THE DEVELOPMENT OF THE EMBRYO-SAC AND EMBRYO OF AGROSTEMMA GITHAGO.*

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Agrostemma githago L. was selected as a plant for comparison with Claytonia virginica, they usually being placed in the same order but in different families.

The ease with which the material can be prepared and the regularity of the development of the embryo-sac and embryo make it a very desirable plant for study.

METHODS.

The material was killed and fixed in Flemming's solution and in chromo-acetic, passed through the alcohols, imbedded in paraffin, cut on a Minot microtome and stained in aniline safranin and gentian violet, and also in Hadenhaine's haematoxylin and iron alum. For the development of the embryo-sac the safranin and gentian violet combination was most desirable, but for the development of the embryo either stain was satisfactory.

The young buds were killed entire, but the sepals and petals were removed from the older ones. In the very youngest stages it is desirable to cut the sections thin, but when the embryo-sac has reached the eight-celled stage it must be cut thick, otherwise important structures may be lost. The orientation was very simple; by cutting the ovaries transverely the sac will be cut longitudinally.

The archesporium may originate as one, but more frequently as two or three, hypodermal cells (Figs. 1, 2, 3). These increase in size (Fig. 2), and one eventually absorbs the others. Many specimens were examined, but in all cases only one cell developed into an embryo-sac. This single archesporial cell now divides by transverse divisions into three cells, of which the lowest develops into the functional megaspore (Fig. 4.) The two (Fig. 5), four (Fig. 6), and eight (Fig. 7) celled stages of the embryo-sac are formed in the usual manner. The sac increases in size very slowly up to this time, and the nuclei of the sac are of practically the same size (Figs. 6 and 7), except that antipodals are slightly smaller than the other nuclei.

After the formation of the megaspore the ovule begins to enlarge, and a very pronounced growth of the nucellus and integuments on the micropolar side projects from the micropyle. The embryo-sac is thus left deeply imbedded in the nucellus (Figs. 4 and 23). By the time the sac has reached the two-celled stage the nucellus shows two well-defined zones (Figs. 5 and 23). The inner zone surrounding the sac is made up of thin-walled cells,

* Contribution from the Botanical Laboratory of Ohio State University. XI.

which degenerate for the enlargement of the sac which occupies the entire inner zone in the eight-celled stage (Fig. 7). The walls of this inner zone were so delicate that it was difficult to get good preparations of the eight-celled stage. The outer zone is made up of thicker walled cells, which are more permanent and which are in more or less regular rows, which radiate from the inner zone. The inner zone is connected with the micropolar end of the ovule by two or three rows of elongated cells, which degenerate to form the path for the pollen tube (Figs. 5 and 23). After fertilization the part of the nucellus projecting through the micropyle degenerates and the integuments come together at that point.

Lyon* describes an enlargement of the ovule similar to that in Euphorbia corollata, except that there is no zone-like structure, and the cells which break down for the passage of the pollen tube are larger and looser than the surrounding tissue.

After the conjugation of the polar nuclei the sac enlarges on one side and at right angles to its long axis (Fig. 24). The endosperm nucleus passes down into this pocket, divides and eventually forms a peripheral endosperm (Fig. 18). One case was observed where the endosperm nucleus had failed to divide, although the embryo was in its five-celled stage. At the lower end of this newly formed pocket a mass of endosperm is formed, which probably hastens the absorption of the nucellus at that point (Fig. 19). At this time the egg has enlarged considerably; the synergids remain about the same size and disappear very early; in only one case was a synergid observed to persist until after the formation of the first transverse wall in the embryo. By the enlargement of the sac in the new direction the antipodals are left in a small pocket (Fig. 24*a*); they degenerate, sometimes by fragmentation, and eventually disappear.

The pollen tube was observed a number of times, always following the canal formed by the absorption of the cells previously described, but in no case was I able to observe the act of fertilization.

EMBRYO.

