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DEVELOPMENT OF THE EMBRYO SAC AND EMBRYO OF BATRACHIUM LONGIROSTRIS.*

LUMINA COTTON RIDDLE.

Batrachium longirostris (Godr.) F. Schultz is one of the white water Ranunculaceae. By many authorities it is included in the genus Ranunculus but Britton in his Manual separates them into two genera on the character of the achene, that of Batrachium being transversely wrinkled. He distinguishes B. longirostris from B. divaricatum and B. trichophyllum with which the first is often confused, by the length of the beak of the achene. Prantl in his classification of the Ranunculaceae in the "Pflanzenfamilien" includes Batrachium in the Genus Ranunculus but divides the genus into seven sub-genera placing Batrachium in the third, Marsypadenium. This sub-genus he further divides into five super-species of which the first is Batrachium and the second Xanthobatrachium. Under this he places Ranunculus delphinifolius Torr. (R. multifidus Pursh) making the following distinctions:

Batrachium, Honigbl. weisze, Nektarium in einer Grube; Fr. runzelig.

Xanthobatrachium, Honigbl. gelb, Nektarium oefters mit seitlichen Lappen; Fr. nicht runzelig.

The writer had the privilege of studying three dozen excellently prepared and carefully selected slides of Ranunculus delphinifolius and some close resemblances were noted to Batrachium longirostris which will be referred to later in the discussion.

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* Contributions from the Botanical Laboratory of the Ohio State University, XX.

The subject has proved very interesting because of the large number of closely related plants which have already been studied and the accumulated literature which was easy of access either in the original publications or through brief reviews and abstracts. The writer became thoroughly familiar with her own material before making any comparisons in order to avoid having preconceived ideas of what ought to be expected.

Material for study was collected at Licking Reservoir in 1901 by Professor J. H. Schaffner and at Sandusky Bay, Lake Erie in the summers of 1902–1903 by the writer. The usual methods of killing, imbedding, sectioning and staining were employed. Thickness of the sections varied from 8–20 microns the older material being cut thickest.

The development of the carpel is almost identical with that of Ranunculus abortivus as described by E. A. Bessey (1). The rounded pyramid of the receptacle first appears from which numerous conelike projections arise (Fig. 1). Those nearest the base develop into the stamens. Near the summit of the receptacle the arrangement of parts is spiral but approaches the cyclic among the outer stamens. The number of stamens found by actual count varied from 17–21 while the number of carpels was approximately half as great. A lamina or flap develops from the distal side of the young carpel enveloping the inner portion which begins to grow away from the receptacle (Fig. 2). This lamina thins out as it meets the axillary placenta and traces of the integuments can be seen (Fig. 3). As the nucellus develops it describes an angle of 180° and when the gynoecium is mature the tip of the nucellus is directed downward while the opening of the micropyle is towards the receptacle. (Fig. 4). Only a single integument develops. The outer cells of the integument nearest the placenta are large and glandular and seem to function in conducting the pollen tube to the micropyle (Fig. 5). After the closing of the carpel an elongated style develops having finger like, glandular cells on the stigma which afford a lodging place for the pollen.

The microsporangium develops a plate of four or five hypodermal archesporial cells which divide by periclinal walls to form primary wall and primary sporogenous cells (Fig. 6). The primary wall cells then divide and the inner cells develop into the tapetal layer (Fig. 7). The outer cells may divide once or twice forming two or three distinct layers between the epidermis and the tapetum (Fig. 8). The layer next to the epidermis forms the endothecium with thickenings in the angles of the walls, exactly as were found in the endothecium of R. delphinifolius. Further divisions by anticlinal walls occur in both tapetal and wall layers and later the tapetum becomes binucleate by karyokinesis, without forming walls, instead of by fragmentation of the nucleus (Fig. 10–11).

