

BOTANICAL GAZETTE

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ON THE ENDOSPERM AND EMBRYO OF PEPEROMIA
PELLUCIDA.

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(WITH PLATE 1)

In the spring of 1899 I began the study of the Piperaceæ, with well-preserved material of the genera *Peperomia*, *Heckeria*, and *Piper*, collected by the late Professor J. E. Humphrey on the unfortunate expedition to Jamaica in 1897. It was soon discovered that seeds were needed for sprouting in order to complete the work, and it was therefore laid aside temporarily and my time given to work on the related genus *Saururus*.

The recent paper by Professor Campbell ('99) on *Peperomia pellucida* announced results differing from those I had obtained, and led me to reexamine my slides, with the result that I was satisfied with the essential correctness of my former observations, and noted several interesting features in addition. As the intended detailed study of the group may be deferred for some time, an outline of the most important observations thus far made on *Peperomia pellucida* Kunth is given here.

The flower consists of two stamens, and a carpel sessile in the axil of a top-shaped bract (*br*, *fig. 1*). The ovule is single, basal, and orthotropous (*fig. 1*), with a single integument and one archesporial cell. The development of the flower as far as followed agrees with the account given by Schmitz ('72), and the development of the macrospore in the nucellus is as described by Campbell.

The primary archesporial cell cuts off a tapetal cell above, and then immediately forms the definitive macrospore below. The tapetum divides to three or four tiers of cells, which finally form, together with the outer layer of cells of the nucellus, a persistent plug of yellowish thick-walled cells, directly under the micropyle (*tp*, *figs.* 1, 2, 9, 13).

The first four nuclei formed from the large macrospore nucleus are located at the periphery of the pretty dense protoplast of the embryo-sac, arranged like the spores of a tetrad and connected by strands of granular cytoplasm (*es*, *fig.* 2). Soon after this we find eight ellipsoidal peripheral nuclei, imbedded in the cytoplasm surrounding the enlarged vacuole of the embryo-sac. Up to this stage the sequence of phenomena has not been very different from that in the normal angiosperm embryo-sac, except for the lack of the bipolar grouping usually found. But now each of the eight nuclei divides again, as Campbell has shown, to form an embryo-sac with sixteen similar nuclei, pretty uniformly distributed in the peripheral layer of cytoplasm (*fig.* 1). A little later than this, before the pollen tube reaches the embryo-sac, the cytoplasm begins to get denser about one of the nuclei at the top of the embryo-sac, and finally a definite limiting membrane surrounds this and forms the oosphere (*o*, *fig.* 1). This egg is not directly under the micropyle, but is pushed aside slightly by the aggregation of a smaller amount of cytoplasm about a second nucleus at the top of the embryo-sac to form the single *synergid*, as we may call it from its position (*sy*, *fig.* 1). The position of spindles in certain cases seems to indicate that this is a sister cell to the oosphere. Sometimes other nuclei are found near the egg, but often not, and in no case was there seen a definite massing of cytoplasm about any of these. At first the synergid does not have a definite wall, but later on, as it persists, at and after fertilization, a distinct wall can be seen (*sy*, *figs.* 3, 7, 9, 11, 12).

At about the time the pollen tube enters the egg certain of the remaining fourteen peripheral nuclei begin to move together to form a compact group, of usually eight nuclei, surrounded by

cytoplasm. This group may appear at the lower end of the embryo-sac, in the middle near the wall, or center, or above, and near or even in contact with the egg (*espn*, *fig. 3*).

The remaining peripheral nuclei retain their position near the wall, at first as naked nuclei in the thin layer of cytoplasm, but later each of these and a small portion of cytoplasm is separated from the great mass of cytoplasm in the embryo-sac by a flat saucer-shaped wall (*pn*, *figs. 3, 7, 9, 11, 13*). Nowhere was there noticed any tendency of a number of these to collect in a basal position, or anything in their behavior to suggest their homology with the antipodals of the typical angiosperm embryo-sac.

