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## THE EMBRYO-SAC AND EMBRYO OF NELUMBO.*

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Since the publication of Lyon's studies on Nelumbo and Cook's work on Castalia and Nymphaea, the systematic position of the Nymphyaeaceae has again become a prominent question. Owing to the variety of opinions held in regard to the classification of this group, it was thought desirable to continue the study of the life history of Nelumbo lutea, although this plant has been described more or less completely a number of times.

Material was collected during July and August, 1902, in Sandusky Bay, near the Ohio State University Lake Laboratory, at Sandusky, Ohio. Flemming's stronger and weaker solutions and chromo-acetic solution were used as killing and fixing agents. On examining the ovules, it was found that in most cases the tissues had not been properly penetrated by these fluids. In the summer of 1903 more material was collected, near the place already mentioned, which was killed and fixed in Kleinenberg's picro-acetic and picro-sulphuric solutions and was found to be preserved in good condition. The ovules were passed through the alcohols, imbedded in paraffin, and serial sections were cut 10–12 mic. thick. For staining several reagents were used : Delafield's heamotoxylin, Heidenhain's iron alum haemotoxylin, analin safranin and gentian violet. All of these stains were successful, the last named stain giving the best results. Considerable difficulty was experienced, in that a great many ovules had failed to develop embryo-sacs and others had not been fertilized. Quite a large number of slides were prepared and most of the points mentioned were observed a number of times.

This work was commenced under the direction of Prof. Mel. T. Cook in the De Pauw University Botanical Laboratory and

*Contributions from the Botanical Laboratory of Ohio State University. XVII.

completed under Prof. John H. Schaffner in the Botanical Laboratory of Ohio State University, to both of whom I wish to express my sincere thanks.

In Nelumbo, the carpels are situated in deep pits of the top-shaped receptacle. The stigma and the narrow canal which traverses the short style are covered with glandular cells which secrete a mucilagenous fluid at the time of pollination (Fig. 8). The ovule is suspended from the summit of the ovulary (Fig. 1). Some time before the integuments begin to develop, the growth of the ovule is more rapid at one side (Fig. 1) and anatrophy is well marked when the incipient seed-coats make their appearance. A single hypodermal archesporial cell can be easily distinguished from the adjacent cells by its larger size and more granular cell contents (Fig. 2). Very early in its development, it divides by a transverse wall into an upper cell, the primary parietal cell and a lower cell, the megasporocyte (Fig. 3). By a series of divisions of the primary parietal cell, a large parietal tissue of twelve cells, arranged in three tiers of four cells each, is formed (Fig. 5).

The megasporocyte expands almost equally in all directions. The divisions of the megasporocyte were not followed, but four megaspores are formed. The lowest one becomes the functional megaspore while the others degenerate (Fig. 6). By the further division of the parietal tissue and the epidermis at the tip of the nucellus, the functional megaspore becomes deeply placed in the ovule (Fig. 7.) The nucleus of the functional megaspore now divides into two (Fig. 9), four (Fig. 10), and eight nuclei respectively, producing the eight-celled embryo-sac (Fig. 11). Frequently great irregularities in the development of the embryo-sac were present. In many cases two or more imperfect sacs were observed. Usually there was one complete sac with one or more imperfectly developed sacs. By the appearance of the preparations, it seems that the extra sacs are derived from sister megaspores, rather than from independent megasporocytes (Fig. 15.)

The embryo-sac developes very rapidly and is usually straight. It enlarges principally in the direction of its longer axis, destroying the parietal cells above and encroaching on the ovular tissue below. The antipodals are small (Fig. 11) and usually disappear before the conjugation of the polar nuclei. In only a few instances could any trace of them be found after the polar nuclei had conjugated. The synergids are small. They become slightly enlarged from their original condition, and are elongated transversely to the longer axis of the sac. They degenerate about the time of fertilization or soon after (Fig. 12). The egg becomes quite large and usually is placed considerably to one side of the sac (Fig. 13).