The primary sporogenous cells continue to divide and apparently give rise to the axial layer of tapetal cells. The origin of the peripheral layer from the wall cells and the axial layer from the sporogenous, seems to accord with their origin in R. delphinifolius, both from examination of the slides and from the observations recorded in Coulter's Life History of Ranunculus (3). This refers the origin of the tapetum to the primary archesporium instead of referring the axial layer to the inner tissue of the androecium. Frequently a splitting was observed between the sterile wall layers and the tapetum but quite as often it could be seen between the tapetum and the sporogenous tissue (Fig. 9), and sometimes seemed separated from both. As the stamen matures the cells are forced past each other and misplaced, making it extremely difficult to determine the origin of the tapetum unless a careful study of a series of stages has been made.

The primary sporogenous cells then divide a number of times so that a central cross section shows sometimes as many as twelve microsporocytes (Fig. 8), while a longitudinal section shows from three to four rows (Fig. 11). The tapetal layer does not disintegrate early but is still quite well organized after the separation of the tetrads.

The microsporocyte divides to form four microspores (Fig. 12–13). No cases of more were found as has been reported in Ficaria (4) and other Ranunculaceae but in some cases the separation is incomplete. This is shown in one of the pollen grains in Fig. 25. In many cases the microspore never germinates (Fig. 14), in fact scarcely one to four. The tube nucleus and the generative nucleus lie close together. Just before pollination the generative cell becomes lenticular and divides to form the sperm nuclei (Fig. 15). These are not readily seen because of the abundant starch granules, the deep color which the pollen grain takes, and the crowding of the three nuclei. In the slides of R. delphinifolius there were found similar cases of two male nuclei before the germination of the pollen tube.

Before the lamina has entirely enclosed the nucellus, the archesporium can be distinguished (Fig. 16–17). The occurrence of two or more archesporial cells is not at all unusual and in many cases the struggle for supremacy results disastrously for all concerned. The remains of other archesporial cells can almost always be seen around the megasporocyte. There is no evidence of the cutting off of any primary parietal cell but the reduction division occurs at once. The lower of the two cells divides first and in many cases the division of the upper seemed never to pass beyond the formation of the spindle (Fig. 18–19). This is not unlike the development of the megaspores as reported by Mottier (8). In a few cases there seemed to be two complete sets of megaspores but the writer did not observe any twin embryo sacs though it is

quite reasonable to expect to find them where the archesporium is so commonly multicellular. But in Batrachium, or at least in the material collected for study, the number of megaspores which divide and the number of embryo sacs that develop embryos seem very few. In many cases only a few of the ovules matured, perhaps two or three, as was seen in the ripened carpels and also in the material sectioned.

The functional megaspore passes through the two, four and eight celled stages and the nuclei arrange themselves normally (Fig. 20–23). The two synergidae stain much darker than the egg cell and the antipodals than the polar nuclei. After the conjugation of the polar nuclei the resulting definitive nucleus was very readily distinguished by its enormous size (Fig. 24). While the polar nuclei are approaching each other the antipodals enlarge and seem to take on definite walls, and the embryo sac begins to widen below. At the time of fertilization the antipodals are situated in an elevated crater-like pouch (Fig. 24). The lengthening of the embryo sac is greater on the distal than on the proximal side and extends beyond the chalaza near where the antipodal pouch is situated. The antipodals are typically those of Ranunculaceae resembling almost exactly those of R. delphinifolius. The nuclei sometimes divide (Fig. 27) but usually only three were present. They persist for a long time staining quite deeply and can be distinguished even in quite mature ovules (Figs. 31 and 33).

The entrance of the pollen tube into the embryo sac and the actual phenomenon of fertilization was not observed in Batrachium. The pollen tube was traced well down into the stigmatic tissue, found emerging in the cavity of the ovulary and again seen among the glandular cells of the shorter integument and traced into the micropyle. One might expect anything since Overton (10) reported parthenogenesis in Thalictrum purpurascens, Coulter (3) found the second sperm cell much disorganized at the time of its discharge in Ranunculus septentrionalis and Miss Thomas (14) discovered double fertilization occurring in Caltha palustris. Double fertilization is also reported by Nawaschin (11) for Delphinium elatum and by Guignard (7) in Ranunculus flammula, R. cymbalaria, Anemone nemorosa, Helleborus foetidus, Nigella sativa and N. damascena. In Batrachium, the fact that so few ovules develop and the traces of the pollen tube found in those that do, seems to set aside entirely the occurrence of parthenogenesis. The peculiar pale nucleus shown in Fig. 26 may be the second sperm nucleus. In one slide there was what might be taken for double fertilization, but the evidence was so unsatisfactory that the writer prefers to leave the question unsettled.

