

EMBRYOLOGY OF ORCHIDACEÆ

BY

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ORCHIDACEÆ comprising 450 genera and 7,500 species constitute a specialised group among angiosperms. In spite of their complex life-cycle only about 175 species (included under 75 genera) seem to have received the attention of morphologists, since the publication of the first available paper on the embryology of *Orchis* by Muller (1847). In the present article the author proposes to give a brief review of the scattered information on the embryological aspects of orchidaceous members.

MICROSPOROGENESIS AND MALE GAMETE

Our knowledge concerning the microsporogenesis and the development of the male gametes is scanty and confined to the study of a few plants, and does not explain the organogeny of stamens, differentiation of parietal layers, tapetum, etc.

With the exception of *Paphiopedilum*, *Selenipedium* and *Cypripedium* most of the forms studied show only simultaneous divisions in the pollen-mother-cell. In the three above-mentioned forms successive type has been noticed. In *Epidendrum raniferum* Hoffmann first suspected a successive type of development; but later confirmed it as a simultaneous type.

Details of the division of the uninucleate microspore into the tube and generative cells are incompletely understood. Recently investigations on the microsporogenesis of *Cymbidium* (Swamy, 1941) and *Eulophea* (Swamy, in press) have clarified many doubtful points. The nucleus of the microspore divides into an inner tube cell and an outer lenticular generative cell and a definite wall is laid down between them. After a time the separating wall disappears and gradually the plasma of the vegetative cell engulfs the tube nucleus which is surrounded by a small quantity of cytoplasm; ultimately the tube nucleus becomes spherical with a characteristic clear space around and comes to lie within the plasma of the vegetative cell. Such a type of "movement of the generative cell", as it is termed, has been reported in very few plants and it would be of great interest to discover if similar cases occur in other members of this family.

The mature pollinia of certain terrestrial orchids as *Orchis*, *Calanthe*, *Neottia*, *Calopogon*, etc., are in the form of small aggregates known as "massulæ" in contrast to almost all epiphytic forms where the entire pollen is massed up in the form of pollinia.

In most of the species the pollen is two-nucleate at the time of pollination. In *Cymbidium*, *Eulophea* and *Calopogon pulchellus* (Pace, 1909) the individual microspores of the quartet do not get separated, and germinate *in situ*. It is only in certain species of *Cypripedium* that they are seen to round off individually. The generative nucleus divides in the pollen tube into two male nuclei. The tube nucleus in *Cymbidium bicolor* exhibits a remarkable behaviour in that it elongates in the tube nearly ten times its original length before degenerating.

Zeuxine sulcata (Seshagiriiah, 1941) exhibits some interesting peculiarities during the microspore formation. The "second mitotic division seems to be suppressed; hence 'dyads' of microspores are formed". These divide into vegetative and generative nuclei but such pollinia are not functional. In some pollinia the author reports the formation of abnormal spindles during the heterotypic nuclear division as a result of which supernumerary nuclei are formed.

FEMALE GAMETANGIUM

The primary archesporium is subepidermal and single-celled, which directly functions as the megaspore mother-cell. Formation of the parietal layers is unknown in the family. Differentiation of two megaspore mother-cells which are either superposed or placed side by side, have been noticed occasionally in *Calopogon pulchellus* and species of *Cypripedium* and in stray cases in *Gastrodia elata*. In *Calopogon pulchellus* some of the twin mother-cells were provided with individual covering of the nucellar epidermis. In *Oncidium prætextum* (Afzelius, 1916) the terminal epidermal cell of the archesporium has the appearance of the embryo-sac mother-cell.

In *Gastrodia elata* the embryo-sac is organised even before pollination. In many terrestrial orchids, however, especially, the

group-Habernarieæ, the ovaries show only the archesporial initials at the time of pollination; and their further development is dependent upon pollination. In some of the epiphytic orchids (*Cymbidium*, *Dendrobium*, *Cottonia*, *Vanda*, etc.), the archesporial cell is differentiated in the nucellus only after pollination.*

TABLE I

Showing the Orchids that exhibit the *Allium* and *Adoxa* types of development of Embryo-Sacs

