Development of the Embryo Sac in Some Japanese Members of *Cephalanthera* (Orchidaceae)

By

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The genus *Cephalanthera* L. C. RICHARD has not received much attention from the embryological point of view, although some observations on the embryology of the genus were reported by HAGERUP (1945), SAVINA (1968) and SATÔ (1976). The writer intended to investigate the development of embryo sacs of three members of the genus, *C. erecta* (THUNB.) BLUME var. *erecta*, *C. erecta* var. *subaphylla* (MIYABE et KUDO) OHWI and *C. falcata* (THUNB.) BLUME, and he extended his observations of the ovule of *C. longibracteata* BLUME which he has mentioned in his previous paper (1976).

The results obtained will be reported in this paper and the features found in the developmental process of the embryo sac of *Cephalanthera* will be compared with those of *Epipactis* (BROWN and SHARP, 1911; VERMOESEN, 1911; SATÔ, 1974) which is regarded as a closely related genus (MAEKAWA, 1972).

Materials and Method

A good many ovaries of *C. erecta* var. *erecta* were collected at the foot of Mt. Funagata in Miyagi Prefecture during June in 1973 and 1974, and those of var. *subaphylla* were collected at Sahoyama in Sendai City in the same Prefecture from May to June in 1974. From several plants of *C. falcata* growing wild on the campus of the Yokohama National University in Yokohama in Kanagawa Prefecture, many ovaries were collected from May to June in 1975.

The ovaries collected were immediately fixed in formalin-acetic-alcohol (FAA). They were embedded in paraffin (m. p. 56-58 C) after dehydration in ethyl alcohol series and sectioned serially at 8-12 μm in thickness. The sections were stained with Heidenhain's iron alum hematoxylin and fast green.

Observation

Ovule: The ovaries of these three orchids begin to grow soon after pollination as in many other orchids. The ovary wall is dotted with somewhat large cells containing crystals of calcium oxalate and has cells with granules stained strongly with hematin near the placenta and at the conspicu-

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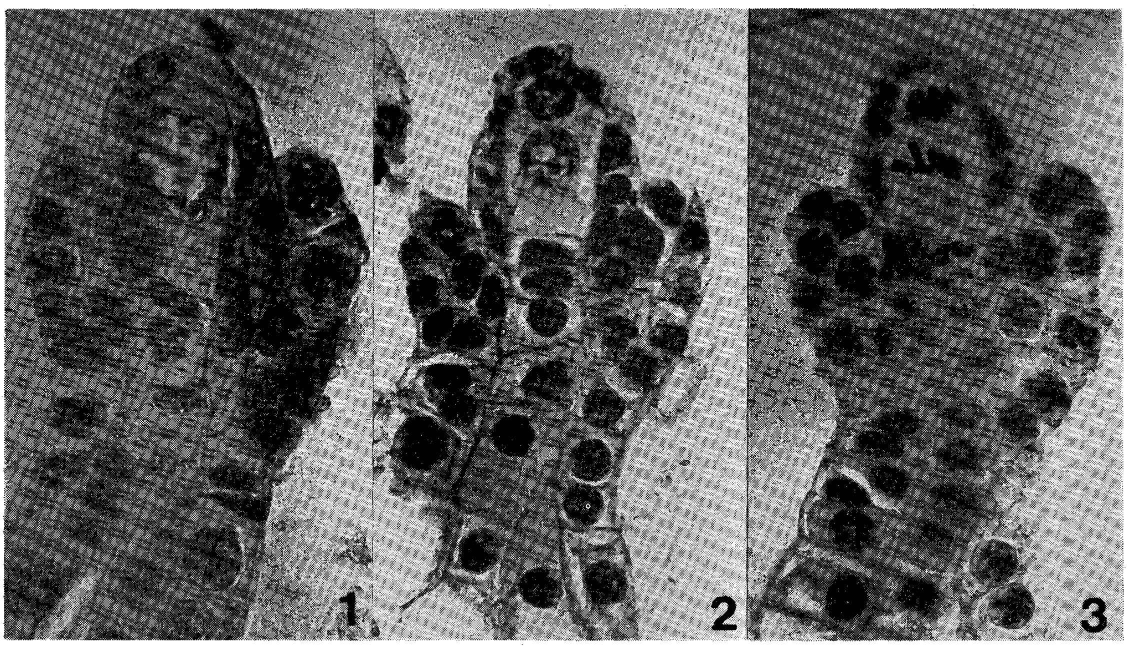
ously bi-lobed placental ridges. But these granules are never observed in cells composing a body of the ovule.

