GAMETOGENESIS AND EMBRYOGENY OF *EULOPHEA EPIDENDRAEA* FISCHER.

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Eulophea epidendraea Fischer is a terrestrial orchid, growing wild in the scrub jungles of South India. Of the several genera in the sub-tribe Eulophieae (Tribe Vandae), *Eulophea* is the only genus reported from India (Hooker, 1894).

The vegetative activity of the plant commences soon after the outbreak of monsoons in July. The plant possesses large conical pseudobulbs, which are greenish in early stages and later turn greyish brown, with persistent leaf bases. The inflorescences, usually 1 to 2 per pseudobulb, spring up laterally and attain a length of nearly 3 feet, bearing pale greenish flowers in racemose fashion from September to December.

Flower.

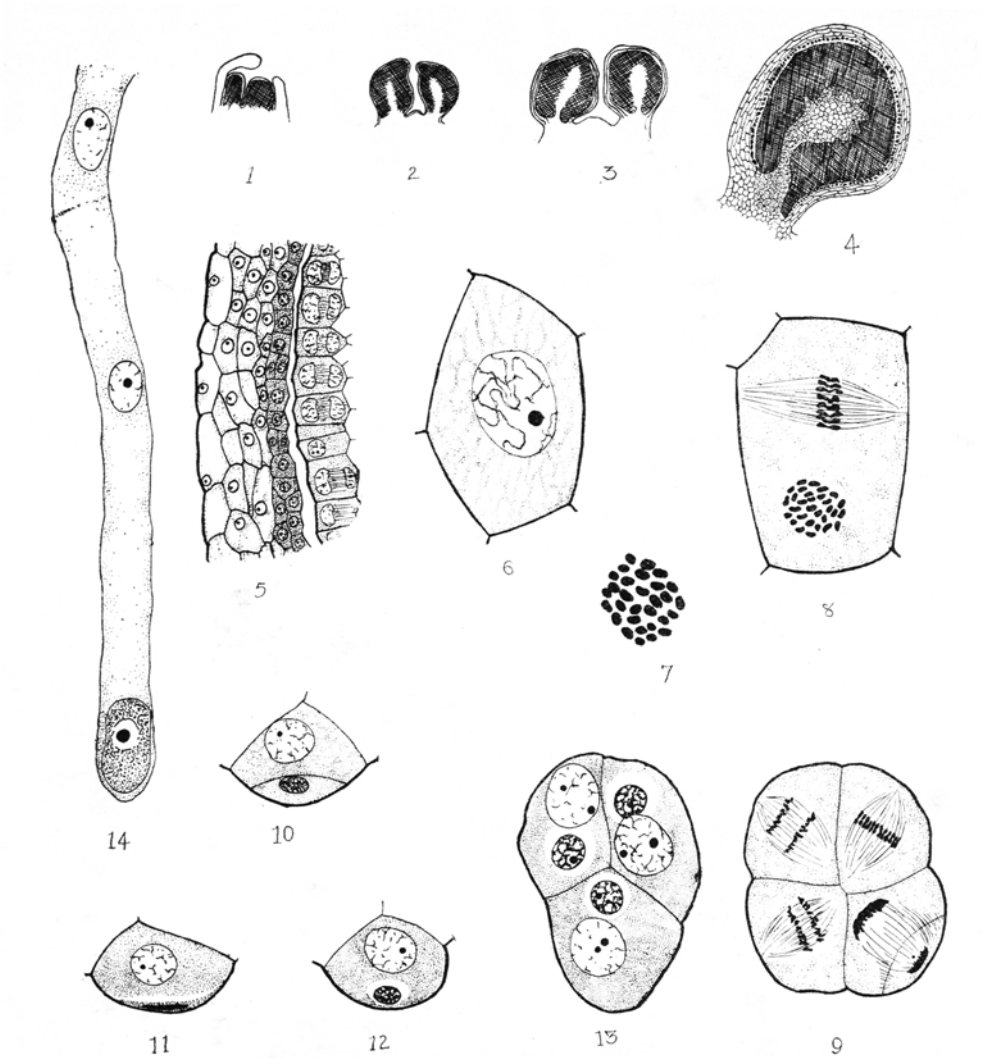
The sepals and petals are free and spreading. The spurred lip is erect and arises from the base of the column, which is up to $\frac{3}{4}$ inch in length. The sepals, petals and lip are marked with red crested nerves. The anther is two-celled and occupies a terminal position (Figs. 15 and 16). The short-stalked pollinia are attached to the discoid gland in the rostellum.