Plant	Author	Remarks
Allium-type		
<i>Achreanthus monophyllus</i>	Stenar (1937)	
<i>Cymbidium bicolor</i>	Swamy (1942)	
<i>Cypripedium spectabile</i>	Pace (1907)	
<i>C. parviflorum</i>		
<i>C. pubescens</i>		
<i>C. candidum</i>		
<i>Epipactis latifolia</i>	Vermoessen (1911)	Only some-times
<i>E. pubescens</i>	Brown and Sharp (1911)	Only some-times
<i>Gyrostachys cernua</i>	Pace (1914)	Only some-times
<i>G. gracilis</i>		
<i>Neottia nidus avis</i>	Mo-kilewski	Very rarely
<i>Orchis sambucina</i>	Afzelius (1916)	
<i>Oncidium pratectum</i>	" "	
<i>Paphiopedilum insigne</i>	" "	
<i>P. Leeatum</i>	Francini (1930)	
<i>P. spicerium</i>		
<i>P. barbatum</i>		
<i>P. villosum</i>		
<i>P. venustum</i>		
Adoxa-type		
<i>Bletia shepherdii</i>	Sharp (1912)	Very occasionally
<i>Epidendrum variegatum</i>		
<i>Epipactis pubescens</i>	Proan and Sharp (1911)	Occasionally
<i>Epipogon nutans</i>	Swamy (unpublished)	
<i>Gyrostachys cernua</i>	Pace (1914)	Sometimes
<i>G. gracilis</i>		

In *Cymbidium bicolor* female archesporium is differentiated 3-4 days after pollina-

tion; in *Eulophea epidendrea* in about a week's time and in certain species of *Vanda* after more than ten days. In *Dendrobium anosum* the megaspore mother-cell was observed to be in division 76 days after pollination.

FEMALE GAMETOPHYTE

Monosporic eight-nucleate Normal-Type is the predominant course of development of the embryo-sac. Orchids showing other types of development are listed in Table I. *Epipactis pubescens* and two species of *Gyrostachys* are very variable, showing both Bisporic and Tetrasporic types of development. This variability in *Epipactis pubescens*, "may be due", according to the authors (Brown and Sharp, 1911), "however, to some condition such as nutrition, which is external to the megaspores, and is probably not due to potentialities inherent in the various megaspore nuclei; The most reasonable conclusion would seem to be that different courses of development are due to conditions external to the nuclei, and that the fate of the nucleus will depend on its position". Sharp (1912) who reports Adoxa type as of occasional occurrence in *Epidendrum* and *Bletia* also lays much stress on the position of the spindles at the first two divisions, as the determining factor in influencing the further course of development and his conclusion is in accordance with the one for *Epipactis*.

Among the orchids investigated, the megaspores of the linear tetrad show a difference in their individual sizes soon after their formation, the three micropylar ones being very much smaller than the chalazal one. The upper cell of the dyad in many instances remains without any further development. The chalazal megaspore by further development forms the mature embryo-sac. It may be mentioned here that Chodat (1913) in *Ophrys apifera* and *O. botteroni* finds sometimes that the two lowermost megaspores resolve into embryo-sacs.

Differences in the number of antipodals are of common occurrence, and are often exhibited in different ovules of one and the same ovary. The retardation or even the suppression of nuclear divisions at the antipodal end is often responsible for the development of 5-, 6- or 7-nucleate embryo-sacs. Fusion of the daughter nuclei following division of the antipodals at the chalazal

* Recent investigations have revealed that for the development of the female gametophyte, the transference of actual pollen on to the stigmatic surface is not necessary in certain species of orchids to initiate the development of the megasporangium but a mere application of the pollen extract in any suitable solvent on to the stigma does not only induce the enlargement of the gynostegium but also initiate the development of ovules and ovary. But it is obvious that no fertilisation takes place due to the absence of the male element. According to Laibach (1932) the orchid-pollen-substance is capable of stretching *Avena coleoptiles* and he is of the opinion that this active substance is related to the other growth-promoting auxins.

end which has been observed in *Gastrodia elata* (Kusano, 1915), *Cypripedium guttatum* (Prosonia, 1930), etc., ultimately results in an embryo-sac with less than eight nuclei. According to Sharp (1912) the presence of 6-nucleate embryo-sacs in certain of the species investigated by him "seem to show a tendency towards a further reduction of the vegetative portion of the gametophyte".