Ovular primordia of the three orchids are composed of single axial row of cells covered with one layer of epidermal cells (Fig. 4). The uppermost cell of the row becomes a single archesporial cell with a distinct nucleus and dense contents of cytoplasm, and then it becomes larger and functions directly as a megspore mother cell (Figs. 5, 16, 28), so that it lies directly below the epidermis of the nucellar tip. The ovule with a megaspore mother cell has an elongated funicle and has already attained to nearly an anatropous condition (Figs. 5, 28). As the ovule develops further, it becomes bitegmic (Figs. 8, 19, 31). An inner integument begins to differentiate at an early stage of the ovular development and elongates rapidly to form a micropylar canal, while an outer one initiates much later than the inner. The outer grows up to the level of the micropyle (Fig. 39) and is arrested to develop further, but after fertilization it elongates again greatly to form a seed coat.

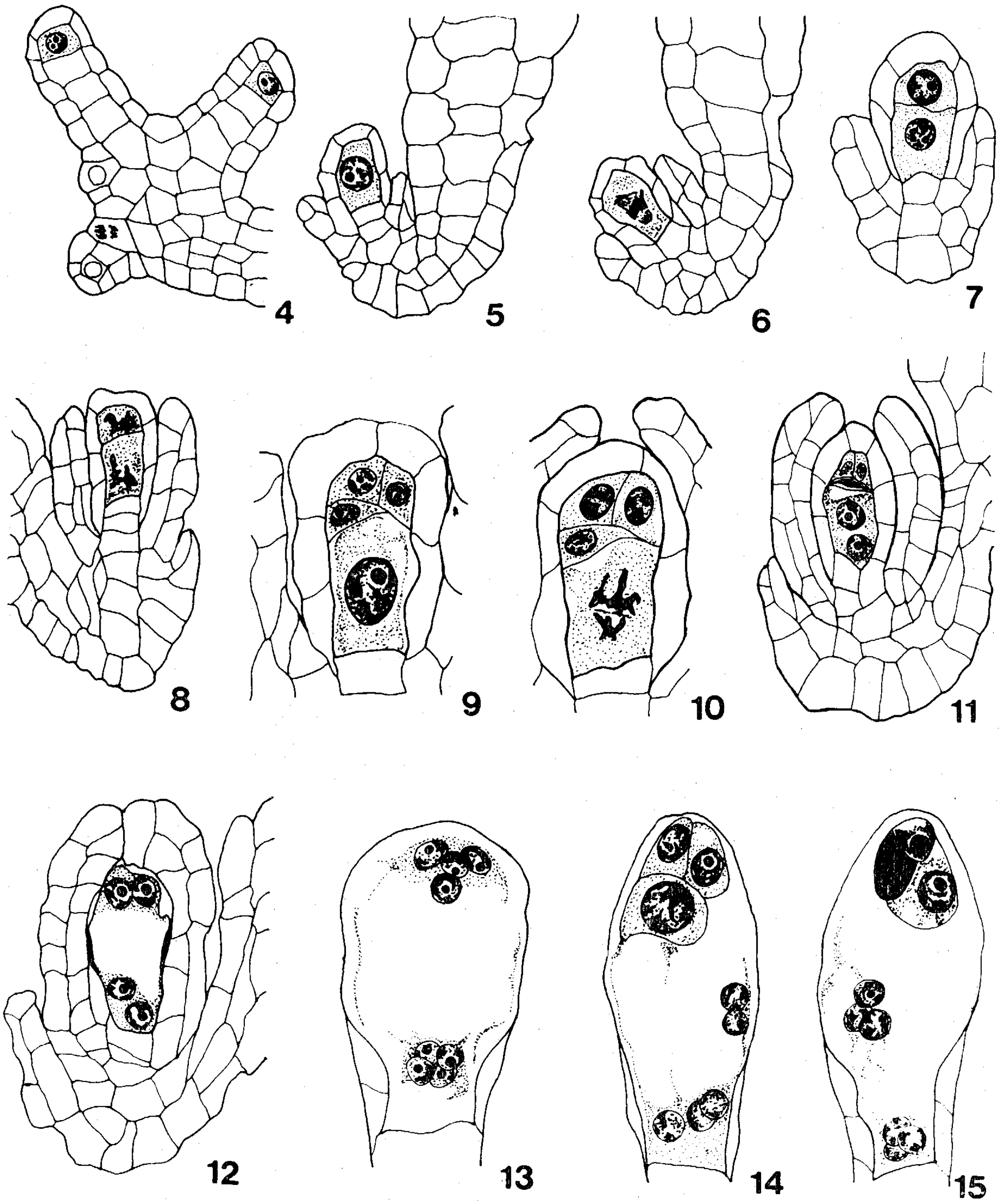
But the ovule which has much shorter funicle is frequently observed in *C. falcata* (Figs. 1, 2, 3). It continues to be nearly erect and unitegmic during megasporogenesis, and then it begins to bend along with the elongation of the funicle and the initiation of a second or outer integument, attaining to nearly an anatropous condition at the time when fertilization takes place. The same phenomenon is rarely observed in some ovules of *C. longibracteata.*