After fertilization the oospore begins to elongate and soon divides into a two celled embryo (Fig. 26). Before the first longitudinal division there is evidently another transverse one (Fig. 28). These two suspensor cells later divide in both directions varying considerably in method and order of divisions (Figs. 30 and 35.) The suspensor is short and does not seem to function long especially after the formation of the endosperm. The dermatogen is cut off by a series of periclinal walls from the octant and later divides by anticlinal walls. The cotyledons are small compared to the hypocotyl and the embryo is straight (Fig. 37). In the literature consulted there were but few of the Ranunculaceae in which the mature embryo was described. In Delphinium exaltatum Miss Dunn (15) finds a small heart shaped embryo with rudimentary suspensor and short hypocotyl. This seems to be the typical embryo in the Ranunculaceae.

The definitive nucleus divides immediately after fertilization and when the four celled embryo was found there was a single layer of endosperm completely lining the embryo sac (Fig. 29). These nuclei were not enclosed in cell walls but showed faint radiations (Fig. 31). In later divisions however, walls are formed and the entire embryo sac is filled with endosperm cells of varying shapes and sizes. Those in the antipodal region are large and rounded, those near the embryo wedge shaped or rhomboidal, and the peripheral layer is flattened. (Fig. 32.) The cells store up an abundance of starch (Fig. 34) which nourishes the young embryo. The cells are arranged in a radiate manner and as the young embryo enlarges the surrounding cells are emptied of their store (Fig. 33).

The inner wall of the carpel is made up of a layer of elongated cells which are longest in the plane at right angles to the axis of the carpel. Next to these cells there are four or five layers elongated at right angles to the first and rather crescentic (Fig. 38). As the ovule matures these cells develop thick perforate walls while the cells beneath the epidermal layer become somewhat separated to form a delicate spongy tissue (Fig. 39). These cells seem to contain some starch. The thickened cells make it a difficult matter to section the mature ovule so as to obtain good sections of the fully developed embryo.

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DESCRIPTION OF PLATES.

For the drawings a Bausch and Lomb camera lucida and microscope were used with oculars 2, 1 and ½ and objectives 2-3, 1-6 and 1-12; Figs. 1, 8-11, 16-24, 26, 28, 30, 34, 36, 38 and 39 were drawn under the 1-inch ocular and the 1-6 objective; Figs. 2, 3 and 37 with the 2-inch ocular and the 1-6 objective; Figs. 4, 5 and 25 with the ½-inch ocular and the 2-3 objective; Figs. 6, 7 and 14 with the ½-inch ocular and the 1-6 objective; Fig. 12 with the ½-inch ocular and the 1-12 objective; Fig. 13 with the 1-inch ocular and the 1-12 objective; Figs. 29 and 33 with the 2-inch ocular and the 2-3 objective.

Fig. 1—Section of the receptacle showing two stages in the development of the carpels.

Fig. 2—Section of young carpel showing the lamina.

Fig. 3—Section of carpel showing tip of carpellary leaf folded to enclose the nucellus.

Fig. 4—Section of carpel showing cavity closed and integument well developed. Megasporocyte divided.

Fig. 5—Longitudinal section of carpel showing elongating style and stigma and the micropyle.

Fig. 6—Section of young androecium showing division of archesporium into wall cells and sporogenous cells.

Fig. 7—Wall cells divided to form parietal tapetum. Outer wall cells beginning to divide.

Fig. 8—Delayed division in one primary wall cell. Sporogenous cell dividing to form axial tapetum.

Fig. 9—Central section showing tapetum fully developed and a splitting between the peripheral tapetum and the sporogenous tissue.

Fig. 10—Section near tip of stamen showing binucleate tapetum and spindle.

Fig. 11—Longitudinal section of more mature stamen showing the same.

Fig. 12—Microsporocyte dividing.

Fig. 13—Tetrads.

