

Plant Embryology

CERTAIN PECULIARITIES IN THE EMBRYOLOGY OF ORCHIDS

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A comparative study of orchids of temperate zones in the USSR (*Orchis incarnata*, *O. maculata*, *O. militaris*, *Listera ovata*, *Ophrys insectifera*, *Cypripedium calceolus*, *Epipactis atrorubens*, *Cephalanthera longifolia*) showed variations in the structure and different degrees of reduction of the embryo sac. *Listera ovata* and *Ophrys insectifera* exhibited maximum reduction (6-4 nuclei).

The fertilization process varies in the species studied and several endosperm nuclei may be formed (*E. atrorubens*, *C. longifolia*, and *C. calceolus*) or the second sperm may fail to fuse with the fusion (secondary) nucleus (*O. insectifera*, and *L. ovata*).

The analysis of published data and author's own observations made it possible to conclude that within the orchid family the species with more reduced embryo sac have more frequent abnormalities in double fertilization. Among the species studied, various types of fusion of sexual nuclei were found which, according to Gerassimova-Navashina's (1954) classification, may be classified as pre- and postmitotic types.

Orchids are suitable for embryological studies, particularly for the study of fertilization because of its long duration, large size of sexual nuclei, and numerous ovules at different stages of development in the same ovary. The process of nuclear fusion during fertilization, as well as the successive stages of embryogenesis can be studied easily.

At the same time, there are specific peculiarities in the embryology of these plants which are of much interest. The variability and reduction observed during development of female gametophyte, abnormalities in double fertilization, and suppression of endosperm formation are characteristics of orchid embryology.

The orchids of temperate zones of the USSR (*Orchis incarnata* L., *O. maculata* L., *O. militaris* L., *Listera ovata* R. Br., *Ophrys insectifera* L., *Cypripedium calceolus* L., *Epipactis atrorubens* (Hoffm.) Schult., *Cephalanthera longifolia* (L.)] have been studied.

The orchids are characterized by the reduction and variability of the number of nuclei, and divisions during megasporogenesis. The megasporogenesis may be completed with the formation of tetrad of megaspores, a row of three cells (triad), or dyad of two cells. The tetrads and triads are characteristic of the subfamily Monandreae. The formation of dyads is characteristic of the subfamily Diandrae. The Occurrence of triads in orchids is twice as much as in tetrads (Abe, 1972).

There is a tendency towards reduction in the number of embryo sac nuclei from 8 to 4. This can be observed in the same species (Figs. 1, 2, 3). The reduction is essentially due to various irregularities in the behaviour of the chalazal nuclei of

the embryo sac, by due to reduction in the number of nuclear divisions in the chalazal region, by fusion of spindles of the dividing chalazal nuclei, or by fusion of nuclei in the central cell of embryo sac. The maximum reduction (6-4 nuclei) of embryo sacs occurs in *Listera ovata* and *Ophrys insectifera*.

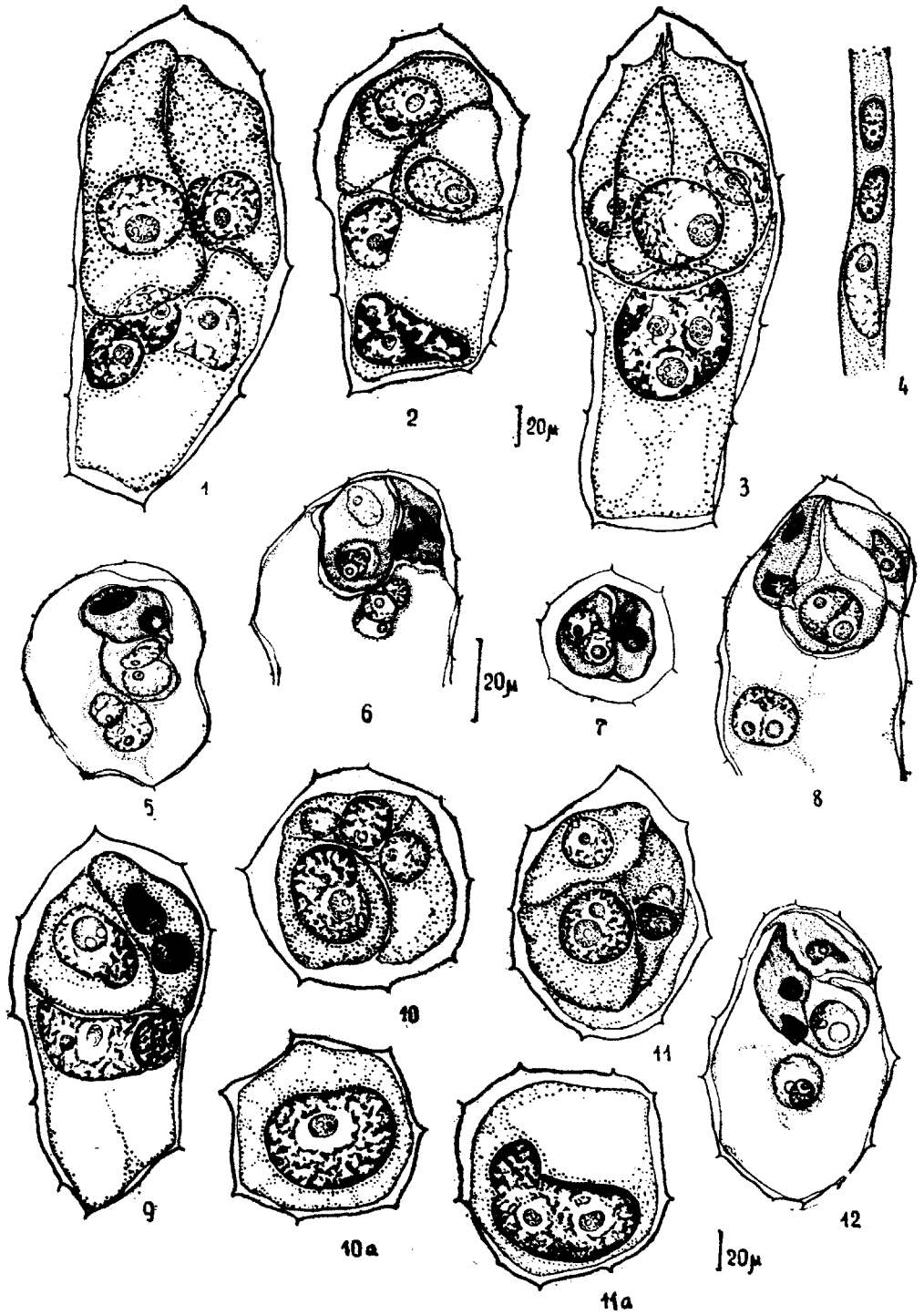
The antipodal apparatus in orchids has a tendency not only to the reduction of its components, but also to the change of organization from the cellular to the nuclear. In the 8-nucleate embryo sacs the antipodals are usually represented by 3 cells; in the 5- and 6- nucleate embryo sacs 1 or 2 free nuclei are usually present at the chalazal end. Sometimes, they approach the polar nucleus, forming a polar-antipodal (polar-chalazal) complex (Fig. 1). In certain representatives the antipodals of the same species, and even of the same individual, may organise into cells or remain as free nuclei (*Epidendrum variegatum* — Sharp, 1912; *Orchis morio* — Afzelius, 1916; *Platanthera tipuloides* and *Phajus minor*—Abe, 1972 b). The number of elements composing the antipodal apparatus may be unstable varying within the limits of the same species.