The polar nuclei begin to conjugate about the time the flower opens and the fusion is not complete until after fertilization. In

approaching each other, the lower polar nucleus travels much farther than the upper one and the fusion usually occurs near the egg or even in contact with it (Fig. 13). Quite a number of examples of a triple fusion were found. In many of the preparations in which the pollen tube had appeared, two of the nuclei were about the same size while the third one was smaller (Fig. 14). Several other examples were found where there were three conjugating nuclei, almost equal in size and similar in appearance even before the pollen tube had appeared. It seems that in the first instance where fertilization had occurred, the small nucleus of the three conjugating nuclei represents the second male cell and that there is here a true case of what has been called double fertilization; while in the second instance the conjugating nuclei were embryo-sac nuclei, since the pollen tube had not yet entered the sac.

Soon after the eight-celled sac is formed it begins to grow very rapidly in the direction of the longitudinal axis of the ovule. The cells of the tissue below the antipodal region of the sac become greatly enlarged and between them are large intercellular spaces. Usually there is a single row of cells very rich in cytoplasm, which becomes very prominent in the preparations because of its deep stain. This row extends downward from the base of the sac toward the lower end of the ovule (Fig. 14). The cells surrounding this axial row become much larger in size and then disintegrate, leaving a large space filled with thin cytoplasm (Fig. 18). The cytoplasm of the embryo-sac extends down to the axial row of cells (Fig. 14). These central cells are present some time after the adjacent cells have disappeared, and since they are rich in cytoplasm, it seems that they serve as a conducting passage for food from the lower ovular tissue to the cytoplasm above, which in turn carries the food to the egg apparatus. After fertilization the axial row begins to degenerate and then disappears entirely, leaving a cavity reaching far back into the tissue of the ovule (Fig. 21) Sometimes the nuclei of the axial row of cells become active and divide (Fig. 20), and are afterwards found massed together in the lower part of the cavity after their walls have disappeared (Fig. 21). The cavity formed by the disintegration of the cells below the antipodal region enlarges greatly while the embryo is developing and into it the two basal lobes of the embryo are rapidly extended, their outer surface lying in contact with the walls of the cavity.

The first division of the definitive nucleus occurs about the time of the formation of the two-celled embryo and a very delicate wall is formed between the two daughter nuclei which divides the embryo-sac into two chambers. A division of one of the two endosperm nuclei thus formed takes place and a second wall is formed across the sac so that there are then three superposed

compartments (Fig. 24). It seems that all three of the daughter nuclei continue to divide until the whole sac is filled with endosperm extending far down into the space formed by the dissolution of the tissue of the ovule below the base of the embryo-sac (Fig. 27). The development of the endosperm, after the three-celled stage, begins at the upper end, but there is no large vesicular cell developed at the lower end of the sac, as Cook reported for Castalia odorata. At first the endosperm cells are quite large, but as the division continues the cells become much smaller, walls continue to be formed between the dividing nuclei until the endosperm is fully developed, no free cell formation taking place, so far as observed, at any stage of the process.

The history of the embryo as followed is the same as reported by Lyon. After fertilization, the oospore continues to occupy the same position as the oosphere and it enlarges somewhat before it divides (Fig. 13). Although no two-celled embryo was observed, it is evident that the first division of the oospore is by the formation of a transverse wall. Then by the formation of a longitudinal wall in each of the two cells, a quadrant is formed (Fig. 23). Although this is the typical course of development, very frequently the divisions are different. The lower cell often divides by a transverse wall, thus forming a tier of three cells in the proembryo (Fig. 22). By the formation of longitudinal walls in the quadrant, the embryo passes into the octant stage. In case of a more irregular development, the three cells of the embryo arranged in a row, divided by longitudinal walls, making a six-celled embryo (Fig. 25). Whether the early development is typical or irregular, a series of divisions follows by which a spherical embryo of several hundred cells is formed (Figs. 26–29.)