Fig. 14—Disintegrating microspore and two celled pollen grain.

Fig. 15—Mature pollen grains showing tube and two sperm nuclei.

Fig. 16—Nucellus and archesporial cell.

Fig. 17—Double archesporium.

Fig. 18—Megaspores.

Fig. 19—Nucellus showing megaspores and division in epidermal layer.

Fig. 20—Two-celled embryo sac.

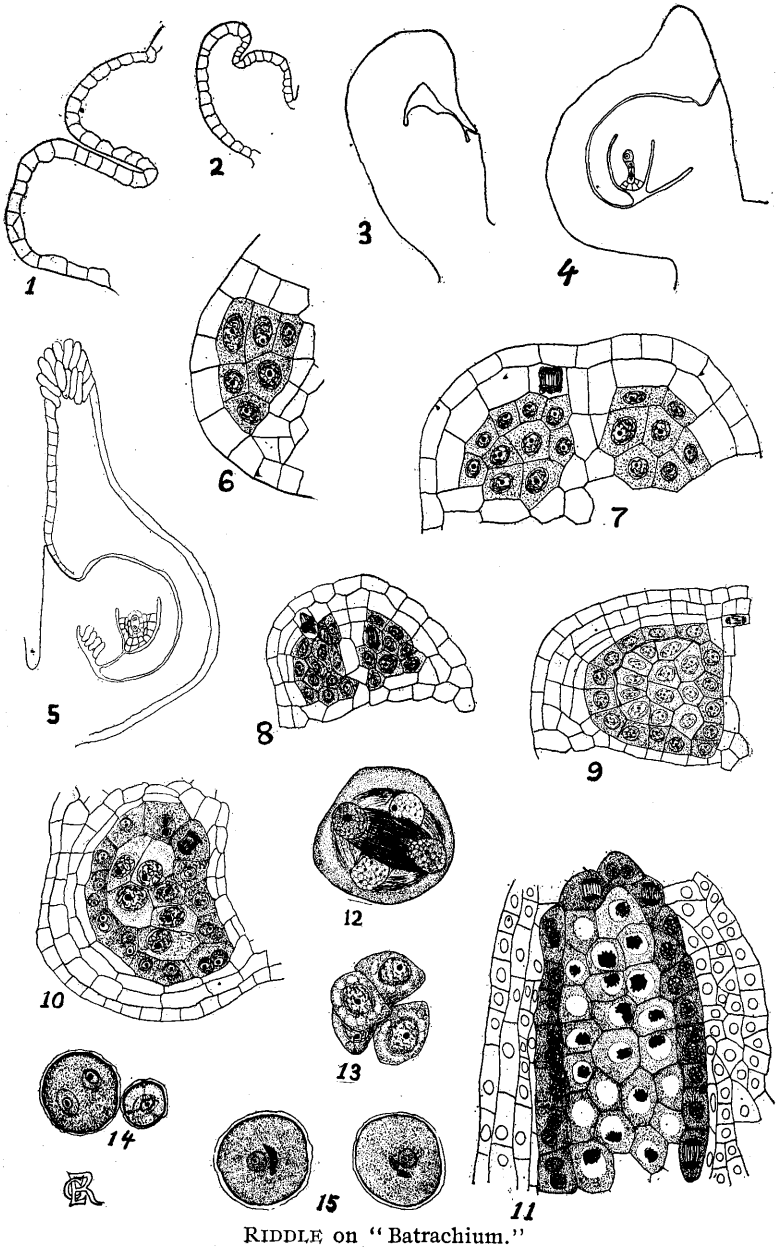
Fig. 21—Four celled embryo sac.

Fig. 22—Eight celled embryo sac.

Fig. 23—Eight celled embryo sac showing synergidae and oosphere, conjugating polar nuclei and antipodals.