Megasporogenesis and Megagametogenesis: A megaspore mother cell (Figs. 5, 16, 28) undergoes the first division of meiosis (Figs. 6, 17, 29) to form two



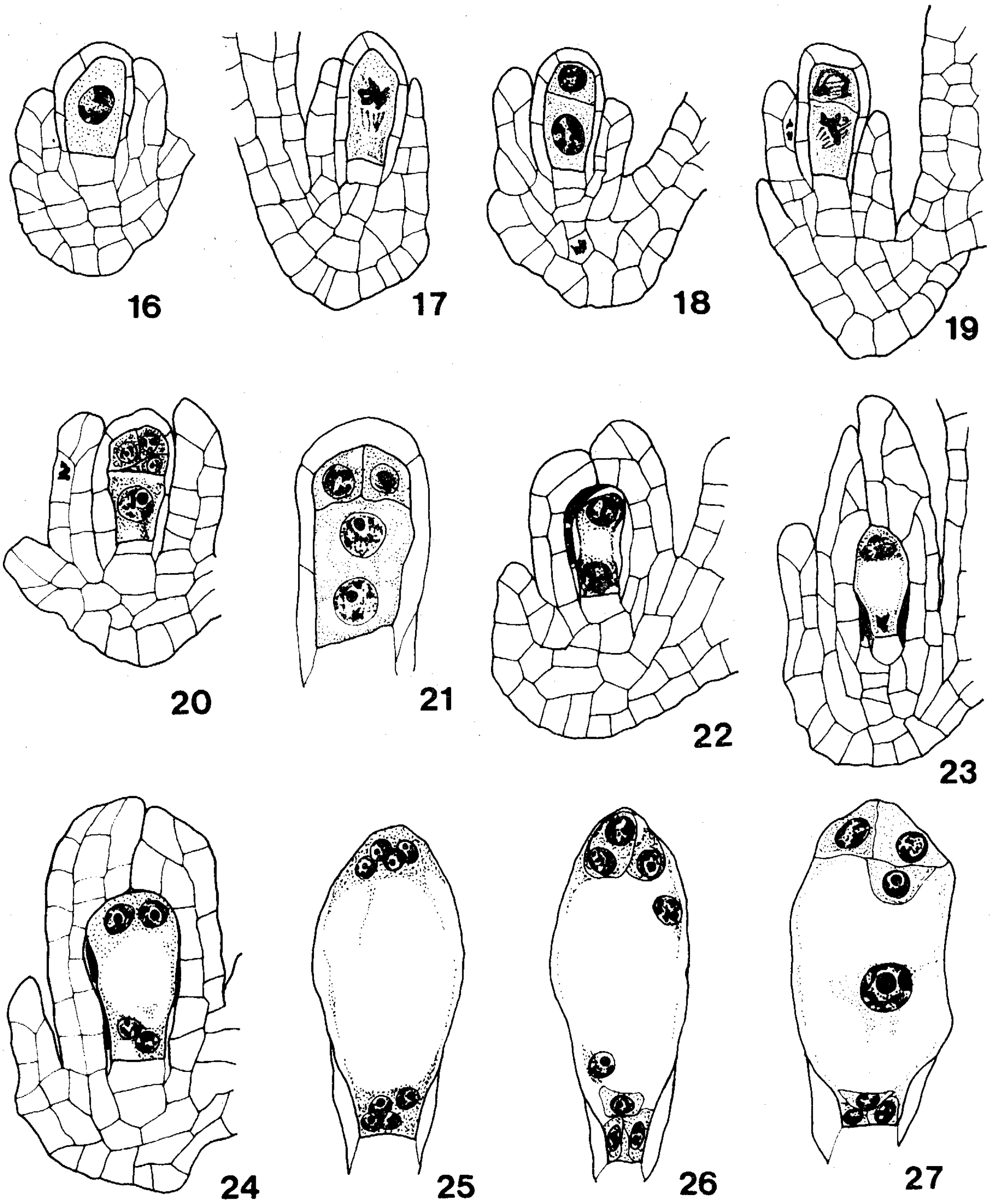
Figs. 1-3. Ovule of *Cephalanthera falcata,* continuing orthotropous and unitegmic condition. 1. Ovule with megaspore mother cell (×250). 2. Ovule with two dyad cells (×250). 3. Ovule with two dyad cells in second division of meiosis (×250).

dyad cells (Figs. 7, 18, 30), of which the micropylar is about a half of the chalazal in size. The micropylar dyad cell undergoes the second division of meiosis (Figs. 8, 19, 31) to form two micropylar cells of a tetrad. The par-