No suspensor cell is present; so the young embryo lies against the nucellus at the micropylar end and is almost surrounded by endosperm tissue (Fig. 27). When the spherical embryo has reached its maximum growth, it becomes flattened at the outer end by the development of a collar-like ridge extending about two-thirds of the way around (Figs. 30, 31 and 32). This is followed by the outgrowth of a small protuberance from the flattened side about parallel with the apex of the ovule. After the formation of the crescent-shaped ridge, the development continues at the opposite side, giving rise to the two "cotyledonary" lobes of the embryo (Fig. 33). The two lobes grow downward very rapidly outside the endosperm, the tissue of the ovule rapidly disappearing before them. In the meanwhile, the endosperm has formed a sac-like mass of tissue around the embryo and extends down into the cavity of the embryo-sac to the disorganizing tissue below. In the meantime the growth of the plumule has been very slow, being a dome-shaped projection of tissue occupying a central position between the lobes but to one side of the axis of

the embryo (Fig. 33.) Both the cotyledonary ridge and the incipient stem tip come from the outer end of the embryo and probably represent terminal structures, but the stem tip represents the more central mass of cells. On account of the spherical condition of the embryo it is practically impossible to trace the origin of any set of cells which appear at the outer end of the more mature embryo, and the cotyledonary ridge may be lateral.

After the cotyledonary lobes have become greatly enlarged the incipient plumule continues its development. It grows downward, forcing its way into the center of the mass of endosperm which lies between the two cotyledonary lobes. The first leaf and stem tip develop side by side from the terminal mass of cells in the protruberance. The leaf arises on the side opposite the cotyledonary ridge (Fig. 34). The second leaf arises on the side of the plumule opposite the first and develops more slowly than the first leaf. The comparative growth and manner of development may be seen from Figs. 35–40. The radicle has its origin at the base of the plumule. It is a vestigial organ and does not develop on the sprouting of the seed. It can only be seen at a late stage of development and is enclosed by an outgrowth from the surrounding tissue (Fig. 40).

The homology between the development of the embryo of Nelumbo and other monocotyledonous embryos is very striking in many respects. In its early development the embryo of Nelumbo is very similar to those of Aglaonema, Diffenbachia, and Lysichiton. In these forms the oospore does not cut off a suspensor cell but builds up a spherical embryo as is formed in Nelumbo. In the forms described by Campbell, the egg may segment, first, by two transverse divisions before any vertical division, or a regular quadrant may be formed, which is likewise true in Nelumbo. The development of the "cotyledonary" ridge shows a striking resemblance to the hypocotyledonary expansion of various Helobiae. The mature embryo may thus be compared with those of Halophila, Ruppia, Zostera, and Phyllospadix. In these forms there is a broad expansion of tissue below the plumule. In Halophila, Ruppia, and Zosteria, the hypocotyledonary lobe is continuous, while in Phyllospadix the structure is somewhat lobed if one may judge from the published figures and descriptions. The plumule with the so-called cotyledon is attached near the center. It is probable that the broad two-lobed expansion of tissue in the Nelumbo embryo commonly known as the cotyledons, is a true hypocotyledonary body as in the forms just mentioned. It bears a rather close resemblance to the hypocotyledonary expansion of Phyllospadix. If such a comparison is correct, the first leaf of Nelumbo is homologous with the so-called cotyledon in Ruppia and Phyllospadix, and the plumule and cotyledon of these forms may arise as terminal structures side by side, as do the plumule

and "first leaf" of Nelumbo and the similar structures of the Araceae mentioned above. A careful study of all the Helobiae with "macropodous" embryos, as well as other monocotyledonous types, will probably be necessary before a definite conclusion can be reached.

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## EXPLANATION OF PLATES.

The figures were drawn with the aid of an Abbe camera and the following combination of oculars and objectives: Figs. 2–7, 9–13, 20, 22, 23, 25 and 26, Bausch & Lomb $\frac{1}{12}$ obj., Leitz oc. 4; Figs. 15, 28–32 and 34, Leitz $\frac{1}{7}$ obj. and oc. 4; Figs. 14, 16–19 and 33, Leitz $\frac{1}{7}$ obj. and oc. 2; Figs. 21, 24 and 27, Bausch & Lomb $\frac{1}{12}$ obj. and oc. 2.

## PLATE XVI.

Fig. 1. Young carpel before the integuments appear on the ovule.

Fig. 2. Nucellus with archesporial cell.

Fig. 3. The megasporocyte and primary parietal cells.

Fig. 4. Megasporocyte and three parietal cell.

Fig. 5. Megasporocyte and twelve parietal cells, six of which show in one plane.

Fig. 6. The four megaspores, the lowest enlarging as the functional megaspore.

