Proceedings of the Iowa Academy of Science

Volume 66 | Annual Issue Article 11

1959

Flower Development and Gametogenesis in Oenothera laciniata Hill

R. L. Hulbary
State University of Iowa

A. Nagaraja Rao
State University of Iowa

Flower Development and Gametogenesis in *Oenothera laciniata* Hill

By R. L. Hulbary and A. Nagaraja Rao

Abstract. Calyx, carpels, stamens, and corolla arise in acropetal succession. The wall of the anther has four layers of cells. The tapetum is binucleate and is of glandular type. Tricolpate pollen grains are triangular in outline and are binucleate at the time of shedding. The cells of the endothecium have spiral thickenings. Development of the ovule is described. The synergids have filiform apparatus with prominent hooks. Pollen tube enters porogamously and destroys one of the synergids during its entry. A hypostase is organized at the chalazal end of the ovule. The present observations are discussed in relation to the previous literature.

The genus *Oenothera* has been well investigated embryologically. The embryo sac development is the monosporic tetranucleate type. Ishikawa (1918) and Maheshwari (1948) have reviewed the previous literature on the embryology of this genus. Johansen (1930*a*, 1930*b*, 1931*a*, 1932, and 1934) has described the morphology of the different American genera of the Onagraceae. Though many members of this family have been investigated, certain aspects of the development of the flowers and the male gametophyte still remain to be explained. A detailed account of inflorescence and floral development is presented, in addition to the development of the male and female gametophytes.

Materials and Methods

Oenothera laciniata is a small herb growing in open, sandy places. The material for the present study was collected in late July from a sandy hillside near Coggon, Iowa, and later in October, on a sandy blow-out near Muscatine, Iowa. On both collecting dates plants were in full bloom. The flowering season for this species seems to be from June to October. The flowers are axillary and densely pubescent. Inflorescence and flower buds were fixed in F.A.A. A tertiary butyl alcohol series was used for dehydration. Sections were cut at 10-14 μ and stained in iron alum haematoxylin, with eosin as counterstain.

Observations

Development of the flower. Longitudinal sections of the inflorescence apex show that the bract is a lateral outgrowth in the axil of which arises the floral primordium. The floral primordium differentiates soon into a much broadened dome (Figures 1 and 2). Calyx initials are the first to develop and then the carpels (Figure 3). After the carpellary initials become prominent the initials of the stamens arise, followed by corolla lobes (Figures 4-8). Though cor-

olla lobes are the last to form, they overgrow the calyx lobes soon and are conspicuous with a creamy yellow color.

The floral parts are tetramerous (Figure 11). The ovary is inferior and from its upper region the floral parts are initiated (Figures 6 and 7). Lawrence (1951) designated this region of initiation of floral organs as the hypanthium. Early in floral development the calyx lobes grow rapidly and envelop the other floral parts. However, the upper portions of these calyx lobes are free (Figure 8). At the point where the calyx lobes meet each other above the floral parts, a number of uni- and multi-cellular hairs are formed (Figure 9). When the stamens and the style with its four stigmatic lobes become prominent, the calyx lobes are separated and the corolla lobes soon outgrow the sepals. Raphide bearing cells are present in almost all parts of the flower including the style and the stigmatic lobes. Well developed epidermal hairs are present on the ovary, the floral tube, and the calyx lobes.

Microsporangium and male gametophyte. Stamens are eight in number and in two whorls (Figure 11). The anthers are borne on long filaments. The transverse section of the young anther shows that the wall of the anther is made up of epidermis, endothecium, and two middle layers (Figure 12). The tapetal cells are binucleate and are of glandular type. Pollen mother cells undergo meiosis to form the dyads and the tetrads of microspores. The microspore tetrads show tetrahedral, isobilateral, and decussate arrangement.

A young microspore has a centrally situated nucleus embedded in dense cytoplasm. Subsequently the microspore nucleus moves to the periphery and divides to produce a small generative nucleus and a large tube nucleus. The mature pollen at the time of shedding is binucleate (Figure 14). The anther wall at the time of dehiscence has only epidermis and endothecium, and the cells of the latter show conspicuous spiral thickenings (Figure 13).

The tricolpate pollen grains are triangular in outline (Figure 13). The exine as well as intine are smooth and thin, but the latter becomes very thick at the region of the colpi (Figures 13 and 14). Few pollen grains reach the stage of maturity. Others abort at various stages of development particularly at the stage of tedrad formation.

Ovary. The ovary is inferior, four carpelled, four loculed, with an indefinite number of anatropous, bitegmic, crassinucellate ovules arranged on an axile placenta (Figure 10). The wall of the ovary is made up of 8-10 layers of cells and the epidermal cells bear a number of unicellular hairs.