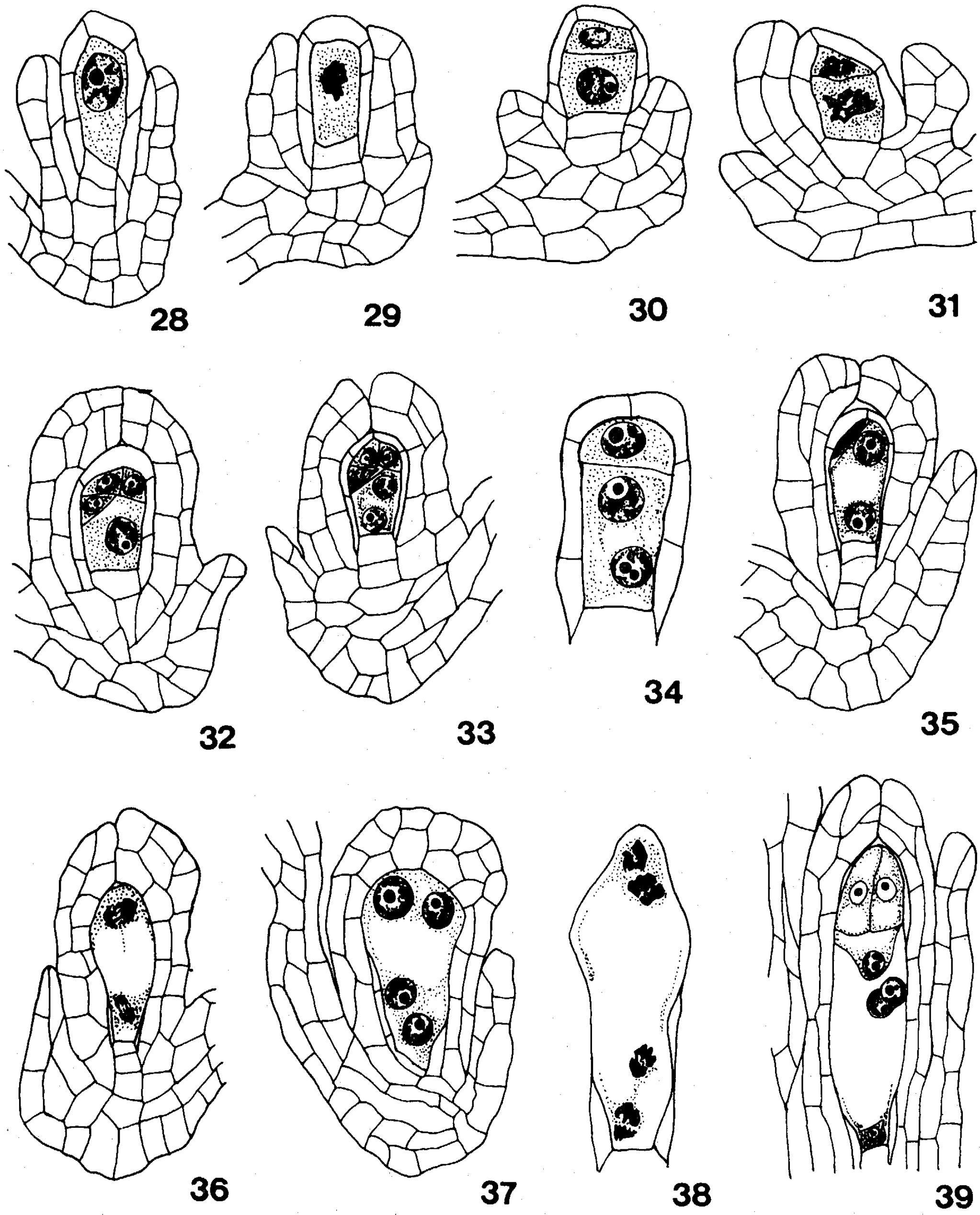
Figs. 4-15. Development of embryo sac of *Cephalanthera erecta* var. *erecta*. 4. Ovular primordia with archesporial cells (×220). 5. Ovule with megaspore mother cell (×200). 6. Ovule with megaspore mother cell in first division of meiosis (×200). 7. Ovule with two dyad cells (×240). 8. Ovule with dyad in second division of meiosis (×200). 9. Nucellus with tetrad (×240). 10. Nucellus with functional megaspore in nuclear division (×240). 11. Ovule with two-nucleate embryo sac (×200). 12. Ovule with four-nucleate embryo sac (×200). 13. Eight-nucleate embryo sac (×240). 14. Organized embryo sac; antipodals remain in free nuclei (×240). 15. Embryo sac, in which triple fusion is about to take place (×240).

tition wall separating these two cells always lies vertically to the wall formed after the first division of meiosis (Figs. 9, 20, 32). These two cells are frequently persisting still after the first nuclear division of functional megaspore



Figs. 16-27. Development of embryo sac of *Cephalanthera erecta* var. *subaphylla*. 16. Ovule with megaspore mother cell (×200). 17. Ovule with megaspore mother cell in first division of meiosis (×200). 18. Ovule with two dyad cells (×200). 19. Ovule with dyad in second division of meiosis (×200). 20. Ovule with T-shaped tetrad (×200). 21. Two-nucleate embryo sac derived from chalazal dyad cell (×240). 22. Ovule with two-nucleate embryo sac (×200). 23. Ovule with embryo sac containing two nuclei in division (×200). 24. Ovule with four-nucleate embryo sac (×200). 25. Eight-nucleate embryo sac (×200). 26. Organized embryo sac (×240). 27. Organized embryo sac with secondary nucleus (×240).