The fertilized egg divides by transverse wall, the lower cell enlarging into a large basal cell (Fig. 8). The upper cell now divides by transverse division (Fig. 9). This is followed by a series of transverse divisions, the order of which I could not determine, resulting in a filamentous embryo of five, six or seven cells, with one large basal cell (Figs. 19, 11, 12). When the embryo has reached this condition the cell next to the upper cell divides by a longitudinal wall (Fig. 13). The cell next below now divides in a similar manner, while the two cells next to the

* Florence May Lyon. A Contribution to the Life History of Euphorbia corollata. Bot. Gaz. 25, 6. 1898. pp. 418-426.

top divide again so as to form a quadrant (Fig. 14). The upper cell is the next to divide by a longitudinal wall (Fig. 15), and this is followed by a division of the fourth cell from the top (Fig. 16). Repeated longitudinal divisions now result in the spherical embryo made up of five tiers of cells (Figs, 17, 18, 20). In the meantime the suspensor has elongated by transverse divisions, but the large basal cell remains unchanged (Figs. 18, 20, 21, 22).

This spherical embryo now enlarges by both longitudinal and transverse divisions in the different tiers until the appearance of the cotyledons, when it begins to elongate (Figs. 21, 22 and 25).

The cotyledons develop in the typical dicotyl manner on opposite sides and at the summit of the spherical embryo, and with the plumule between. At the same time the calyptrogen begins to develop in the row of cells next to the suspensor, giving rise to a well-developed root-cap. The embryonic tissues are quite distinct; the dermatogen, periblem and plerome being easily recognized. At about this time the suspensor disappears, and the embryo elongates and becomes very much curved in the embryo-sac, the inner cotyledon being slightly shorter than the outer one (Fig. 26).

It will be easily seen that there is very little similarity between the development of the archesporium, the ovules or the embryo of Agrostemma githago and Claytonia virginica, the embryonic development being entirely different. The embryo of A. githago resembles in general appearance the embryos of Cruciferae as represented by Capsella and Alyssum.

I wish to express my thanks to Prof. J. H. Schaffner, of the Ohio State University, for many valuable suggestions in this study.

CONCLUSIONS.

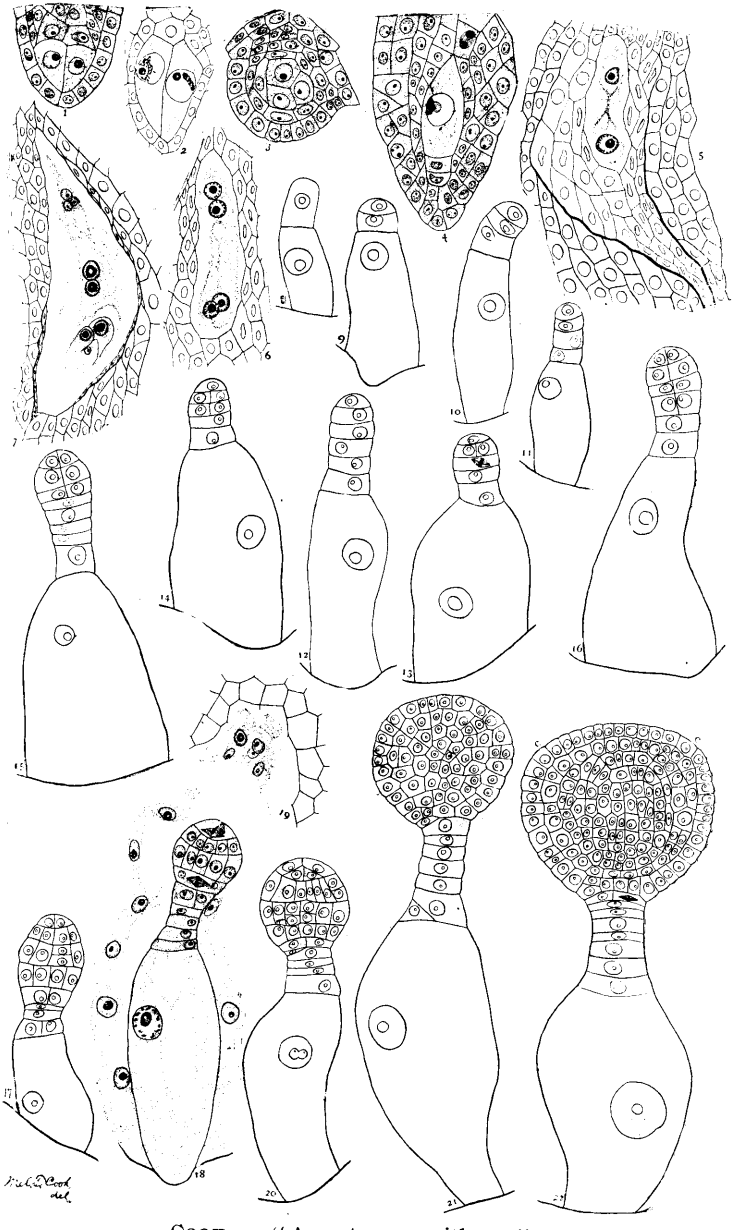
1. The archesporium develops as one, two or three cells, of which all but one are absorbed.