Megasporangium and female gametophyte. The ovular primordium is initiated as a conical outgrowth (Figure 15). Soon the in-

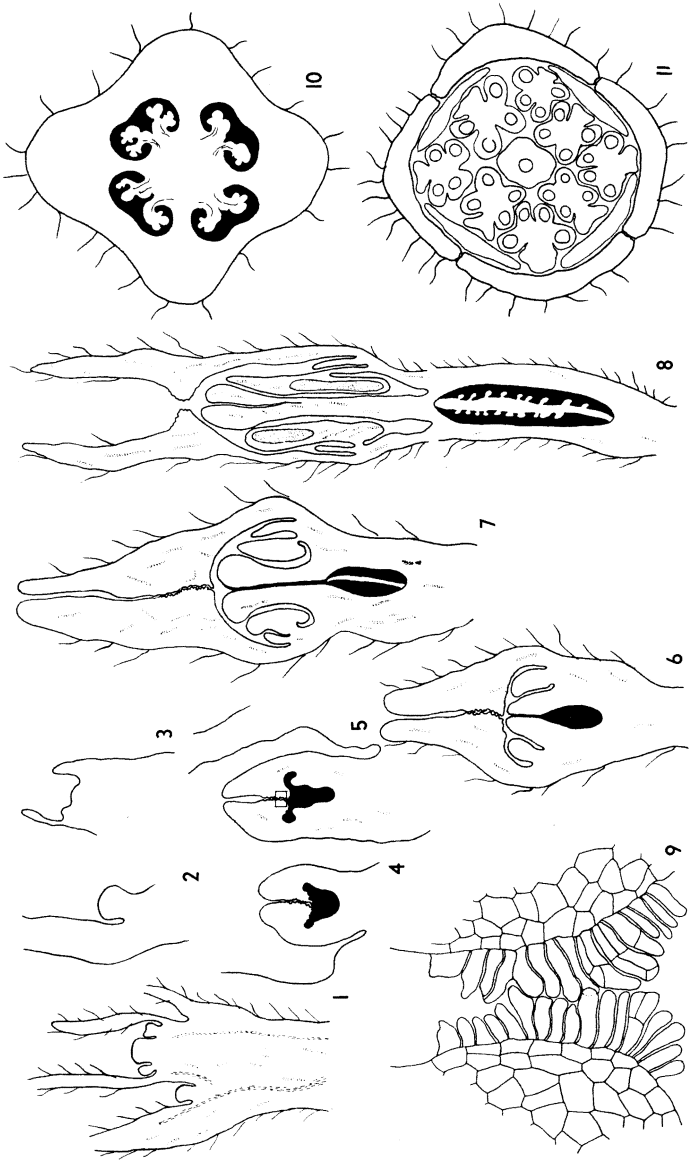
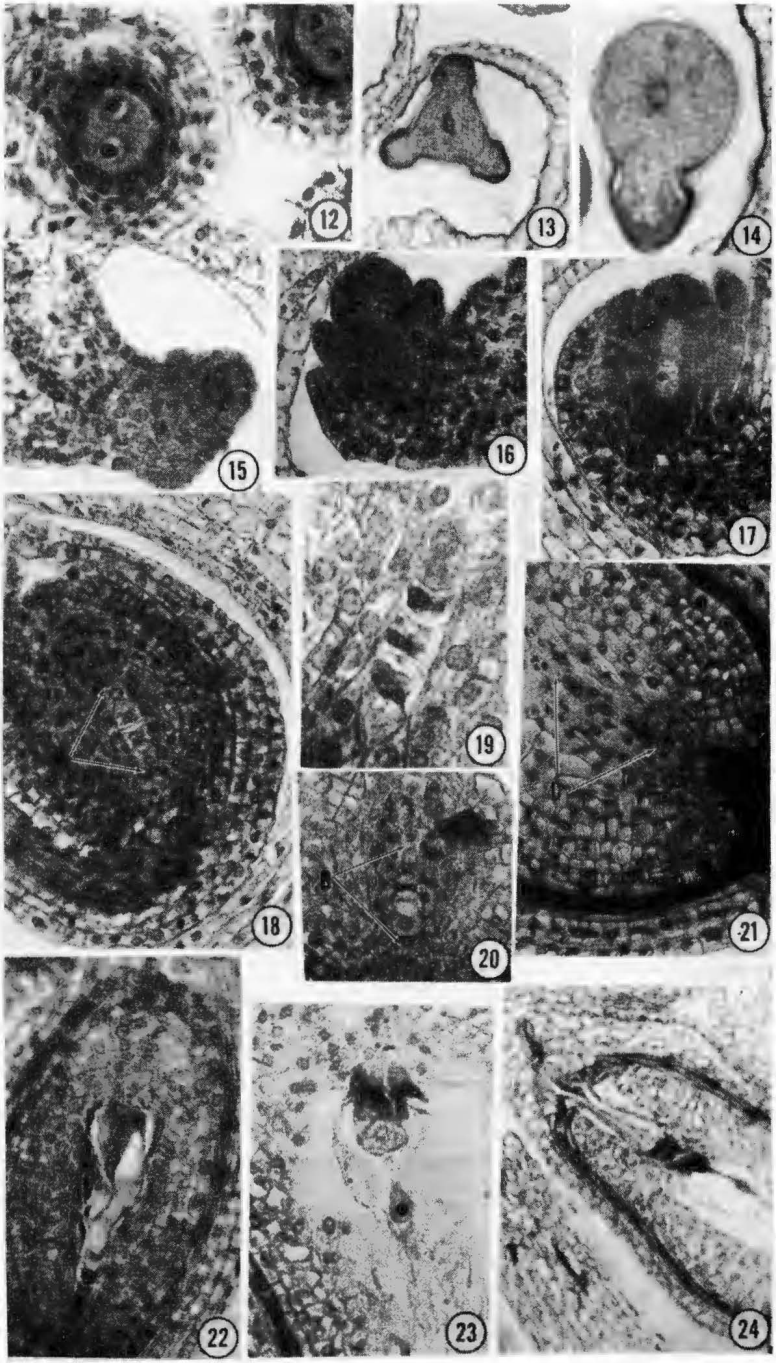