The genus *Cypripedium* has raised a keen controversy in the orchid embryology. Pace (1907) studied four species of this genus in which she first described the "*Cypripedium*-type". According to her the lower dyad cell divides twice resulting in a pair of nuclei at each end of the embryo-sac. The upper pair constitutes the synergids; the lower forms the egg and the single polar nucleus. During the time of fertilisation one of the synergid nuclei descends down and takes part in the triple fusion with the second male nucleus. This was subjected to severe criticism by Rutgers (1923), who demanded a confirmation of Pace's work. Prosonia (1930) investigated *Cypripedium guttatum* and found the development to be very much similar to the *Allium*-type but with certain amount of reduction at the chalazal end of the embryo-sac, as a result of which 5- or 6-nucleate condition of the mature embryo-sacs arose. Francini's (1930) investigations on other species of *Cypripedium* corroborate Prosonia's findings. Carlson (1940) has re-investigated *Cypripedium parviflorum*. Unfortunately her work has not so far resulted in any substantial contribution to the megasporogenesis but she hopes to study the same in light of the previous work on the genus. According to her the mature embryo-sacs in the species studied by her contains 8 nuclei though "more evidence is required to settle this point".

In this connection the type of development in *Gastrodia elata* (Kusano, 1915) may also be considered. In this plant the chalazal megaspore of the tetrad develops into a mature embryo-sac of 4 nuclei; two synergids, one egg and one polar, similar in organisation to the one described by Pace for *Cypripedium*. The author (Kusano, 1915) also found in *Gastrodia elata* at the time of fertilisation one synergid leaving its position and taking part in triple fusion. "Perhaps influenced by the work of Pace", as Schnarf remarks, Kusano reports the above condition; but in the opinion of the

present author a reinvestigation of the form might reveal only a normal type of development, probably with reduced antipodal nuclei.

FERTILIZATION AND ENDOSPERM

The time taken by the pollen tube from the time of pollination to actual entry into the embryo-sac is highly variable. The shortest period so far recorded is four days for *Gastrodia elata* (Kusano) and the longest in the case of *Vanda suavis* as observed by Guignard. Fertilization is strictly porogamous; double fertilization has been observed in most of the species investigated.

In the majority of forms examined, the development of endosperm proceeds upto the stage of triple fusion. Even this might be incomplete due to the fact that the polars remain just apposed and do not fuse.

The division of the secondary endosperm nucleus up to 2-3 divisions has been recorded so far only in five forms, viz.,

<i>Cypripedium spectabile</i>	} (Pace, 1907).
<i>C. parviflorum</i>	
<i>C. pubescens</i>	
<i>Paphiopedilum insigne</i>	} (Afzelius, 1916).
<i>Chæmæorchis alpina</i>	

Sharp (1912) referring to the work of Pace on *Calopogon pulchellus* states that this plant is the only known case of endosperm formation among orchids. But a careful perusal of Pace's paper by the writer, however, revealed no such statement made by Pace herself about the endosperm. In fact only its stages upto double fertilisation have been described by her. In 1900 Nawaschin attributed the lack of endosperm to the failure of complete fusion of the polars or the second male nucleus. But Strasburger in the same year showed in several European orchids, that in spite of the complete fusion of the polar nuclei and the second male nucleus, endosperm did not develop. How it is that in this particular family the endosperm tissue is not seen in evidence, it is still difficult to determine.

EMBRYO

A most pronounced feature of the family is the undifferentiated embryo in the mature seed. The highest differentiation so far recorded is in *Sobralia macrantha* (Treub, 1879) where a clear differentiation into cotyledon and hypocotyl region is

observed and *Platyclinis* (Rendle, 1930) with a "terminal green cotyledon".

The zygote divides as a rule by a transverse wall; it is only in *Cymbidium bicolor* that the first wall may be sometimes laid down either vertically or obliquely to the long axis of the embryo-sac. A proembryo of a chain of three cells is formed, the terminal cell of which undergoes further divisions to form the actual embryonal mass. The basal cell usually develops into a suspensor whose shape and structure is most variable.

