintegrate. Traces of them may be seen during enlargement of the functional megaspore (Fig. 40) and during subsequent development of the embryo sac (Fig. 41).

Just as different stages of development are found at different levels in the male inflorescence, it is likewise found that ovules at different levels are found in different stages in the same ovulary. In this case, however, the more advanced stages are found in the basal instead of in the upper part of the ovulary. In one example, ovules in the upper end of the ovulary show the

first contraction of the mother cell nucleus. Ovules in the basal end are found to contain the daughter cells of the first meiotic division and stages intermediate between these two occur in the middle parts of the ovulary. No correlations are here observed between differences in development and differences in function.

3. *Development of the Embryo Sac*

When the functional megaspore enlarges as the one-nucleate stage of the developing embryo sac (Fig. 40), a large vacuole develops below the nucleus, in the chalazal end of the sac. In the two-nucleate stage this vacuole diminishes and a new and much larger vacuole occupies the central part of the sac between the two nuclei. From this stage on, the embryo sac consists chiefly of a large central vacuole, a thin parietal layer of cytoplasm and, at each end, larger masses of cytoplasm in which the nuclei are contained.

By the time the embryo sac has reached the four-nucleate stage (Fig. 41), it has not only increased in size; it has changed in shape. The micropylar end has greatly widened and the antipodal end is narrowed into a sort of pocket. The nuclei at each end are arranged accordingly. In the micropylar end, the nuclei are lined up transversely to the longitudinal axis of the sac; in the antipodal end the orientation is obliquely longitudinal. Early eight-nucleate stages before the spindle fibers had disappeared and before the mature embryo sac had been organized, were also observed.

The mature embryo sac is of the socalled normal type (Fig. 42), according to the terminology employed by Schnarf (1931). There are eight nuclei distributed among the seven cells. The two polar nuclei lie in the central part of the sac. They are approximately equal in size and are the largest nuclei in the embryo sac. The egg nucleus is second in size, and, as long as it is unfecundated, it is nearly as large as a polar nucleus. A synergid nucleus has a diameter two-thirds, and an antipodal one-third of the diameter of a polar nucleus. Male nuclei that have just escaped from the pollen tube into the embryo sac are found to measure in diameter about half as much as a polar nucleus.

A specialized tip of the embryo sac is formed by the terminal portions of the two synergids together (Figs. 42 and 45). The cell wall separating the synergid cells proper, extends on through this specialized apex in a manner that imparts to it approximate bilateral symmetry. At the very tip, a number of striations extend from the median cell wall a short distance down each side. The region bearing striations is stained a bright orange when treated with Flemming's triple stain. Below the striated orange-colored area is a clear zone which is sharply marked off from the main cytoplasmic mass of the synergid cells.

The egg cell does not extend to the very apex of the embryo sac as do the synergids. In a profile view of the egg apparatus this feature is seen as well as the fact that the egg cell extends farther in the direction of the chalazal end than do the synergids.

The fusion of the polar nuclei frequently occurs before a pollen tube enters

the embryo sac. In fusing, the two polars which had been lying merely in contact, become flattened against each other (Fig. 43). Then the nuclear membranes dissolve at the point of contact while part of the outline of each nucleus remains distinct (Fig. 44) until the fusion is more complete. The nucleoli contributed by the polars usually fuse into one large nucleolus and the fusion nucleus is larger than either of the polars (Fig. 45).

The antipodals are usually organized into definite cells, although in some instances cell walls are not discernible between the antipodal nuclei of the mature embryo sac. Typically, one of the antipodal nuclei is slightly larger than the others. The contents of the three nuclei may become quite homogeneous and show signs of disintegration in the mature sac.

Starch grains are found in some embryo sacs, scattered throughout the cytoplasm,—under what particular conditions has not been ascertained. They do not occur in as large numbers or in as compact masses as in the pollen spores. They are also frequently larger and especially are they more elongated than those in the pollen spores.

4. *Anomalous Ovules*

A small number of what may be termed anomalous embryo sacs are found. In some of these sacs no nuclei are found at the ends of the sac. All the nuclei are in one clump in the central part. In other cases the egg apparatus is disintegrating and the polars are separated from each other.