Fig. 7. The nucellus with cap of tissue developed from the epidermis.

Fig. 8. Carpel with two celled embryo-sac in the ovule, showing the stylar canal lined with glandular cells.

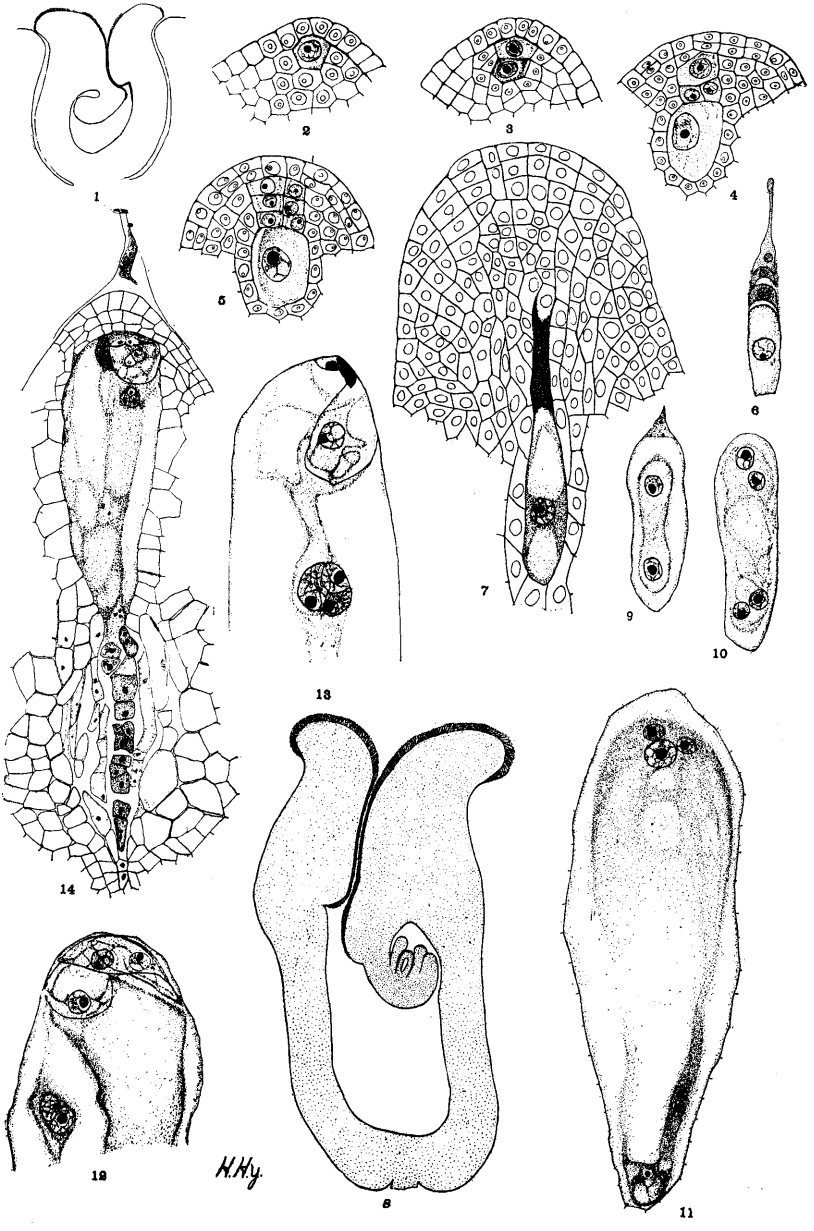
Fig. 9. Two-celled embryo-sac with remains of the three potential megaspores.

Fig. 10. Four-celled embryo-sac.

Fig. 11. Eight-celled embryo-sac, showing conjugation of the polar nuclei and disorganization of the antipodals.

Fig. 12, Upper end of embryo-sac, showing the oosphere, synergids and conjugating polar nuclei.

Fig. 13. Upper end of embryo-sac, showing tripple fusion, the egg, remains of pollen tube, and synergid.




YORK on "Nelumbo."