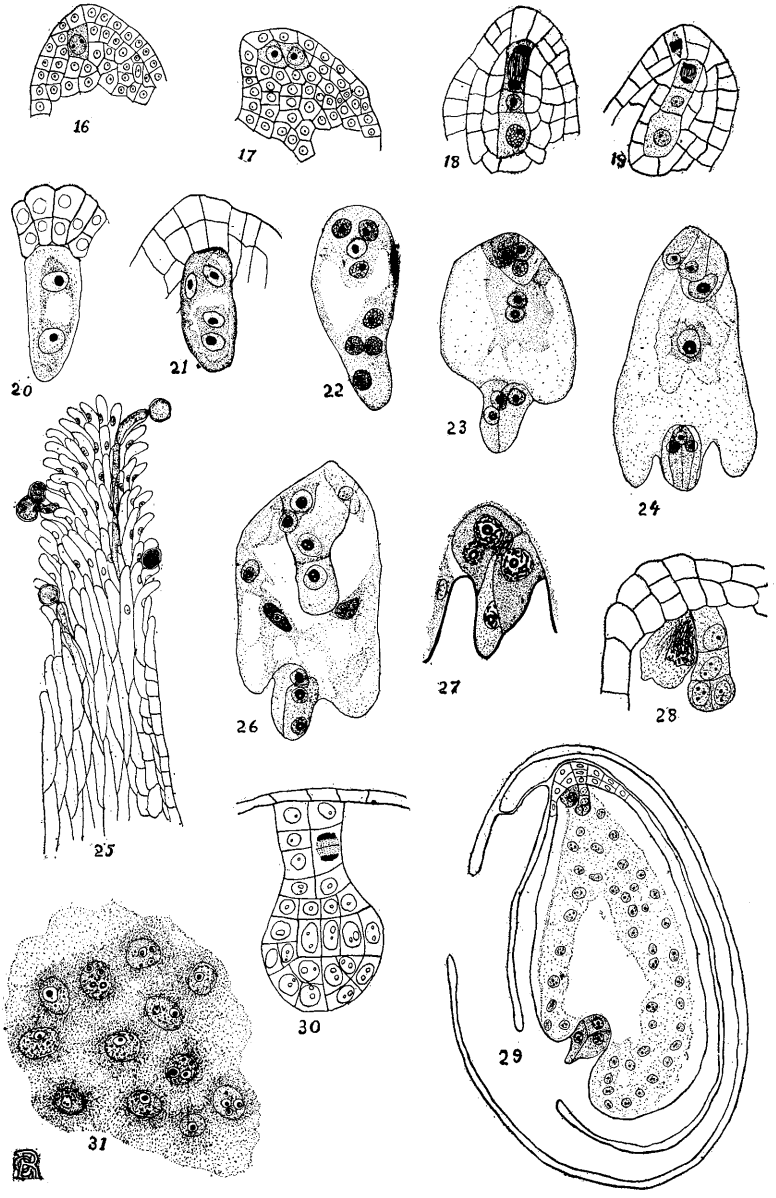
Fgi. 24—Seven celled embryo sac showing egg apparatus, definitive nucleus and antipodals. Embryo sac enlarging in antipodal region.