Double ovules were found in two instances. Mottier (1895) found that two embryo sacs to the ovule sometimes occur in *Delphinium tricorne*. Figur 9 of his paper shows two embryo sacs side by side without any intervening nucellar tissue. Ferguson (1908) describes and figures two embryo sac mother cells in one ovule of *Lilium longiflorum*. In this case a single layer of nucellar cells separates the young embryo sacs. Young (1922) found ovules containing two embryo sacs in the Irish potato, *Solanum tuberosum*. His figure shows several layers of cells lying between the sacs. He states that the vascular strand supplying the ovule, branches in the funiculus, sending a branch toward either embryo sac, the larger branch going toward the better developed embryo sac. In each case found by Young, one embryo sac was better developed than the other.

Both of the double ovules found in *Vallisneria spiralis* were sectioned transversely. However, in each case all parts of the entire ovule could easily be observed section by section.. One ovule (Fig. 46) was sectioned slightly obliquely. In this ovule each of the two embryo sacs is surrounded by a nucellus; but the two nucelli are fused to a large extent. Each integument is single and both are common to the two nucelli.

The second double ovule (Fig. 47) has two embryo sacs, two nucelli, two inner integuments but only one outer integument. It would seem that this ovule is double in almost as many parts as is possible without forming two separate ovules. In each of these double ovules the two embryo sacs are supplied with separate conducting strands which are traceable through the double funiculus (Fig. 48), into the wall of the ovulary where they are found to

arise separately from a larger conducting strand which supplies separate strands to other ovules. This suggests that each of the double ovules really consists of two ovules which arose from the wall of the ovulary so close together that they were engrafted from the start.

In one of the double ovules (Fig. 46) both of the embryo sacs appear typical and approximately equally so. In the other double ovule the condition resembles that found by Young. One of the sacs appears typical and functional; the other is in a degenerating condition.

C. Fecundation

Hall (1902) in his study of *Limnocharis emarginata* believed that fertilization occurred the night following pollination. To test this matter in *Vallisneria spiralis,* pistils were marked and artificially pollinated. Most of these pistils were fixed close around twelve hours after pollination. The study of the material showed that in this time ovules are already supplied with pollen tubes and fecundation has usually been completeed.

In a pistil fixed three hours after pollination, the pollen tubes are found in the stylar canal and the ends of most of them have reached the upper part of the cavity of the ovulary. In a pistil fixed six hours after pollination, pollen tubes have entered some of the embryo sacs but no fecundation was observed. A pistil fixed nine hours after pollination shows fecundation taking place. These few data will be of aid in making a more extensive study of the time elapsing between the pollination and fecundation processes.

The pollen tubes in their course from the stigma through the stylar canal, through the cavity of the ovulary, through the micropyle and finally, through the nucellus to the embryo sac, were not observed to penetrate any tissue except that of the nucellus and one of the synergid cells. The starch grains stored in the pollen spore, as described above, probably contribute to the nourishment for the growth of the pollen tube. These starch grains were observed to pass down into the tube and in some cases were found in the portion of the tube that reached the ovule.

The sperm cells were observed within the pollen tubes at different points between the stigma and the embryo sac. Sometimes they were seen in cystoids or swellings at the ends of pollen tubes that had not reached ovules. Figure 49 shows the two sperm cells and the degenerating tube nucleus in the tip of a pollen tube just about to enter a micropyle. In all of these locations it is clear that the sperms retain their cytoplasm.

Sperms within the embryo sac and just emerged from the pollen tube were observed. These sperms still retain their cytoplasm. In the material thus far studied it is not possible to show anything further concerning the fate of the cytoplasm of these male gametes. The male nucleus was observed within the cytoplasm of the egg and very near to its nucleus.

The actual process of fusion of sperm and egg nuclei is represented in figure 50. The two nuclei are still largely distinct in this stage. They are flattened against each other and the nuclear membranes between them have partially dissolved. Although the male nucleus, prior to fecundation, is not

vermiform as in *Lilium candidum,* described by Mottier (1898), the actual fusion of the gametic nuclei resembles this process as illustrated in Mottier's figure 24.

Later stages in the fusion of sperm and egg were observed. An irregularity in the outline of the nucleus of the zygote or a line of contact between the two masses of chromatin may indicate such stages. These evidently represent temporary conditions following closely the actual process of fusion.

After the fecundation process has been completed the zygote nucleus differs from that of the unfecundated egg in a number of features. It is slightly larger. The nuclear membrane is heavier. The chromatin content is denser. It contains more nucleolar material,—either a larger nucleolus or two nucleoli. These features do not constitute a reliable test for identification, but they may very frequently be recognized as subsequent checking with other conditions in the embryo sac confirms.