Figure 1. L. S. of floral apex showing the developing floral primordia.
Figures 2-8 Development of flower; dotted lines show the distribution of raphides.
Figure 9. An enlargement of the portion marked in Figure 5 showing uni- and multicellular hairs.
Figure 10. T. S. of young ovary with placentae.
Figure 11. L. S. of flower bud showing arrangement of floral parts.


12
13
14
15
16
17
18
19
20
21
22
23
24

itials of the inner integument appear, followed by those of the outer (Figure 16). The outer as well as the inner integument is made up of two layers of cells, except at the region of the micropyle where they consist of 3-4 layers of cells (Figure 24). The cells of the inner layer of the inner integument have dense contents that take deep stain (Figures 21 and 24).

The hypodermal archesporial cell differentiates in the nucellar primordium before the appearance of the integuments (Figure 16). It divides periclinally forming the megaspore mother cell and parietal cell. The latter divides many times forming the parietal tissue, and as a result of it the megaspore mother cell becomes deep seated (Figures 16 and 17). Multiple archesporium and megaspore mother cells are observed. The megaspore mother cell undergoes two more divisions to form a linear tetrad of megaspores (Figure 18). Of these, usually the micropylar megaspore develops further, and the other three degenerate forming an inverted tetrad (Figure 19). In two other cases observed, the megaspores of the same tetrad show variation in development. In one, the first and third megaspores are developing, whereas the second and fourth are degenerating (Figure 20). In the other, the first and second are developing whereas the third and fourth have already degenerated (Figure 21). In two ovules the development of more than one megaspore in a tetrad was observed, and these had reached the binucleate embryo sac stage. Such variations with regard to the development of the megaspores are reported in *O. pycnocarpa, O. nutans* (Ishikawa, 1918), and *O. odorata* (Subramanyam and Govindu, 1948).

The nucleus in the developing megaspore moves toward the micropyle by the formation of large vacuoles (Figure 22) and undergoes two divisions to form four nuclei. Subsequently, the embryo sac becomes greatly elongated and broadened at the micropylar end. The four nuclei organize into an egg apparatus and a single polar nucleus. The synergids are elongated with deep indentations and they develop prominent hooks as a result of this change in shape. A filiform apparatus is conspicuous. The egg is somewhat spherical in outline. The polar nucleus is oval in shape and comparatively large (Figure 23). Development of the embryo sac is thus typical of the monosporic four nucleate type (Maheshwari, 1950).

Figure 12. T. S. of young anther wall.
Figure 13. Mature anther wall and tricolpate pollen grain.
Figure 14. Two nucleate pollen grain at shedding stage.
Figure 15. L. S. of young ovular primordium.
Figures 16 and 17. Ovule development and enlarging megaspore mother cell.
Figure 18. Arrows from (A) show position of dyads in division.
Figure 19. Linear tetrad with enlarging micropylar megaspore and other three degenerating.
Figure 20. Linear tetrad with first and third developing megaspores; second has degenerated, and fourth is in process. Arrows from (B) show position of megaspores.
Figure 21. Linear tetrad with first and second developing megaspores, and third and fourth degenerated. Arrows from (C) indicate position of megaspores. Note the position of hypostase and inner integument.
Figure 22. Two nucleate embryo sac.
Figure 23. Mature embryo sac.
Figure 24. L. S. of ovule showing entry of pollen tube.