(Figs. 11, 33). The chalazal dyad cell also divides almost simultaneously with the micropylar one (Figs. 8, 19, 31) to form two chalazal cells of the tetrad. Usually, the wall separating these two chalazal ones lies obliquely to the



Figs. 28–39. Development of embryo sac of *Cephalanthera falcata*. 28. Ovule with megaspore mother cell (×200). 29. Ovule with megaspore mother cell in first division of meiosis (×200). 30. Ovule with two dyad cells (×200). 31. Ovule with dyad in second division of meiosis (×200). 32. Ovule with tetrad (×200). 33. Ovule with two-nucleate embryo sac (×200). 34. Ovule with two-nucleate embryo sac derived from chalazal dyad cell (×300). 35. Ovule with two-nucleate embryo sac (×200). 36. Ovule with embryo sac containing two nuclei in division (×200). 37. Ovule with four-nucleate embryo sac (×200). 38. Embryo sac with four nuclei in division (×230). 39. Portion of ovule with organized embryo sac (×180).

longitudinal axis of the dyad (Figs. 9, 32). The chalazal megaspore, which is largest and farthest from the micropyle, of the tetrad (Figs. 9, 20, 32) always becomes functional and takes part in the formation of an embryo sac.

The functional megaspore undergoes the first division of its nucleus (Fig. 10) to form a two-nucleate embryo sac (Figs. 11, 22, 33, 35). The two nuclei divide almost simultaneously (Figs. 23, 36) to form a four-nucleate embryo sac (Figs. 12, 24, 37). Some epidermal cells at and near the nucellar tip begin to degenerate, and as the embryo sac develops further, only its chalazal end is supported by some nucellar cells. The four nuclei of the embryo sac always divide once more (Fig. 38) to form an eight-nucleate embryo sac (Figs. 13, 25). These eight nuclei differentiate into an egg cell, two synergids, two primary polar nuclei and an antipodal apparatus (Figs. 14, 26, 39). The synergids of *C. falcata* continue to develop conspicuously and occupy about one-fourth of the embryo sac (Fig. 39). The antipodal apparatus is always formed by three cells except *C. erecta* var. *erecta*, in which it is usually composed of three free nuclei (Fig. 14, 15). The antipodal apparatus of the three orchids usually persists still after fertilization. Thus, the embryo sac of these three orchids is usually organized according to the monosporic eight nucleate type of development.

But, rarely, a nucleus of a chalazal dyad cell divides without the formation of a separating wall (Figs. 21, 34) and an embryo sac may be formed from the chalazal dyad cell. This mode of embryo-sac formation, which is referred to as the bisporic eight nucleate type, exists together with the monosporic one in these three orchids.

Double fertilization takes place in these three orchids, that is, one male gamete fertilizes with an egg cell, and the other may simultaneously fertilize with two primary polar nuclei (Fig. 15) or may fertilize with a secondary nucleus (Fig. 27). In particular, the former, which is referred to as the triple fusion, is frequently observed in *C. erecta* var. *erecta*.

Discussion

It has been presumed by MAEKAWA (1972) that *Epipactis* was derived from *Aphyllorchis*, which is a saprophytic genus, through *Cephalanthera*.

No embryological informations of *Aphyllorchis* have yet been reported to date. In *Epipactis*, the developmental type of embryo sac is principally of the monosporic eight nucleate type, and the bisporic eight nucleate type rarely exists together with it (BROWN and SHARP, 1911; VERMOESEN, 1911; SATÔ, 1974). The embryo sac of *C. longibracteata* (SATÔ, 1976), *C. erecta* var. *erecta*, *C. erecta* var. *subaphylla* and *C. falcata* is principally formed according to the monosporic eight nucleate type of development, and rarely formed according to the bisporic eight nucleate type.