Microsporogenesis and male gametophyte.

The primordium of the anther originates as two adjacent protuberances (Fig. 1) consisting of actively dividing cells. At an early stage, the central cells become elongated and highly vacuolated, forming a core of sterile cells in the centre. The rest of the cells lying between the core and the epidermis remain highly protoplasmic and form the sporogenous tissue (Figs. 2 to 4).

The parietal tissue in the anther consists of 2 to 3 layers of cells towards the distal and 5 to 6 layers towards the basal end. Some of the tapetal cells divide periclinally so as to form a 2-layered tapetum at places here and there (Fig. 5).

The microspore mother cells (Fig. 6) undergo the usual stages of reduction division. Thirty bivalents were counted in the metaphase plate of the first division (Fig. 7) and the same number of univalents in the second division (Fig. 8). The resulting quartets may be either isobilateral (Fig. 9) or tetrahedral (Fig. 13).



Figs. 1 to 4—Diagrams representing the differentiation of the sterile core of cells and the sporogenous mass of the anther, × *ca.* 267. Fig. 5—A portion of the anther showing wall layers, tapetum and dividing microspore mother cells, × 840. Fig. 6—Microspore mother cell, × 1200. Fig. 7—Metaphase plate of division I, × 1200. Fig. 8—Metaphase of division II, × 1200. Fig. 9—Microspores of an isobilateral quartet dividing, × 1200. Fig. 10—Division of the microspores, × 1200. Figs. 11 and 12—Stages in the movement of the generative cell, × 1200. Fig. 13—A tetrahedral quartet at the time of shedding, × 1200. Fig 14—Pollen tube with two male nuclei and the degenerating tube nucleus, × 1200.

In the division of the microspore, the generative cell is always cut off towards the exterior (Fig. 10). The resulting nuclei and cells are unequal in size almost from the very beginning, the generative cell and nucleus being

smaller than the vegetative but displaying a greater avidity for stains than the latter. After a time the wall between the two cells disappears and its place is taken by a clear space (Fig. 11). The generative cell changes its original shape and becomes spherical, its cytoplasm becoming so hyaline and transparent as to look like a clear space. The plasma of the vegetative cell gradually engulfs the generative and completely surrounds it (Fig. 12). At this stage the position of the engulfed generative cell varies greatly even in the same quartet and gives no indication of the original place where it was cut off (Fig. 13). Pollinia are shed at this two-celled stage of the male gametophyte.

The microspores do not separate even at maturity. Pollen tubes are put forth simultaneously from all the four cells of the quartet. In most cases the tube nucleus is the first to enter the pollen tube but in a few instances the reverse may happen. Eventually the tube nucleus occupies the tip of the tube and becomes slightly hypertrophied before degeneration sets in. The actual division of the generative nucleus was not noticed. The two male nuclei are ovate in shape and lie behind the tube nucleus (Fig. 14).

The pollen tubes pass through the stylar canal along the large vacuolate cells that form the core of the column and reach the ovary about a week after pollination. Within the ovary they proceed along on either side of the three placental ridges (Fig. 17) and then distribute themselves to the ovules. Successful pollination is externally indicated by the enlargement of the gynostegium.

Ovule.

The ovule becomes completely anatropous at a very early stage of development. The origin and growth of the integuments can be followed from figs. 18 to 20. The inner coat consists of two layers of cells while the outer is single-layered and remains so till the end. During post-fertilisation stages the inner integument, together with the few nucellar cells at the chalaza, becomes completely disorganised so that the developing embryo is in contact with the outer integument. During later stages (when the proembryonal cells are elongating) the nuclei of the outer integumental cells also disintergrate, their cell walls thicken and become translucent. It may be mentioned here that because of this feature many of the post-fertilisation stages are very suitable for microscopic examination when whole mounts of the ovules are prepared in lactophenol or chloral hydrate.