Brightly staining bodies usually occur in the end of the pollen tube after the sperms have been discharged. Sometimes they occur farther up in the tube or even in the embryo sac just outside of the tube. They are very commonly two in number. It is noted that Wylie (1923) derives these bodies from the tube nucleus.

D. The Development of the Seed

1. *The Proembryo*

Before the first division of the zygote occurs, it elongates in the direction of the clalaza. Figure 51 shows a stage in the first division of the zygote as well as of the primary endosperm nucleus. The development of the endosperm will be discussed separately further on. The first division of the zygote results in the basal suspensor cell and the embryonal cell. The basal cell is attached to the micropylar end of the embryo sac and does not divide again. The distal cell forms the embryo and a part of the suspensor. By the division of the embryonal cell of the 2-celled stage (Fig. 52), the 3-celled proembryo (Fig. 53) is formed. Burr (1903) working on this species, noted that the 4-celled stage is regularly attained by division of the middle cell. In the material examined by the writer it appears that the middle cell and the terminal cell may in some cases divide simultaneously or the division of the terminal cell may even run ahead somewhat. The 4-celled proembryo (Fig. 54) must be considered as consisting of four tiers with one cell in each tier (1+1+1+1). From this stage on, multiplication of cells occurs in certain of the tiers, and new tiers are added at more or less regular intervals by the transverse division of the cell situated adjacent to the basal suspensor cell.

The 5-celled proembryo (Fig. 55) still consists of four tiers (1+1+1+2). The fifth cell is added by the formation of a longitudinal wall in the terminal cell. The stages following were observed very much as Burr (1903) observed them,—especially with respect to tiers and number of cells in each tier. In figure 55 the cell lying adjacent to the basal suspensor is shown in process of division whereby the 5th tier is added. By doubling the number of cells in

the terminal tier from two to four and in the penultimate tier from one to two, the 9-celled proembryo (1+1+1+2+4) with 5 tiers is formed (Fig. 56).

The 17-celled stage with 6 tiers (1+1+1+2+4+8) is reached by the addition of another tier and doubling the number of cells in each of the three most terminally situated segments. The cell added to form the 6th tier very commonly is cut off obliquely and therefore is out of line as shown in figure 57. This figure shows a 17-celled proembryo in which some of the cells are dividing. This figure also illustrates how the remaining synergid may persist through the development of the proembryo.

A 7th tier is added and there is multiplication of cells in all tiers again except in the three situated most basally. The cell divisions, however, no longer occur is as regular a manner as they did before. Figure 58 shows a section of an embryo sac containing a proembryo of approximately 33 cells (1+1+1+2+4+8+16).

2. *Polyembryony*

One of the embryo sacs sectioned contains two proembryos, both in the micropylar end (Fig. 59). They occur in a position similar to those found by Guignard (1901) in a case of polyembryony in *Naias major*.

3. *The Embryo*

The differentiation of the primary tissues and the development of the parts of the embryo from some of the tiers of cells, do not differ in any important respect from these processes in *Sagittaria variabilis* as described by Schaffner (1897). Figure 60 shows how the tiers of cells formed in the proembryo are now apportioned. The terminal tier forms the cotyledon; the next tier forms the plumular region; two or more tiers go into the hypocotyl; and, three tiers of one cell each go into the suspensor. This figure shows the suspensor broken as Schaffner found was usually the condition at similar stages in *Sagittaria variabilis*. In addition to epidermal and cortical tissues, a plerome is differentiating. The last named tissue is evident in a tier of the hypocotyl adjacent to the plumular region.

After the contour of the embryo is established, the cotyledon and hypocotyl elongate greatly and a conducting strand extends through these regions with a branch to the plumular tip. At this stage it is already apparent that the base of the cotyledon forms a sheath around the plumular tip, leaving an opening only on one side.

4. *The Endosperm*

It has been shown above (Fig. 45) that the polar nuclei may fuse before fecundation. In the few cases in which a third (sperm) nucleus can be observed to participate in the formation of the primary endosperm nucleus, the conditions suggest it may unite with one of the polars just before the two polars fuse (Figs. 61, 62, 63). A more extensive study would probably show some variations in the formation of the primary endosperm nucleus.