The mature embryo sac of *Cephalanthera* is always composed of eight nuclei and the antipodal apparatus are persisting long after fertilization. In

Epipactis (BROWN and SHARP, 1911 ; SATÔ, 1974), on the other hand, the phenomenon of "strike" frequently occurs in the developing embryo sac, by which the mature embryo sac composed of less than eight nuclei is frequently formed. Even if this phenomenon does not occur, the antipodal apparatus, which may not differentiate into three cells, is disorganized and disappears before or soon after fertilization. Thus, the nuclei or cells composing the antipodal apparatus of *Epipacits* may be short-lived compared with those of *Cephalanthera*.

In the process of megasporogenesis of *Cephalanthera*, the micropylar of the two dyad cells formed after the first division of meiosis is about a half of the chalazal dyad cell in size and a four-celled tetrad, of which the non-functional megaspores are long persisting, is formed in almost all of the ovules. In that of *Epipactis* (SATÔ, 1974), on the other hand, the micropylar dyad cell is much smaller than the chalazal one, and the arrangement and number of the megaspore formed after two successive divisions of meiosis show a wide range of variation, and the non-functional megaspores are short-lived. In particular, it is noteworthy that in some ovules of *Epipactis* the micropylar dyad cell disintegrates and disappears before its second division of meiosis is over (SATÔ, 1974). If various conditions of tetrad were derived from a "hypothetical tetrad" which is consisted of four megaspores having equal potential for maturing into embryo sac severally (REMBERT, 1971 ; SATÔ, 1974), the tetrad pattern of *Cephalanthera* may be presumed to be nearer to the "hypothetical tetrad" than that of *Epipactis*.

The megaspore of the two genera is formed by the two successive divisions of the megaspore mother cell and the embryo sac of them is formed by the three divisions of the megaspore nucleus. But earlier disintegration of the non-functional cell or cells of dyad and tetrad, and reduction in the nuclear numbers composing the developing embryo sac occur frequently in *Epipactis*. In these respects, the process of the embryo-sac formation of *Epipactis* seems to deviate from the typical pattern of monosporic eight nucleate type compared with that of *Cephalanthera*.

It is generally considered that, among plants, the angiosperms show the last stage in the reduction of the gametophytic generation. If its direction of specialization is also adapted for that seen among variations within the monosporic eight nucleate type of development, the structure and developmental mode of the embryo sac of *Cephalanthera* seems to be more primitive than those of *Epipactis*.

The phenomenon that attainment to an anatropous, bitegmic condition laggs behind was observed in some ovules of *C. falcata* and *C. longibracteata*. Ovules of the Orchidaceae, usually, show anatropous, tenuinucellate and bitegmic condition, to which they attain relatively soon after pollination. But such saprophytic orchids as *Gastorodia elatu* (KUSANO, 1915 ; ABE, 1976) and *Epipogium aphyllum* (AFZELIUS, 1954) have orthotropous, unitegmic ovules and the ovules of *Lecanorchis japonicu* (TOHDA, 1971), which is saprophytic as well, attain to an anatropous, bitegmic condition after fertilization. In this respect, it may be regarded that the genus *Cephalanthera* retains a saprophytic feature.

MAEKAWA's opinion as to the relationship among *Aphyllorchis, Cephalanthera* and *Epipactis* may be supported from the embryological point of view.

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Summary

The development of embryo sacs in *Cephalanthera erecta* var. *erecta, C. erecta* var. *subaphylla* and *C. falcata* was investigated. In these three orchids the ovules are anatropous, tenuinucellate and bitegmic. In some ovules of *C. falcata* and *C. longibracteata,* it was observed that the anatropous and bitegmic condition was brought much delayed. The largest megaspore of a tetrad, which is farthest from the micropyle, becomes functional and its nucleus divides three times in succession, so that a mature embryo sac is always composed of eight nuclei. The developmental mode of embryo sacs in these three orchids is usually of the monosporic eight nucleate type. The embryological features of *Cephalanthera* investigated were compared with those of *Epipactis,* which is closely related genus of *Cephalanthera.*

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