Embryo-sac and Fertilisation.

A single hypodermal archesporial cell differentiates in the nucellus about a week after pollination. No parietal cells are cut off. Usually a T-shaped tetrad of megaspores is formed (Fig. 21), though linear ones are also frequent. The micropylar dyad cell frequently disintegrates without dividing. The nucellar epidermis becomes disorganised at the 2-nucleate stage of the embryo-sac. The subsequent stages in the formation of the embryo-sac are quite

normal. In many cases the mature embryo-sac is only 7-nucleate, due to the failure of division of one of the chalazal nuclei at the 4-nucleate stage of the

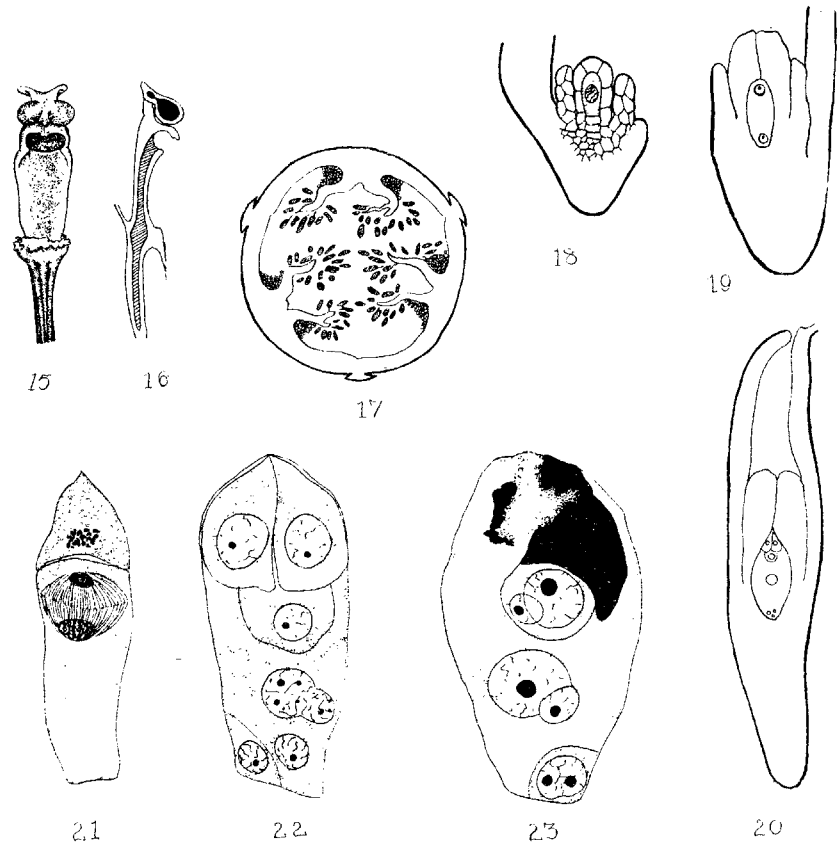


Fig. 15—Gynostegium and ovary; perianth removed, ×*ca.* 3·4. Fig. 16—Optical longitudinal section of Fig. 15, viewed from the cut surface. Pollinium is shown as a black mass. The stylar and the ovarian cavities are shaded, ×*ca.* 3·4. Fig. 17—Transverse section of the ovary, showing the placental ridges and the pollen tubes on either side of the ridges, ×*ca.* 14. Figs. 18 to 20—Stages in the development of the ovule, ×*ca.* 267. Fig. 21—A stage in the formation of the megaspore tetrad, × 1200. Fig. 22—Mature embryo-sac, × 1200. Fig. 23—Double fertilisation, × 1200.

sac (Fig. 22). The egg apparatus is organised as usual at the micropylar end and the polar nuclei fuse just before fertilisation.

The pollen tube enters the sac through the micropyle and double fertilisation takes place normally (Fig. 23). The primary endosperm nucleus, formed after triple fusion, degenerates without any further development.

Embryogeny.