Complete lack of any suspensor has been noticed in *Epipactis pulstris*, *Listera ovata* (Fig. 2), *Cypripedium reginae* and *Zeuxine sulcata*. In *Spathoglottis plicata* the uppermost cell of the proembryo towards the micropyle enlarges and sometimes grows out of the micropyle; a similar case is reported by Treub (1879) in *Goodyera discolor* (Fig. 1). Very frequently in genera like *Orchis*, *Gymnadenia*, *Phajus*, etc., the suspensor consists of a single row of cells. In *Epidendrum ciliare* (Fig. 8) it is very long consisting of several tiers of cells, which are superposed. In several *Habenaria* species (Fig. 9) noticed by the writer (unpublished), the filamentous row of the suspensor cells grows out of the micropyle; and the terminal cell of the suspensor embeds itself in the placental tissue and acts like an aggressive haustorium. The suspensor in a number of cases sometimes branches profusely as in *Serapias lingua* (Fig. 7). In certain species of *Vanda*, the basal cell undergoes four vertical divisions resulting in the formation of eight cells which grow towards the chalaza and engulf the growing embryo (Fig. 5). In *Cymbidium* and *Eulophea* the succeeding few divisions of the zygote after its first transverse division are most irregular; one of the cells situated at the chalaza develops a filamentous row of cells, the terminal two or three cells towards the free end contributing to the actual embryo; the irregularly divided cells grow out and elongate in all directions inside the seed coat but do not reach any nutritive zones (Fig. 4). In *Stanhopea* also the development seems to be similar with some variations. In *Phalaenopsis grandiflora* (Fig. 3) the basal cell is divided by longitudinal walls to form a quadrant and each cell of the quadrant develops conspicuous copious tubular outgrowths which grow towards micropyle and chalaza,