The short top-shaped stamens bear two pollen sacs each. Certain pollen grains (three or four in a section showing fifteen fertile ones) remain with unthickened walls, but apparently are not used for the nourishment of the fertile ones. The nucleus of the finely reticulate-walled pollen grain divides to two at a time soon after the formation of the tapetum in the embryo-sac of the same flower. The pollen grains are shed after the embryo-sac has reached the four-nucleate stage. They lodge on the large abaxial lobe of the carpel (*st*, *fig. 1*), and grow downward through a conical mass of small-celled conducting or nutritive tissue to the fusion canal of the carpel, and thence to the micropyle (*pt*, *fig. 1*). Just when the division of the generative nucleus occurs was not made out with certainty, but in cases where the pollen tube had just reached the embryo-sac the pollen-tube nucleus was seen at its very tip, and a single large generative nucleus, with cytoplasm about it, at the level of the nucellus. In other cases of about the same age there were apparently two generative nuclei.

No indication of a sterile prothallial cell was discovered. The pollen tube often extends some distance into the egg, and after its entrance two nearly similar nuclei are found within the egg. The exact fate of the pollen-tube nucleus and the second generative nucleus was not determined.

For a considerable time after its entrance the male nucleus lies in the egg near or even in contact with the female, but without

fusing with it (*figs. 3, 11*). Its presence in the egg seems, however, to have an important influence on the other contents of the embryo-sac. At the time of its entrance the group of eight nuclei, each with a single nucleolus, is usually found in the central or upper part of the embryo-sac near the egg, surrounded by a considerable mass of cytoplasm, but not separated from each other by cell walls (*espñ, fig. 3*). Soon after this the walls of certain of these nuclei are seen to be flattened against each other (*espñ, figs. 3, 6*), and a little later still the group may consist of one or two larger, elongated, constricted nuclei, each with two nucleoli, and six or four normal-sized nuclei each with a single nucleolus. These larger nuclei have been formed in each case by the fusion of two of the original nuclei of the group. The first one or two of these fusion nuclei form centers to which the other nuclei of the group draw up closely and fuse on to add their bulk to that of the larger nuclei. Finally a very large nucleus is formed, with at first several normal sized nucleoli, and later fewer very much larger ones or a single one (*espñ, figs. 6, 7*). The wall of this large nucleus shows at first several projecting lumps or knobs, each indicating the portion contributed by one of the fusion nuclei (*espñ, figs. 4, 5, 6, 7, 8*—a series of sections of the same group). The line of contact of the walls of the fusing nuclei is at first evident by the darkly stained region where the two peripheral chromatin nets press against each other (*espñ, figs. 5, 6, 7*). Later the lumps on the wall gradually smooth out and the chromatin net becomes evenly distributed about the periphery of the usually transversely elongated nucleus, which lies in a pretty dense mass of cytoplasm just below the oospore (*espñ, figs. 7, 8*).

From this time on this nucleus behaves like the endosperm nucleus of the typical angiosperm embryo-sac. It sometimes begins its development before any activity is noticed in the fertilized egg, except that the wall of the latter becomes more distinct and the sexual nuclei flatten against each other (*fig. 11*). In other cases the sexual nuclei fuse during the fusion of the endosperm-forming nuclei (*fig. 7*). The endosperm nucleus

divides by mitosis, the first spindle being approximately transverse. The number of chromosomes here is seemingly very large, compared with that in other spindles found in the embryo-sac or in the nucellus, but they were so densely packed in all the cases seen that it was impossible to make accurate counts.

It is certain, however, that the amount of chromatin in this and its daughter spindles is greater than that found anywhere else in the plant (*espn. figs. 9, 11*). A cell plate is formed in the typical way at the middle of each spindle, the fibers of the latter being stretched out laterally to a surprising extent (*espn, fig. 10*). The new cell-wall thus formed stretches from the oospore to the base of the embryo-sac and cuts the latter completely in two, forming thus two endosperm cells. Each of these divides further, forming a cell-wall immediately at each division, till in the oldest seeds seen there are forty or more endosperm cells, each with a large nucleus, several nucleoli, and dense cytoplasm, filling up all of the embryo-sac not occupied by the embryo and synergid and flattening the degenerating peripheral nuclei against the wall of the embryo-sac (*esp, figs. 12, 13, 14, pn, figs. 9, 13*).