Once the primary endosperm nucleus is formed, it descends from the

central position it had occupied in the embryo sac, to the antipodal region. Here it divides, simultaneously with the first division of the zygote (Fig. 51). The two nuclei thus formed may be termed, following the terminology of Schaffner (1897), the upper endosperm nucleus and the lower endosperm nucleus. A wall cuts off the lower endosperm nucleus which was not observed to undergo any further divisions. It should be noted here that an early microscopic botanical investigator, Hofmeister (1859), described cases of division of the embryo sac by a transverse wall.

The upper endosperm nucleus returns to the middle region of the embryo sac (Fig. 52) where it divides. By repeated divisions in the parietal layer of cytoplasm and by scattering of the nuclei thus formed, there develops an endosperm which for some time is non-cellular (Fig. 58). In a number of cases the divisions in the endosperm were observed to occur with approximately the same frequency as in the proembryo.

The cytoplasm of the endosperm is divided up into cells at the time when a stem notch is forming in the embryo as shown in Fig. 60, *n*. From this stage the endosperm develops more slowly as compared with the development of the embryo. At each end of the embryo sac the endosperm forms a mass several cells thick; along the sides, however, the endosperm remains one cell-layer in thickness. For a time there is considerable space between the endosperm and the growing embryo. Eventually the embryo sac is filled up by the embryo.

The lower endosperm cell enlarges greatly as does also its nucleus. It contains a dense cytoplasm and the nucleus becomes lobed. Activity in this cell appears to be at a maximum soon after the stem notch of the embryo is formed. The antipodal cells also appear to be associated with this activity. They are distinct at this stage and appear to form an important connection between the lower endosperm cell and the chalaza. As the embryo grows, the cotyledon eventually comes to encounter the lower endosperm and the antipodals, whereupon they immediately exhibit signs of disintegration.

5. *The Mature Seed*

While the embryo is developing, the cells in the outer layer of the outer integument form numerous starch grains. By the time the seed is mature these starch grains have disappeared and the cell walls of this layer contain conspicuous thickenings in a reticulate arrangement. The outer walls of the cells have now formed protrusions which are roughly arranged in rows encircling the seed and which give the seed coat a rough outer surface (Fig. 64). The outer cell walls of the inner integument develop thick protective layers. The nucellus and endosperm have disappeared except for thin membranous non-cellular vestiges which persist between the embryo and the remains of the inner integument.

As is characteristic of the Helobiae, the embryo fills the seed. Campbell (1930) has pointed out that this is one of the features in which these plants differ from much the larger number of monocotyledons in which a small embryo and an abundant endosperm occur. In *Vallisneria,* food, especially

in the form of starch, is stored in the cotyledon and in the large hypocotyl. The lower end of the hypocotyl is root in nature but there is no root cap in this organ.

The plumule consists of a stem tip and the first leaf (Fig. 65). The two structures, one on either side of the plumule are squamules, such as have been found by Arber (1923, 1925) to be characteristically present among leaf-bases of the Helobiae.

IV.—Summary

1. Flowers of the male inflorescence develop and mature in a basipetal direction.

2. The two stamens originate from a common primordium which contains a single group of hypodermal archesporial cells.

3. Two loculi, transversely oriented, are ordinarily formed in the anther. However, the number of loculi may vary from one to four.

4. The antheridial cell is cut off by a definite wall at one side of the microspore. This wall disappears and the cell migrates into the interior of the microspore.

5. The division of the antheridial cell is effected in such a manner as to form two spindle-shaped sperm cells.

6. The megaspores occur in three different types of tetrads,—the linear type, the T-type and the true (tetrahedral) type.

7. Double ovules occur in different degrees of duplicity.

8. Fecundation of the egg may occur approximately 9 hours after pollination.

9. Pollen tubes grow from the stigmas down along the open inner sides of the three carpels.

10. Although the male nuclei are never vermiform as in *Lilium*, the actual fusion of the gametic nuclei closely simulates that process in *Lilium*.

11. The proembryo adds new tiers of cells and doubles the number of cells in the more distal tiers in a somewhat regular manner.

12. A single instance of polyembryony was observed.

13. The two polar nuclei frequently fuse before fecundation. In some instances it appears that a male nucleus fuses with one of the polars before the latter fuse.

14. The primary endosperm nucleus after passing to the antipodal end of the embryo sac, divides, and a cell division results simultaneously with the first division of the zygote. The nucleus of the upper endosperm cell returns to the middle of the embryo sac where subsequent divisions occur.