The first division of the zygote is transverse. The next 3 to 6 divisions are very irregular and take place without any definite sequence, resulting

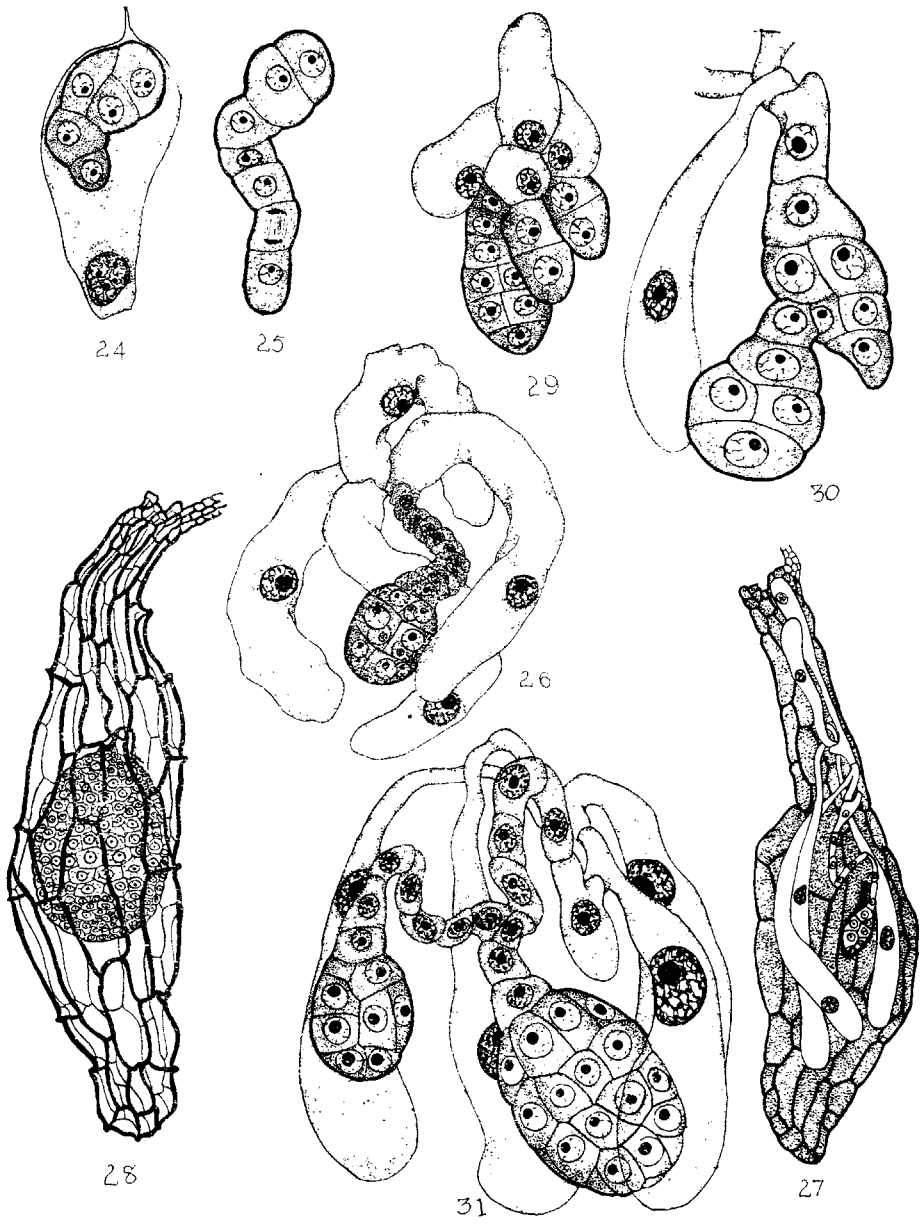


Fig. 24—An early stage in the formation of the embryo, showing the irregularly divided mass of cells, × *ca.* 534. Fig. 25—Formation of the filamentous proembryo, × *ca.* 534. Fig. 26—A typical stage in the development of the embryo ; four of the proembryonal cells have elongated and enlarged, × *ca.* 534. Fig. 27—Optical l.s. of the developing seed showing the relation of the suspensor cells to the surrounding tissues, × *ca.* 107. Fig. 28—Mature seed showing the transparent seed-coat and the embryo inside, × *ca.* 107. Figs. 29 to 31—Stages in polyembryony ; for explanation see text, × *ca.* 534.