The fusion of the sexual nuclei is completed, at the latest, soon after that of the nuclei of the endosperm group, and before many endosperm cells are formed the oospore divides to form the embryo. In the few cases of the early divisions of the embryo seen the first wall seemed to be longitudinal, and the position of the walls in the slightly older embryos, often seen, seemed to confirm this (*em, figs. 12, 13*).

The oldest fruits available, as was evident from their position on the spike were nearly ready to separate from the mother plant, *i. e.*, were nearly ripe. In these the embryo consisted of more than twenty cells, but showed no sign of a definite suspensor and no indication of the organs of the young sporophyte. In fact, the whole structure has much the same shape and but slightly larger size than the one-celled oospore (*osp, fig. 9, em, figs. 12, 13*).

The ultimate fate of the long persistent synergid has not been made out with certainty as yet, but so far as it has been definitely traced it retains much the same size and appearance that it has at the time of fertilization, except that the wall becomes more distinct (*sy*, *figs.* 3, 7, 9, 11, 12). In many cases where the embryo consisted of six or eight cells a single large cell could still be seen beside it, which seemed quite distinct from the endosperm cells that press against the embryo on all other sides, and this is interpreted as the still persistent synergid (*sy*, *fig.* 12), and possibly the cell at the right of the embryo under the tapetal plug in *fig.* 13 is another case of the same sort. In several of the very oldest embryos seen there was a group of cells, smaller than any other cells in the embryo-sac, located in in this same position beside the embryo. The evidence obtained points to these as derivatives of the synergid, but further careful work, including probably the study of the sprouting seed, will be necessary to make this certain and to determine the ultimate fate of these cells.

The absolute size of the embryo-sac in these mature seeds is but little larger than when the egg is differentiated (*figs.* 2, 3, 13—note the magnification of each). The relative size and position with reference to the other parts of the fruit is shown in *fig.* 14. The oblate spheroidal mass of endosperm is about one eighth the length of the whole seed. It is separated from the integument at the top by three or four layers of cells of the tapetum and nucellus. Below is the great mass of perisperm cells developed from the basal portion of the nucellus (*figs.* 12, 14). Each of these cells is finally packed closely with starch, aggregated in several masses (filling up the vacuoles) separated by thin layers of cytoplasm (*psp*, *figs.* 13, 14, 15), in one of which lies the flattened and distorted, but still darkly staining nucleus (*pspn*, *fig.* 15). In these cytoplasm layers are also found large clear, or finely granular, spherical masses of an undetermined chemical nature, but presumably serving as food (*psp*, *fig.* 15.)

The single integument is but two cells in thickness, and both of these take part in the formation of the seed coat or

testa. All the walls of the cells of the outer layer become thickened till the cell cavity is practically obliterated, and the thickness of this layer is about the same over the whole of the seed, or slightly greater toward the base (*int, figs. 14, 15*). The cells of the inner layer thicken the outer walls greatly, especially near the upper end of the seed, where large knobs of the thickening substance project into the cell cavity (*int, figs. 14, 15*). The cavity is never entirely filled as in the outer cells, but considerable space remains which is packed with starch like the cells of the perisperm (*int, figs. 14, 15*). The innermost layer of thickening substance of the outer walls of the cells of this layer is of quite different consistency from the rest of the wall, and shows in sections as a uniform border about all the hollows and projections of the latter.

At the base of the seed several layers of cells of the chalaza thicken their walls, like those of the outer integument layer, to complete the protection of the seed (*fig. 14*).