15. The lower endosperm cell enlarges and persists until it is encountered by the enlarging embryo.

16. Eventually all endosperm is consumed by the developing embryo.

17. The embryo is of the macropodous type. There is no root cap on the radicle end of the hypocotyl.

18. The first squamules are formed between the cotyledonary sheath and the plumular bud.

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EXPLANATION OF PLATES

PLATE 1

FIGURES

1. Long. sec. of young male inflorescence. x 30.
2. Cross sec. of a nearly mature flower. a, adaxial sepal; b, lateral sepal; c, rudimentary petal. x 45.
3. Long. sec. of primordium of male flower. x 275.
4. Long. sec. of young male flower. x 275.
5. Cross sec. of young male flower. x 170.
6. Long. sec. of young male flower. a, primordia of staminodium (right) and rudimentary petal. x 275.
7. Long. sec. of young male flower. x 275.
8. Long. sec. of male flower. x 275.
9. Long. sec. of a developing stamen. x 275.
10. Cross sec. of one loculus of a young anther. t, newly cut off tapetal cells. x 615.
11. Long. sec. of a stamen. t, tapetum. x 275.
12. Cross sec. of an anther containing two united loculi. x 170.
13. Cross sec. of an anther with 3 loculi. x 275.

PLATE 2

14. Cross sec. of both loculi of an anther. x 275.
15. Cross sec. of a loculus containing microspores. x 275.

16, 17, 18. Microspores with antheridial cells. x 615.

19, 20. Developing pollen spores. x 615.

21-25. Stages in the division of the antheridial cell. x 615.

26. A pollen spore containing tube nucleus and the two sperm cells. x 615.
27. Edgewise view of sections of exine. x4,125.
28. Long. sec. of portion of pistil. s, sepal; st, staminodium; c, stylar canal; p, rudimentary petal; o, ovule; w, wall of ovulary; m, mucilage in cavity of ovulary. x 20.

PLATE 3

29. Cross sec. of ovulary. p, paired projections. x 20.
30. Cross sec. of upper part of style. x 20.
31. Primordium of an ovule. x 275.
32. Young ovule with hypodermal archesporial cell. x 275.
33. Young ovule containing megaspore mother cell. Indications of inner (i) and outer (o) integuments. x 615.
34. Long. sec. of young ovule. x 615.
35. Megaspore mother cell with bivalent chromosomes. x 615.
36. Megaspore mother cell in metaphase of first meiotic division. x 615.
37. Linear tetrad of megaspores. x 615.
38. Stage in second meiotic division resulting in the T-type of tetrad of megaspores. x 615.
39. Second meiotic division of megaspore mother cell resulting in the true tetrad. The chromosome groups of the upper division figure are found in adjacent sections and are represented at *a* and *b*. x 615.
40. One-nucleate stage of embryo sac with the three disintegrating megaspores. x 615.