in a mass of irregularly arranged cells (Fig. 24). One of the terminal cells of that irregularly arranged mass divides periclinally to form a filamentous row of 7 to 10 cells (Fig. 25). On the other hand, the cells at the micropylar end—usually 6 to 8—elongate and protrude (Fig. 26) out of the disorganised tissue of the inner integument and come in contact with the cells of the outer integument (Fig. 27). Their nuclei get hypertrophied to 5 or 6 times of their original size. At the same time 2-3 of the terminal cells of the proembryonal filament divide by vertical and oblique walls to give rise to the actual embryonal mass, which occupies a more or less central position in the ovule. The elongated cells of the suspensor wither away and become inconspicuous in the mature seed. The embryo now lies suspended in the cavity of the seed-coat by the degenerated and shrivelled tissue of the inner integument. The fruits open 5 to 6 months after fertilisation.

Polyembryony.

Additional embryos, which are sometimes present, are formed in one of the following ways:—

(1) The zygote divides irregularly to form a mass of cells, some of which lying at the chalazal end develop simultaneously and give rise to multiple embryos (Fig. 29).

(2) The filamentous proembryo becomes branched and an embryo may be formed at the tip of each branch as shown in fig. 31. In one case as many as three embryos were found to have arisen in this fashion.

(3) Buds are given out from the embryo itself and these produce additional embryos (Fig. 30).

Conclusion and Summary.

Certain stages in the development of the male gametophyte show a close resemblance with those in *Cymbidium bicolor* Lindl. (Swamy, 1941). The most important features of resemblance are the remaining together of the microspores of the quartet, the cutting off of the generative cell towards the exterior and its later passage into the vegetative cytoplasm, and the division of the generative nucleus into two sperm nuclei in the pollen tube.

In the development of the embryo-sac there is a reduction in the number of antipodal nuclei, a feature which is not uncommon in the Orchidaceae (see Schnarf, 1931, p. 276). Recently it has also been reported in *Acroanthes* (Stenar, 1937-38) and *Cymbidium* (Swamy, 1942). Sharp (1912) regards this as a tendency towards the reduction of the vegetative portion of the gametophyte.

Except for some minor variations the course of development of the embryo also resembles that of *Cymbidium bicolor* (Swamy, 1942). The filamentous region of the proembryo is curved in the form of a sigma consisting of 10–15 cells. In *Cymbidium*, on the other hand, it is composed of only 5 to 10

cells becoming crushed in later stages between the embryonal mass and the prolongations of the suspensor cells.

Polyembryony originating by budding or proliferation of the sexually produced embryo or proembryo or zygote is termed cleavage polyembryony. This is admittedly a rare phenomenon in Angiosperms. In a previous paper the writer (Swamy, 1942) recorded its occurrence in *Cymbidium bicolor*, the only other form previously recorded for Orchidaceae being *Limnorchis* [1] (Ernst, 1918). *Eulophea epidendraea* is another instance of this type of development.

The author wishes to take this opportunity to express his deep sense of gratitude to Dr. P. Maheshwari of the Dacca University for his able criticism and guidance throughout this investigation.

Postscript.

Since forwarding this paper to the press, A. Ernst's monograph, 'Bastardierung als Ursache der Apogamie im Pflanzenreich (1918)', was available for reference to the author. In it no reference of any sort is made to *Limnorchis* by Ernst. It is quite probable that Webber has overlooked this fact or the original was not accessible to him. In view of these, the present case will have to be considered as the second instance among orchids to exhibit the cleavage type of polyembryony, the first being *Cymbidium bicolor* Lindl. (Swamy, 1942).

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7. Swamy, B. G. L. (1942). Female Gametophyte and Embryogeny in *Cymbidium bicolor* Lindl. *Ibid.*, **15**, 194-201.

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[1] The original paper was not available for reference. The literature has been cited from Webber [*Bot. Rev.*, **6**, p. 578 (1940)] where the plant referred to is printed as *Limnocharis* (Orchidaceae). Since no genus by this name occurs in Orchidaceae, it might probably be *Limnorchis*.