At about the tetrad stage in the embryo sac development, a group of cells in the chalazal end of the ovule become conspicuous with dense cell contents, prominent nuclei, and thick cell walls. This tissue is the hypostase (Figure 21), and it is very prominent at the embryo sac stage. The presence of a hypostase is reported in all other members of this family studied thus far (Johansen, 1928; Subramanyam and Govindu, 1948).

Fertilization. Pollen grains are monosiphonous and they germinate on the glandular stylar hairs. The pollen tube enters through the micropyle and reaches the embryo sac. During its elongation, the course of the pollen tube destroys one of the synergids as it enters between the synergid and the egg. Many dark staining bodies are present as remnants of the pollen tube (Figure 24). Double fertilization has been observed.

Discussion

Johansen has described several aspects of the embryology of many genera in this family. In addition to describing the development of the embryo sac in *Clarkia* (1930*a*), *Stenosiphon* (1930*b*), *Zauschneria* (1931*a*), *Anogra* (1931*b*), and the other American genera, he has also discussed the development and sterility of the ovules; the origin and manifestation of fasciation in the embryogeny of *Clarkia;* the development of funicular hairs and their role in pollination; and the formation of hypostase and epistase and their role in ovule development and physiological activity. The development of the embryo sac in *O. nutans* and *O. pycnocarpa* has been studied by Ishikawa (1918), and that of *O. odorata* by Subramanyam and Govindu (1948). The ovule, hypostase, and embryo sac development in *O. laciniata* is similar to the earlier observations made on other species.

The development of the male gametophyte has not been studied in species other than *O. nutans* and *O. pycnocarpa.* Even here only the nature of the pollen has been described, and there is no mention about the wall of the anther or the nature of the tapetum. But in many members of this family the wall of the pollen and cytology of the pollen mother cell are well studied (Erdtman, 1952). In view of certain variations in the female gametophyte, such as the formation of supernumerary polar nuclei in *Anogra pallida,* the variation in the development of the embryo sac, and the presence of hypostase in most of the genera, further study of the development of the flower and male gametophyte in other genera of this family should prove interesting.

In the present study the development of the flower and the male gametophyte has been described. It is interesting to note that during organogeny of floral parts the corolla is the last to develop. This

variation in the development of floral parts is recorded in few other angiosperms. The formation of uni- and multicellular hairs at the region where the calyx lobes meet deserves special mention. The development of these hairs brings about a compact arrangement of the sepals. Whether this compact arrangement is a device to avoid cross pollination should be decided by further observations. Knuth (1908) did not mention such a feature when describing pollination in *Oenothera,* or in other descriptive accounts of the flower of this genus.

Literature Cited

Erdtman, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. The Chronica Botannica Co., Waltham, Mass.

Ishikawa, M. 1918. Studies on the embryo sac and fertilization in *Oenothera.* Ann. Bot. 32: 279-317.

Johansen, D. A. 1928. The hypostase: its presence and function in the ovule of Onagraceae. Proc. Nat. Acad. Sci. USA 14: 710-713.

———. 1930*a*. Studies on the morphology of Onagracae. II. Emrbyonal manifestations of fasciation in *Clarkia elegans.* Bot. Gas. 90: 75-91.

———. 1930*b*. Studies on the morphology of Onagraceae. IV. *Stenosiphon linifolium.* Bull. Torrey Bot. Club 57: 315-326.

———. 1931*a*. Studies on the morphology of Onagraceae. V. *Zauschneria latifolia,* typical of a genus characterized by irregular embryology. Ann. New York Acad. Sci. 33: 1-26.

———. 1931*b*. Studies on the morphology of Onagraceae. VI. *Anogra pallida.* Amer. Jour. Bot. 18: 854-864.

———. 1932. Studies on the morphology of Onagraceae. VII. *Gayophytum ramosissimum.* Bull. Torrey Bot. Club 60: 1-8.

———. 1934. Studies on the morphology of Onagraceae. VIII. *Circaea pacifica.* Amer. Jour. Bot. 21: 508-510.

Knuth, P. 1908. Handbook of flower pollination, Vol. II. The Clarendon Press, Oxford.

Lawrence, H. M. 1951. Taxonomy of vascular plants. The Macmillan Company, New York.

Maheshwari, P. 1948. The angiosperm embryo sac. Bot. Rev. 14: 1-56.

———. 1950. An introduction to the embryology of angiosperms. McGraw-Hill Book Company, New York.

Subramanyam, K. and H. C. Govindu. 1948. The development of female gametophyte in *Oenothera odorata.* Indian Jour. Bot. 27: 1-5.

Department of Botany
State University of Iowa
Iowa City, Iowa