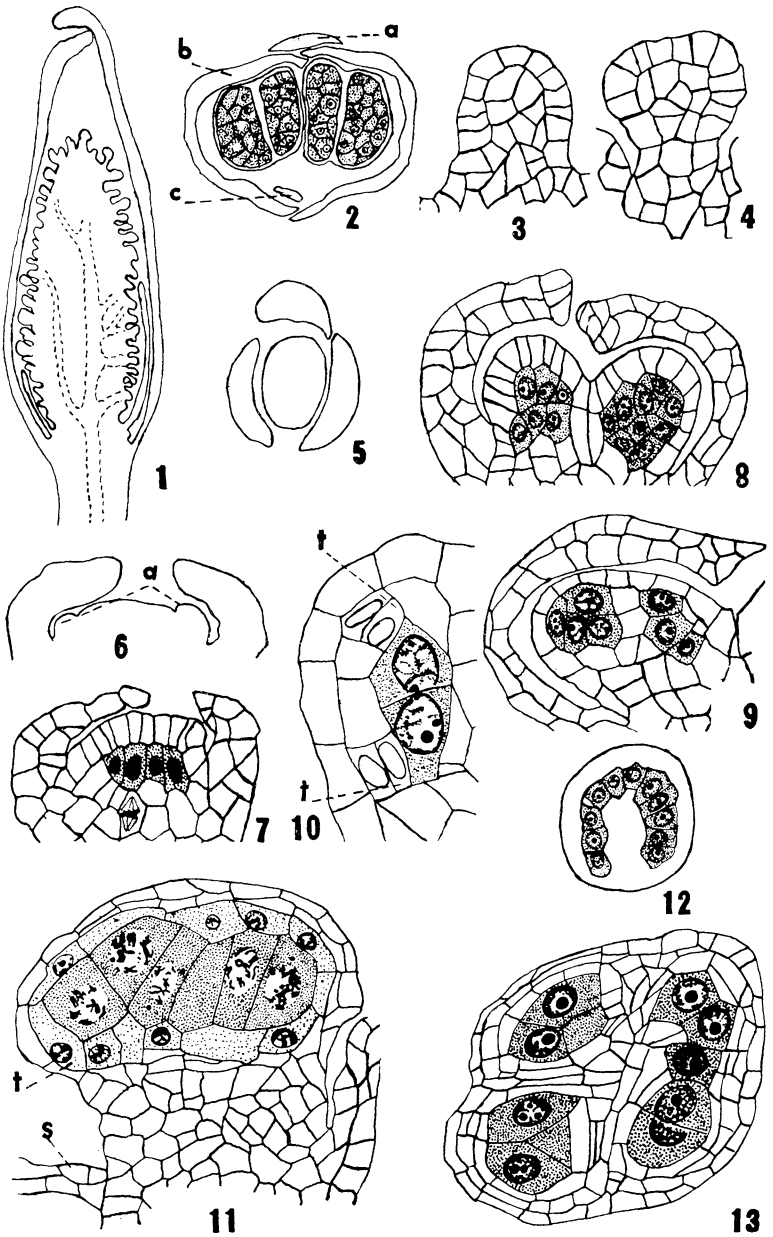
PLATE 4

41. Four-nucleate stage of embryo sac. x 850.
42. A mature embryo sac. x 615.
43. Polar nuclei flattened against each other. x 615.
44. Polar nuclei fusing. x 615.
45. Portion of the embryo sac containing a fusion nucleus. x 615.
46, 47. Cross sections of double ovules. o, outer integument; i, inner integument; n, nucellus; e, embryo sac. x 70.
48. Cross sec. of double funiculus belonging to the ovule represented in Fig. 47. x 70.
49. Tip of pollen tube at entrance of micropyle. x 615.
50. Sec. of embryo sac showing fusion of gametic nuclei. s, remaining synergid; t, pollen tube; p, a polar nucleus. x 615.
51. Parts of an embryo sac showing first division of zygote (e) and of primary endosperm nucleus (p). a, antipodals. x 250.
52. Embryo sac containing a 2-celled proembryo, upper (u) and lower (l) endosperm cells. x 250.

PLATE 5

53. 3 celled proembryo. x 275.
54. 4-celled proembryo. x 275.
55. 5-celled proembryo s, remaining synergid. x 230.
56. 9-celled proembryo. x 275.
57. 17-celled proembryo. x 275.
58. Long. sec of an embryo sac containing proembryo of approximately 33 cells, remaining synergid (s), endosperm proper (e), lower endosperm cell (l). x 120.
59. Two proembryos in one embryo sac. x 230.
60. Young embryo. s, suspensor; p, plerome; n, stem notch. x 130.
61, 62, 63. Stages in fusion of sperm and polar nuclei. x 615.
64. Long. sec. of seed. ch, chalazal end; co, cotyledon; p, plumular region; h, hypocotyl; i, inner integument; o, outer integument. x 20.
65. Plumular region of fig. 64 enlarged. l, first leaf; st, stem tip; sq, squamules. x 70.