The seed does not escape from the carpel, but the latter apparently remains adhering closely to it when the whole falls from the mother plant. At this time the carpel is four or five layers thick, except at the base and in the stigmatic region (*cp, fig. 15*). The outer layer is of large, cuboidal, nearly empty cells, interspersed with knob-like hydathodes. Its cells have unthickened walls, except for the fine striae found quite generally on the outer epidermal walls of the whole inflorescence (*cp, fig. 15*). Next within this layer we find two or three layers of thin-walled flattened cells, with little contents. Closely adherent to the integument is the inner layer of the carpel, made up of large cells of about equal height and meridional length, but elongated equatorially to twice this length. These cells have the basal wall considerably thickened, with comparatively low ridges projecting above this general thickening (*fig. 15*). The lateral and outer walls of these cells have anastomosing ribs surrounding thin spots or pits, forming cells closely resembling those of the velamen of the roots of many epiphytes in structure, and perhaps in function also. The basal or inner end of these cells is

occupied by a granular mass, apparently of some firm substance deposited by the protoplast as an addition to the protective layers of the fruit and seed, or possibly connected with the absorption of water by these cells (*fig. 15*).

The subtending bract increases but little in size after the macrospore is formed, and as the fruit ripens the bract withers and is squashed down by the swelling carpel (*br. figs. 1, 14*).

In comparing the foregoing with Campbell's results it will be seen that my observations confirm his in regard to the origin of the macrospore and its development to a sixteen-nucleate ripe embryo-sac. Campbell thinks that one of the upper of these nuclei goes to the egg, and one to each of the two naked synergids; while eight others, which he interprets as probably antipodals, temporarily collect at the base of the embryo-sac, but later disperse and become indistinguishable. The other five nuclei play no prominent part, there being according to his observations no nuclear fusion analogous to that of the polar nuclei of the ordinary angiosperm embryo-sac.

In my own work I have seen but a single synergid which is long persistent and has a distinct wall. The nuclei of the group which Campbell interprets as possible antipodals I find are ultimately fused together into one endosperm nucleus, there being no special basal (antipodal) group of sterile cells or nuclei.

Again, Campbell says that the at first flattened embryo finally fills the whole embryo-sac and that there is no endosperm whatever, while I find that the embryo is nearly globular at first and is later completely surrounded by endosperm which fills the greater bulk of the embryo-sac.

The meaning of these very striking peculiarities of the embryo-sac of *Peperomia pellucida* (and other species of the same genus) is not easy to determine. The extra division of the embryo-sac is quite unique, and so also is the lack of a basal group of sterile cells or antipodals. Finally the fusion of so large a number of nuclei into one, in forming the endosperm nucleus, is approached only by the cases of fusion, at quite a

different stage of development, of the several nuclei in the endosperm cells of *Staphylea pinnata* and *Corydalis cava*. In these forms, according to Strasburger ('80), when walls appear about the endosperm nuclei several of these are enclosed in a single cell and these later fuse to a single nucleus. The fusion of polar nuclei during instead of before fertilization is found also in *Allium fistulosum* (Strasburger '79, p. 21), and this case may perhaps be considered as analogous to that of the endosperm-forming nuclei in *Peperomia*.

That these peculiarities of *Peperomia* are to be considered primitive rather than higher specializations seems to me unwarranted by the evidence at present available, especially when we consider the fact, which I have ascertained, that such closely related genera as *Piper*, *Heckeria*, and *Saururus* have essentially typical angiosperm embryo-sacs. These latter forms develop a small amount of endosperm in a manner similar to that found in such distantly related and certainly not very primitive forms as the *Nymphaeaceæ*.

Again, the lack of any grouping of the extra peripheral nuclei in the embryo-sac of *Peperomia* fails to give any encouragement from this source to those who look upon the antipodal group in the angiosperms as a second egg-apparatus (Lotsy, '99, p. 106).

So also the fusion of the eight nuclei to form the endosperm nucleus, if we regard it as at all homologous with that of the polar nuclei, seems to indicate that this is a purely vegetative or nutritive process, rather than anything like a sexual fusion as suggested by Mann ('92). Finally, the development of the cell walls in the endosperm directly after nuclear division each time, instead of by the method of free cell formation, as in the prothallus of the higher pteridophytes, is not favorable to the view that *Peperomia* is a transitional form between these forms and the typical angiosperms.