2. The sac is formed from the one remaining archesporial cell in the usual manner. After the formation of the eight-cell stage, the enlargement of the sac is from one side and at right angles to the original long axis.

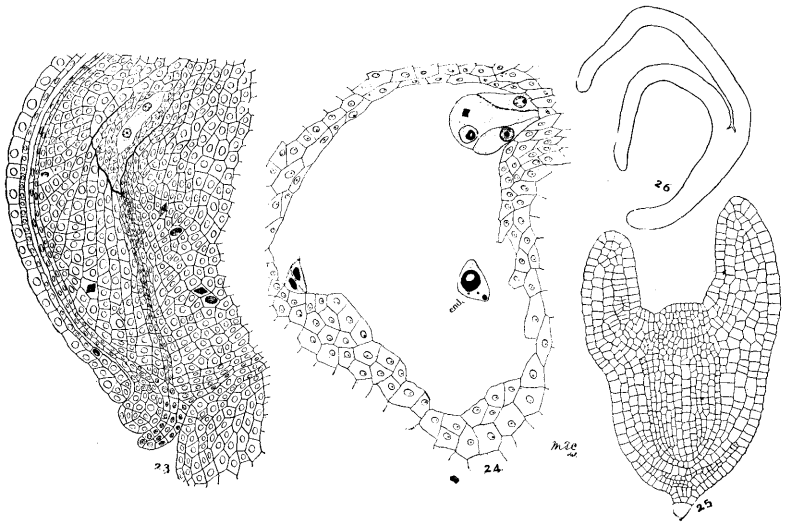
3. With the formation of the sac, the ovule enlarges from the micropylar end, thus leaving the sac deeply embedded in the nucellus. A short beak is formed, which projects through the micropyle. Two or three rows of cells degenerate to form a passage for the pollen tube.

4. The embryo is at first filamentous, the basal cell being very large. The four or five cells next to the apex divide longitudinally, forming the four or five tiers of a large spherical embryo. The cotyledons and the root-tip are formed in the usual dicotyledonous method. Soon after the appearance of the cotyledons the suspensor degenerates.

OHIO NATURALIST. *Plate 7.*



COOK on "Agrostemma githago."



EXPLANATION OF FIGURES.

For the drawings a Leitz stand was used. For Figs. 6 to 7, a No. 6 Zeiss ocular and a 1-12 Bausch and Lomb oil immersion, for Figs. 8 to 24, a No. 6 Zeiss ocular and a No. 7 Leitz objective.

Fig. 1. Two archesporial cells.
" 2. " " "
" 3. Three " " in cross section (slightly oblique).
" 4. Functional megaspore.
" 5. Two-celled embryo sac and inner zone of nucellus.
" 6. Four- " "
" 7. Eight- " " inner zone of nucellus nearly absorbed.
" 8–12. Series of embryos showing transverse division.
" 13. Embryo showing first longitudinal division.
" 14. " " second and third longitudinal divisions.
" 15. " " fourth " "
" 16. " " fifth " "
" 17. " of five tiers of cells and suspensor.
" 18. " " " " " also endosperm.
" 19. Mass of endosperm in basal pockst of embryo sac.
" 20. Spherical embryo of five tiers of cells.
" 21. " "
" 22. " " showing origin of cotyledons (c).
" 23. Ovule showing the two-celled embryo sac, the two zones of the nucellus, the radiating arrangement of the cells of the nucellus, the path to be followed by the pollen tube, and the two integuments.
" 24. Embryo sac enlarging at right angle to the long axis; e, egg and synergids; a, antipodals; end., endosperm.
" 25. Embryo showing the differentiation into cotyledons, calyptrogen, dermatogen, periblem and plerome.
" 26. Diagram of mature embryo.