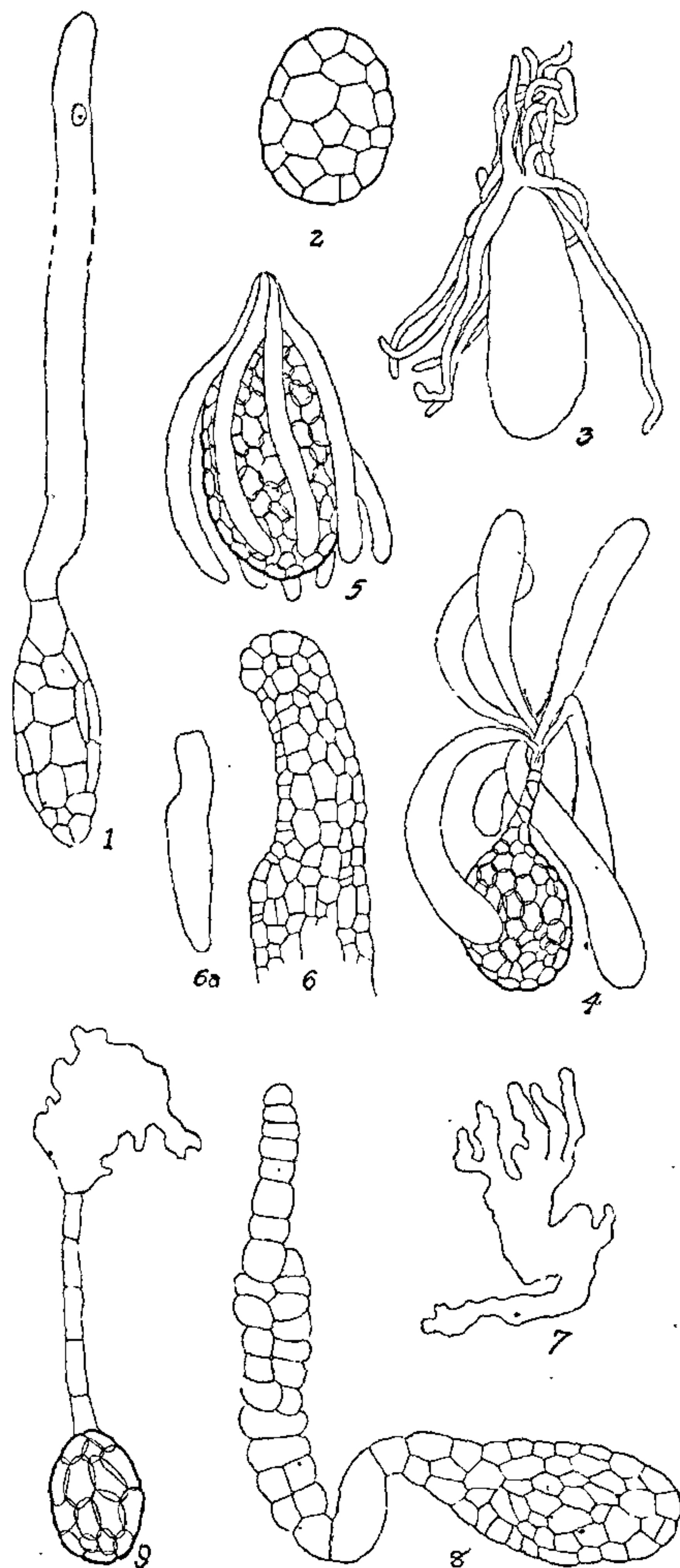


FIG. 1 Embryo of *Goodyera discolor*, showing the unicellular tubular suspensor, $\times 120$. FIG. 2. Embryo of *Listera ovata*, which has no suspensor, $\times 120$. FIG. 3. Embryo of *Phalaenopsis grandiflora*, showing the profusely branched suspensor, $\times 125$. FIG. 4. Embryo of *Eulophea epidendrea*, $\times 100$. FIG. 5. Embryo of *Vanda spathulata*, $\times 120$. FIG. 6. Terminal "cotyledonary" region of the embryo of *Sobralia macrantha*, $\times 120$. FIG. 6a. Outline of the entire embryonal mass of *Sobralia macrantha*, $\times 45$. FIG. 7. Terminal haustorial cell of the suspensor of the embryo of *Serapias lingua*, $\times 85$. FIG. 8. Embryo of *Epidendrum ciliare*, $\times 200$. FIG. 9. Embryo of *Habenaria rariflora*, $\times 120$. FIGS. 1, 2, 3, 6, 6a, 7 and 8, after Treub, 1879.

The function of such elaborately organised suspensor organ is not quite clear. Treub (1879) attributed the function of absorption of nutritive substances to this organ. In some instances, no doubt, they are definitely concerned with the absorption of nutrilites. As against this, in many cases their function as a haustorium has to be doubted. For instance, in many plants, they do not reach any nutritive tissues or possess rich cell contents. The nuclei in these, in fact, degenerate. Such being the case, we might have to modify the statement made by Coulter and Chamberlain (1903) (cited by Kusano, 1915) that "every suspensor is an Haustorium".

POLYEMBRYONY AND OTHER PHENOMENA

Schnarf (1931) has listed a number of species in Orchidaceae that exhibit more than one embryo in a single seed. In many cases the exact origin of the additional embryos has not been traced. In *Gastrodia elata* two embryo-sacs in the same ovule often develop to maturity, separate pollen tubes enter each of these and by further development after fertilisation normal diploid embryos are formed in one ovule. In the same plant, according to Kusano (1915), "at delayed fertilisation of the ovule the synergid tends to develop into an adventitious embryo. In this case fusion between the male and synergid nuclei is highly probable", in case of which the resulting embryo will be diploid (if Kusano's surmise is reliable), and "under a special condition the haploid egg may undergo the nuclear division leading to the generative parthenogenesis, but no cell division is ascertained", in which case the ensuing embryo will be haploid.

The occurrence and origin of haploid embryos in addition to nucellar ones has recently been recorded in *Zeuxine sulcata*. In this plant the megaspore mother-cell after the usual reduction divisions forms a linear row of four megaspores; these upon undergoing anticlinal and periclinal divisions give rise to the haploid embryo.

Apomictic development associated with polyembryony (the origin of all the embryos being similar) is recorded in *Nigretella nigra* (Afzelius, 1923, 1932). In this plant the sexual development of the female gametophyte continues only till the four-nucleate stage. Even though the development of the pollinia is quite normal and

functional fertilisation is not accomplished and all the embryos that arise are from the nucellar epidermis that caps the female archesporium. A very similar type has been described in *Zeuxine sulcata* but the pollen here is completely sterile.

In *Cymbidium* and *Eulophea* cleavage type of polyembryony is recorded and the resulting embryos are monozygotic. In *Cymbidium bicolor* sometimes the first wall laid down in the zygote will be vertical and under such circumstances in some cases the two cells develop independently and produce two embryos. In *Eulophea epidendracea* (Swamy, in press) it is seen that any region of sexually produced embryo proliferates or buds off additional embryos.

Without entering into any discussion as to the cause of polyembryony, it may be stated that a large number of morphologists hold the view that sudden environmental changes might play an important part. Ernst (1918) on the other hand assumes that hybridisation as being the primary cause and nutritional disturbances as one of the probable causes in inducing polyembryony. The cause in *Zeuxine sulcata* "seems that this nutritive disturbance, just prior to the reproductory phase, may set up causes which bring about the phenomenon seen in" this plant, and the author (Seshagiriah, 1941) opines that "Nekro-hormones" which are believed to bring about adventitious embryos in *Oenothera* may act in this particular case also.

(Note.—Only very important references are given here due to the scarcity of space.)

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