I am inclined to believe that the peculiarities of the embryo-sac of *Peperomia* have been secondarily acquired, and are analogous to those found in other angiosperms of peculiar habit, *e. g.*, many aquatic, parasitic, and saprophytic forms.

It is probable that a careful study of the sprouting seed will show the meaning of some of these peculiarities to the plant. I hope soon to be able to determine whether the tissue which I have called endosperm here has the same function as in *Saururus*, of absorbing the perisperm for the benefit of the embryo during the sprouting of the seed. I trust also that a further study of related forms may discover some intermediate type of embryo-sac, that will indicate more definitely the possible derivation of the peculiar one found in *Peperomia*.

In conclusion and summary: the macrospore nucleus of *Peperomia pellucida* forms sixteen free nuclei, of which one goes to the egg, one to the synergid, eight more fuse to form a single endosperm nucleus, while the other five remain sterile and degenerate. The nearly ripe seed contains an embryo of fifteen or more cells surrounded by endosperm cells in which the walls are formed directly from the cell plate of the spindle.

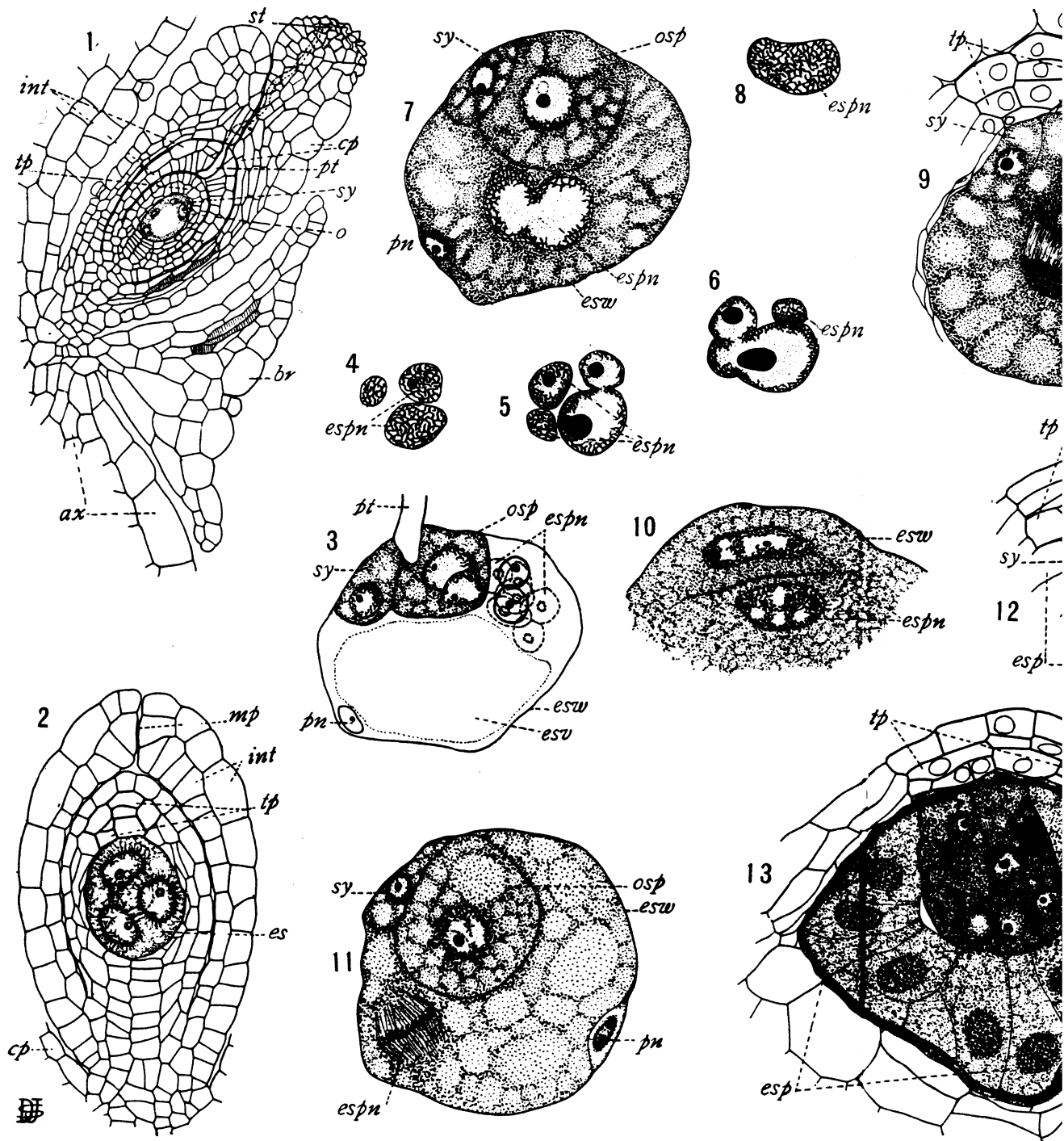
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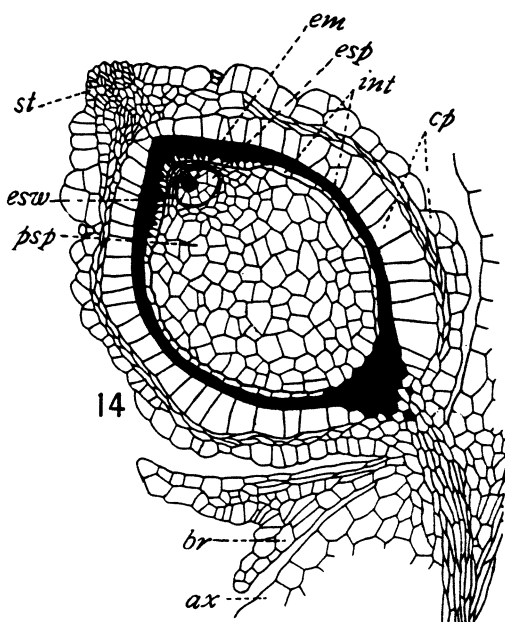
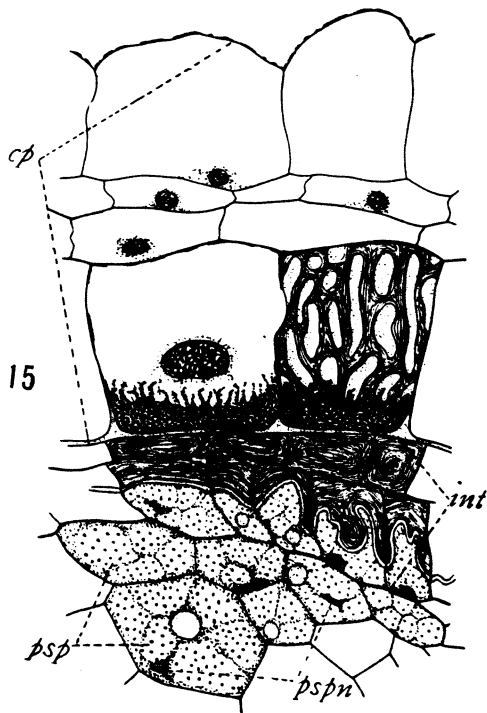
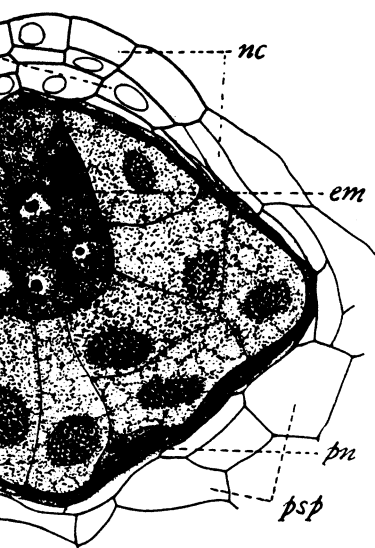
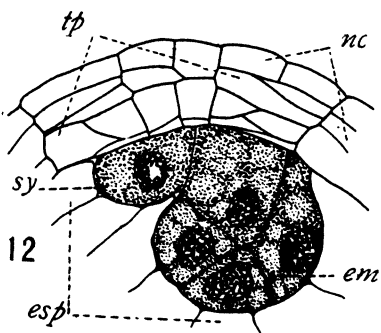
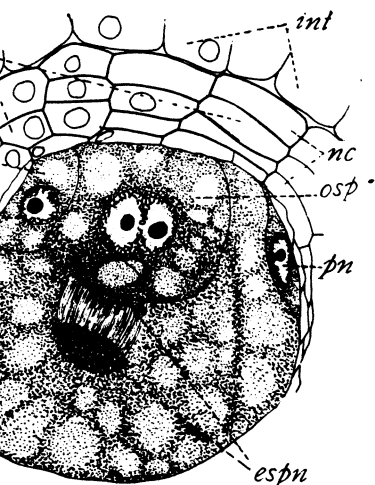
LIST OF WRITINGS REFERRED TO.