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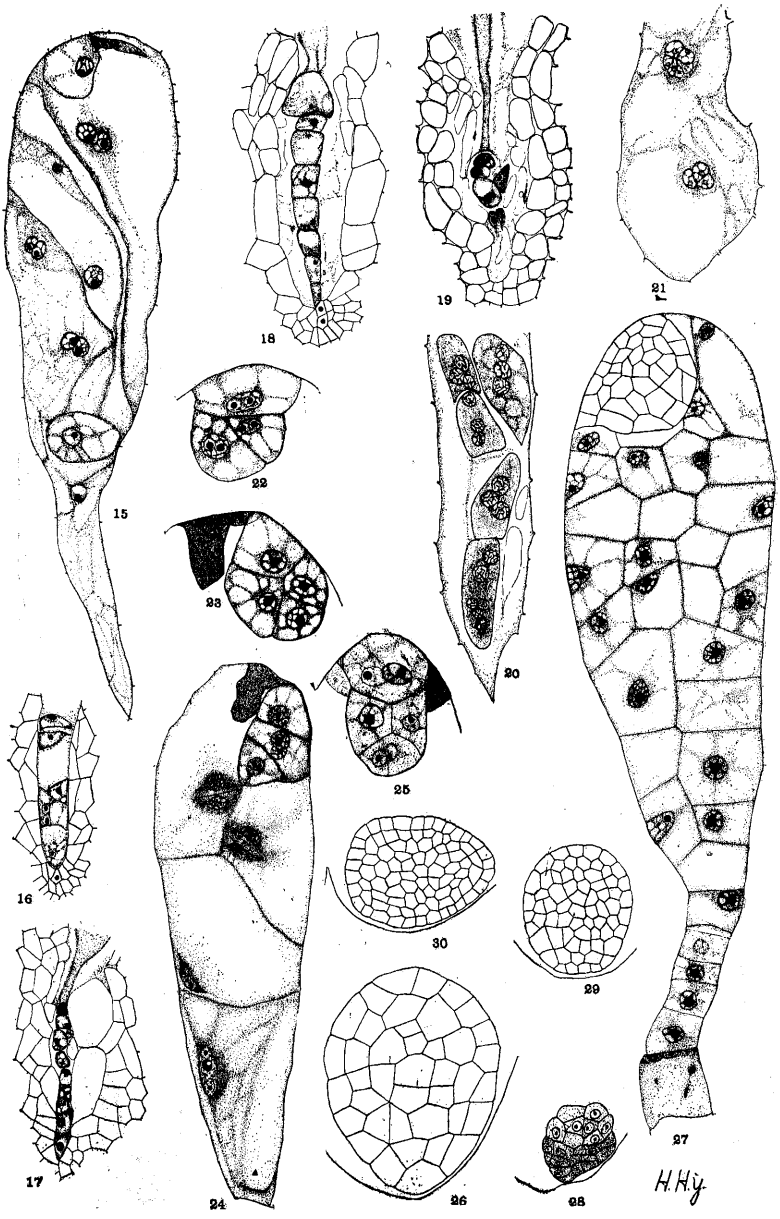