PLATE 1



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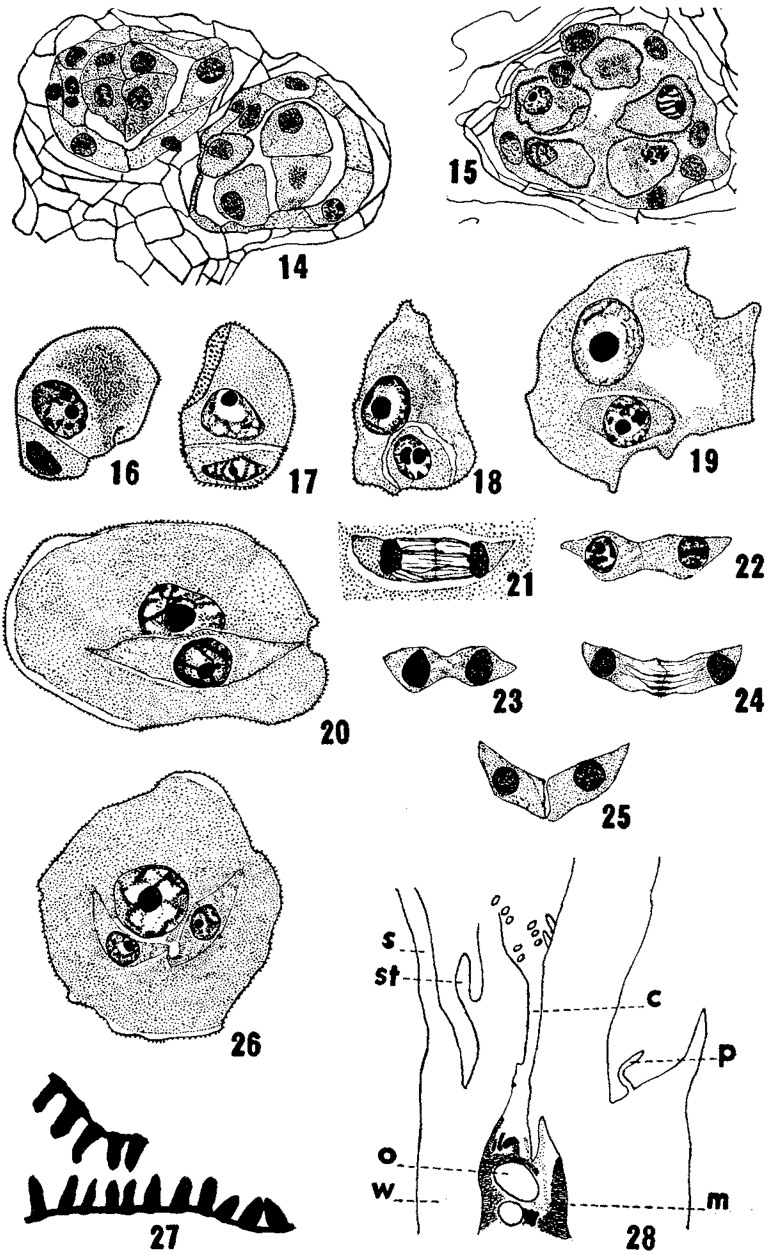