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EXPLANATION OF PLATE I.

Abbreviations used: *ax*, axis of the inflorescence; *br*, subtending bract; *cp*, carpel; *em*, embryo; *es*, embryo-sac; *esp*, endosperm; *espn*, endosperm nucleus; *esv*, principal vacuole of embryo-sac; *esw*, wall of embryo-sac; *int*, integument; *mp*, micropyle; *nc*, nucellus; *o*, oosphere nucleus; *osp*, oospore;





pn, peripheral nucleus of the embryo-sac; *psp*, perisperm: *pspn*, nucleus of perisperm cell; *pt*, pollen tube; *st*, stigmatic lobe of carpel; *sy*, synergid; *tp*, tapetal cells.

All figures are camera drawings from microtome sections.

FIG. 1. Longitudinal section of axis, bract, and carpel containing nearly ripe (sixteen-nucleate) embryo-sac. $\times 150$.

FIG. 2. Longitudinal section of an ovule with a four-nucleate embryo-sac. $\times 440$.

FIG. 3. Longitudinal section of an embryo-sac after the entrance of the pollen tube and male nucleus into the egg, and showing the group of nuclei that fuse to form the single endosperm nucleus (those with dotted outlines are in the next section to the one from which the rest of the figure is drawn). $\times 775$.

FIGS. 4-8. A series of sections of a group of nuclei fusing to form the endosperm nucleus, in an advanced stage of fusion and with large fused nucleoli; in *fig. 7* the other contents of the embryo-sac are shown; the figures are numbered in the order of succession of the sections. $\times 775$.

FIG. 9. Longitudinal section of embryo-sac, showing the fusion of male and female nuclei in the egg, and a spindle of the second division of the endosperm nucleus; the upper nucleus of spindle from another section. $\times 775$.

FIG. 10. Part of a tangential section of embryo-sac, showing beginning of the formation of a cell-wall from the cell plate of the dividing endosperm nucleus. $\times 775$.

FIG. 11. Approximately transverse section of the upper end of an embryo-sac like that shown in *fig. 9*. $\times 775$.

FIG. 12. Longitudinal section of the upper end of an embryo-sac through the synergid and an eight-celled embryo. $\times 775$.

FIG. 13. Longitudinal section through the upper end of nucellus of a nearly ripe seed, containing an embryo of twenty or more cells surrounded by endosperm. $\times 775$.

FIG. 14. Longitudinal section through axis, bract, and a nearly ripe fruit; endosperm and embryo practically as in *fig. 13*. $\times 75$.

FIG. 15. Part of section of carpel, integument, and perisperm from *fig. 14* (at the left from the base of the embryo-sac). $\times 775$.