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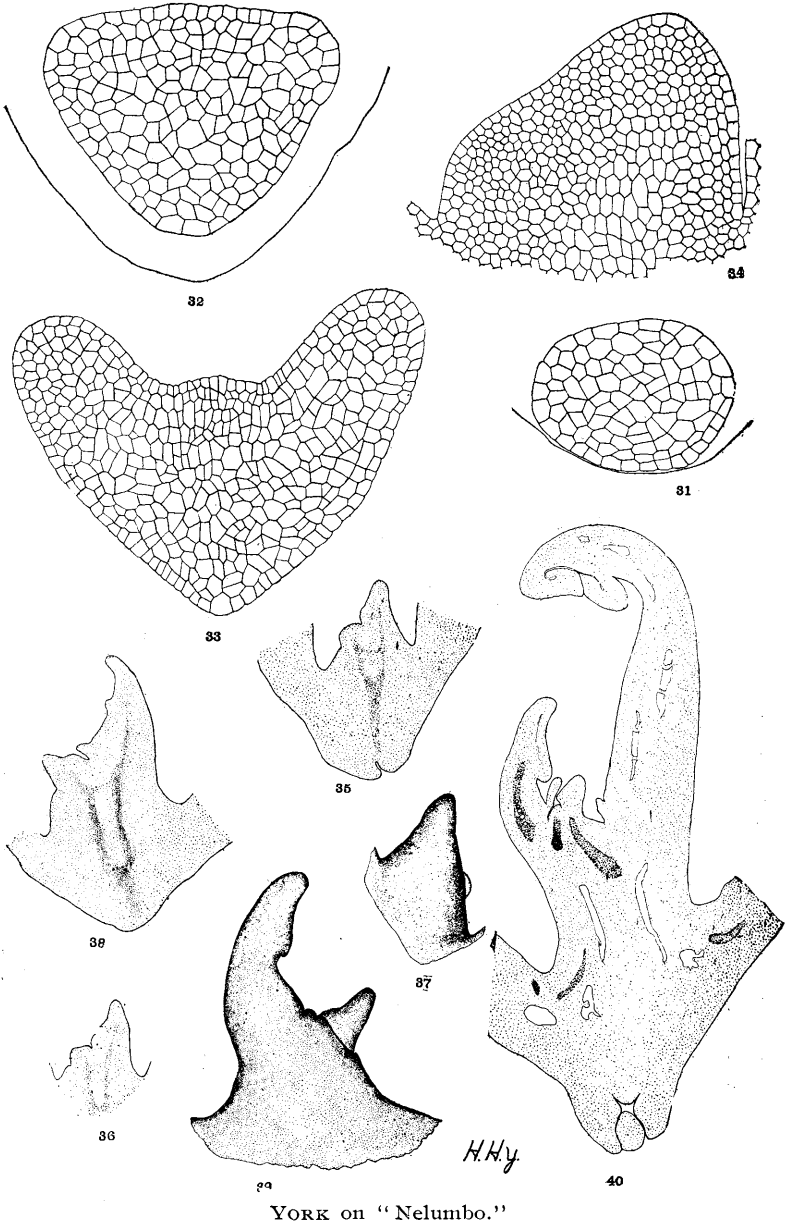


YORK on "Nelumbo."

Fig. 14. Embryo-sac with fusion of the gametes and triple fusion below. Below the sac appears the beginning of the cavity formed by the breaking down of the ovular tissue, with axial row of glandular cells.

PLATE XVII.

Fig. 15. Abnormal embryo-sac, showing three separate nuclear fusions.
Fig. 16. Early stage in development of the axial row of cells.
Fig. 17. Axial row of cells further developed.
Fig. 18. Perfectly developed axial row, surrounded by a cavity formed by the breaking down of the cells of the surrounding tissue.
Fig. 19. The cavity below the sac, with irregularly developed axial row.
Fig. 20. Basal cavity below the embryo-sac, with irregularly developed axial row.
Fig. 21. Basal cavity with groups of nuclei massed together.
Fig. 22. Three-celled embryo.
Fig. 23. Four-celled embryo and pollen tube.
Fig. 24. Four-celled embryo with pollen tube and early formation of endosperm.
Fig. 25. Six-celled embryo with remains of pollen tube and one synergid.
Fig. 26. Section of young spherical embryo.
Fig. 27. Endosperm and embryo somewhat flattened by being in contact with the wall of the ovule.
Fig. 28. Section of embryo, showing difference in staining between the basal and outer parts.
Fig. 29. Section of spherical embryo.
Fig. 30. Section of embryo, showing the flattening due to the development of the incipient "cotyledonary" ridge.

PLATE XVIII.

Fig. 31. Section of the flattened embryo further developed.
Fig. 32. Section of embryo, showing the two sides of the cotyledonary ridge.
Fig. 33. Section of embryo, showing the beginning of the dome-shaped protuberance between the cotyledonary lobes.
Fig. 34. Section of the dome-shaped protuberance, or the incipient plumule, showing terminal origin, side by side, of first leaf and stem tip.
Fig. 35. Outline section of embryo, showing incipient plumule.
Fig. 36. Outline section of embryo, showing the plumule further advanced.
Fig. 37. Surface view of the incipient plumule a little older than in Fig. 36.
Fig. 38. Outline section of plumule, showing the beginning of the development of the leaf blade.
Fig. 39. Surface view of a still older plumule than Fig. 38.
Fig. 40. Outline of section of embryo, showing the position of the first three leaves.