PLATE 3

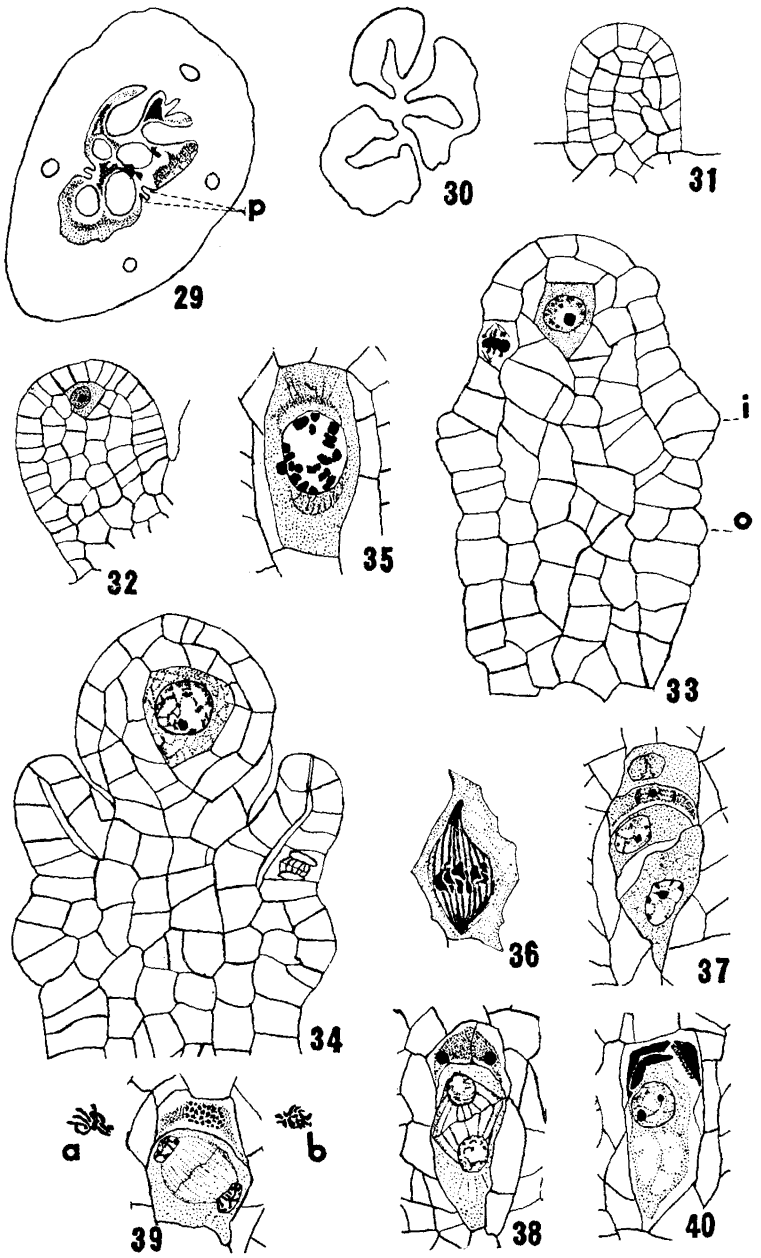

PLATE 4

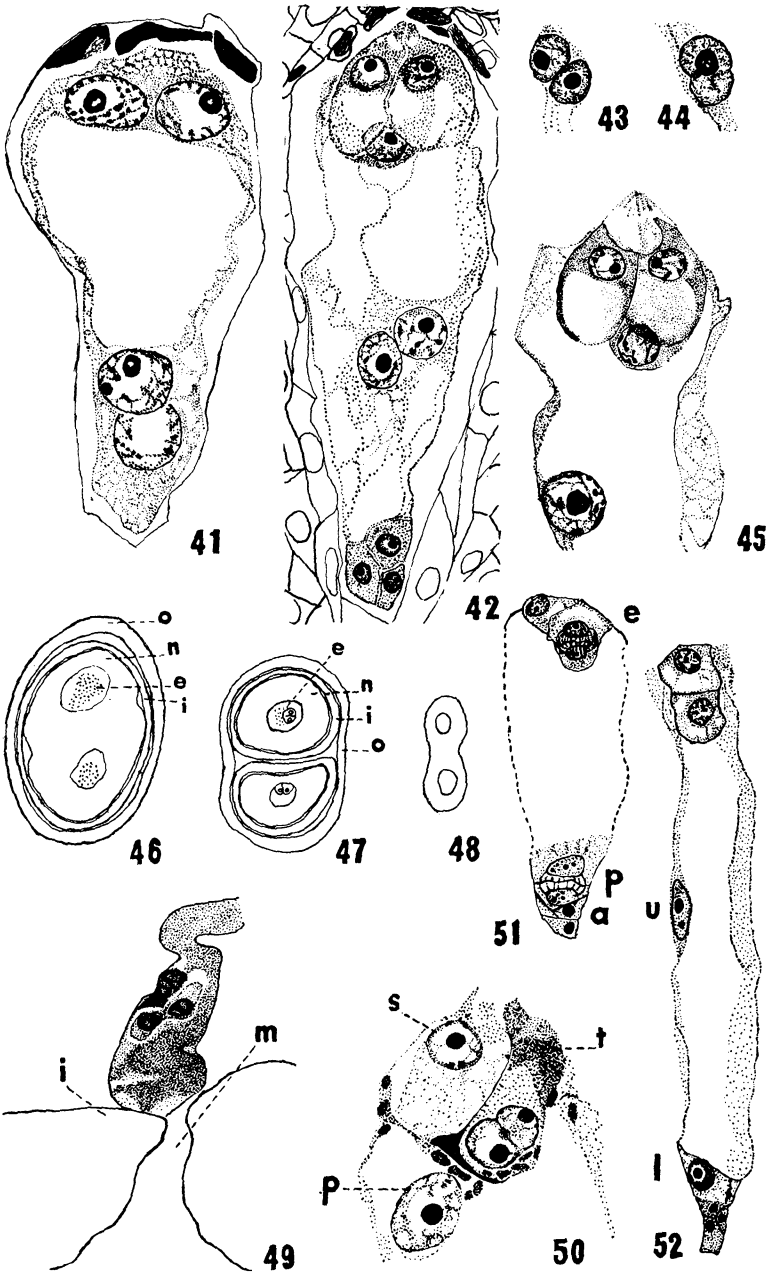

PLATE 5