OHIO NATURALIST. *Plate XXII.*



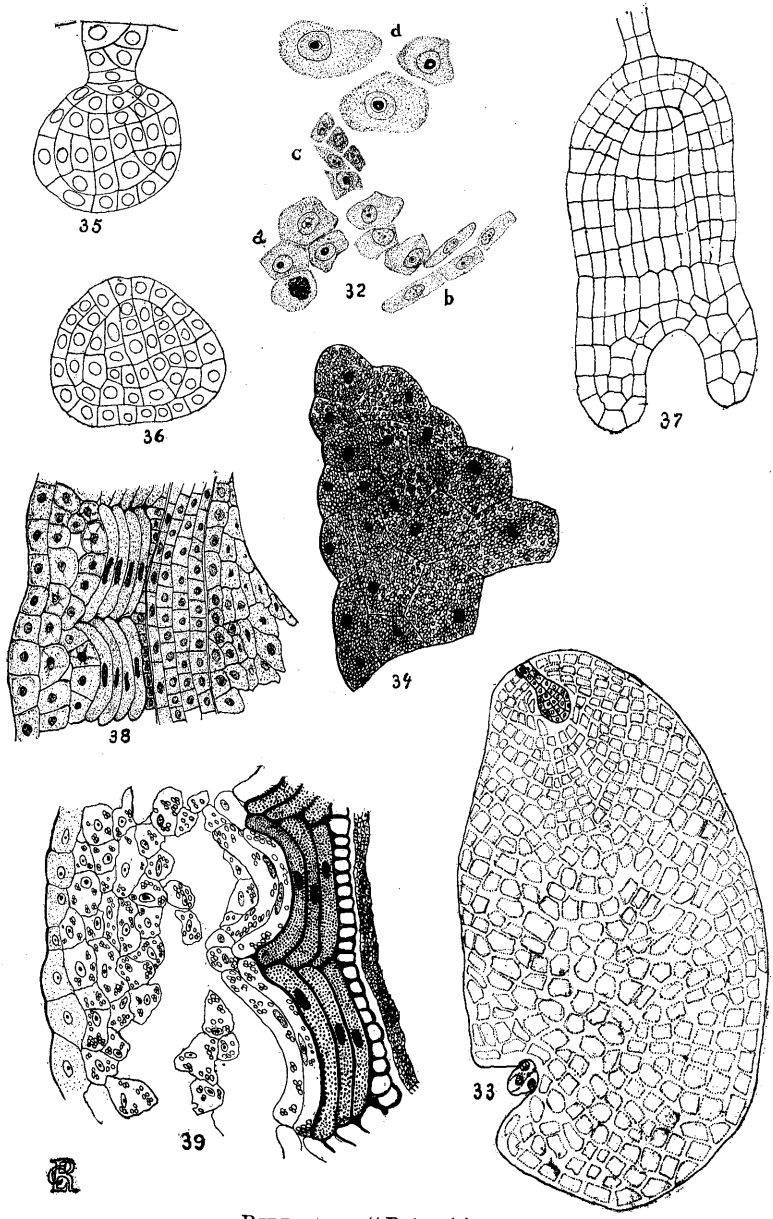
RIDDLE on "Batrachium."

OHIO NATURALIST. *Plate XXIII.*



RIDDLE on "Batrachium."

OHIO NATURALIST. *Plate XXIV.*



RIDDLE on "Batrachium."

Fig. 25—Stigma showing germinating pollen grains.
Fig. 26—Young two celled embryo and endosperm. Possibly the remains of the second generative nucleus and pollen tube.
Fig. 27—Antipodals with five nuclei.
Fig. 28—Four celled embryo and persisting synergid.
Fig. 29—Single integument, nucellus and embryo, the remains of synergid, endosperm, and three nuclei of the antipodals.
Fig. 30—More mature embryo showing dermatogen layer and division in suspensor cells.
Fig. 31—Endosperm from sac of a four celled embryo showing faint radiations.
Fig. 32—Endosperm cells showing variation in shape and size. a-average cells; b–peripheral cells; c–cells near embryo; d–in antipodal region.
Fig. 33—Embryo sac showing the arrangement of endosperm cells and the remains of antipodals.
Fig. 34—Closely packed endosperm filled with starch. From an embryo sac containing a mature embryo.
Fig. 35—Embryo showing rather diagonal division of the suspensor.
Fig. 36—Section of older embryo cut slightly diagonal but showing the beginning of cotyledons.
Fig. 37—Nearly mature embryo showing cotyledons, dermatogen, calyptrogen, pleurome and traces of the suspensor.
Fig. 38—Crescent shaped cells of inner wall of carpel and elongated cells at right angles to the first; integument and nucellus.
Fig. 39—Section of a mature carpel showing these crescent and elongated cells hardened and perforate.

LEAF EXPANSION OF TREES AND SHRUBS. No detailed record of the expanison of leaves was kept for the spring of 1905, but it was noted that the beginning of leafing was seven days earlier than in the spring of 1904. Syringa vulgaris L. came first on March 25, followed by Salix babylonica L., Larix laricina (Du R.) Koch, and L. decidua Mill. Some trees were comparatively earlier than last year and the order of succession was changed in quite a number of species. This was probably due in part to the more uniform advancement of warm weather. Morus rubra L. and Chionanthus virginica L. were the last trees to leaf, coming out on May 6.

J. H. S.