The spermatogenesis in orchids is known to occur in the pollen tube. In the already-formed sperm cell a small quantity of fine-grained cytoplasm is concentrated around the nucleus. The male nucleus is more or less chromatic, gives an intensive Feulgen reaction, and contains a small nucleolus (Fig. 4).

The process of fusion between male and female nuclei varies in different species. In some species (*Cypripedium calceolus*, *Epipactis atrorubens*, *Orchis militaris*, *O. maculata*, *O. incarnata*, *Ophrys insectifera* and *Cephalanthera longifolia*) the male nucleus comes in contact with the egg nucleus and becomes globular; its Feulgen reaction weakens and the size of the nucleolus increases. As a result of these changes the male nucleus looks similar to the egg nucleus (Figs. 5-7). Then, after a while, the Feulgen reaction of the nuclei in contact as well as their chromatic reaction becomes more intense which apparently indicates the onset of mitosis. The gradual dissolution of a wall between them is simultaneously observed (Fig. 8) This type of nuclear fusion is postmitotic type (Gerassimova-Navashina, 1954).

In *Listera ovata* a strongly chromatic male nucleus does not become globular, but starts to fuse with the egg nucleus preserving at first its prolonged shape and intensive Feulgen reaction. The sperm despiralization takes place eventually (Figs. 9-11) and, after a certain period of rest, the zygote enters mitosis. The fusion of nuclei is a slow process and, therefore, convenient to study the successive stages of nuclear fusion. The above-mentioned type of sexual

FIGS. 1-12. 1-3, Mature embryo sacs of *Listera ovata*; 4, Pollen tube of *Epipactis atrorubens* containing two sperms, and vegetative nucleus; 5-8, Successive stages of fertilization in *Epipactis atrorubens*; 9-11, Successive stages of fertilization in *Listera ovata*; 10-10 a, Transections of the same embryo sac; 10, Egg apparatus. One of the sperms fuses with the egg cell nucleus, the second sperm is in the synergid. 10a, Central cell showing the central cell nucleus; 11 & 11a, Transections of the same embryo sac; 11, Fusion of sperm with the egg cell nucleus; note the zygote; 11a, Fusion of sperm with the central cell nucleus has not yet been completed; 12, A rare case of premitotic type of fertilization in *Epipactis atrorubens*.
↑



(FIGS. 1-12)

nuclear fusion can be classified as premitotic (Gerassimova-Navashina, 1954). Occasionally, the premitotic type of fertilization occurs in species where, as a rule, postmitotic type takes place (Fig. 12).

In many orchids double fertilization proceeds normally. At the same time, in some species various deviations from the course of double fertilization were observed. The irregularities occur mainly in the second stage of fertilization, i.e. when the male nucleus and egg nucleus fuse.

In the case when double fertilization takes place, the fate of the primary endosperm nucleus varies in different species. Only in some of the orchids studied, the primary endosperm nucleus divides forming several nuclei (Savina, 1974). In the majority of orchids the primary endosperm nucleus degenerates without division (Swamy, 1949; Abe, 1972a). Various phases of the onset of degeneration of the primary endosperm nucleus have been reported: (a) immediately after fertilization, (b) by the time of first division of fertilized egg cell, and (c) during the formation of the 2- or 4-celled proembryo. Only in a few of the studied species a prolonged existence of the primary endosperm nucleus in the active state, as well as late degeneration was observed (Tohda, 1968; Teriokhin & Kamelina, 1969).

Hagerup (1944) suggested that the polar nuclei are 'dead' by the time of fertilization. Gerassimova-Navashina (1954), and Poddubnaya-Arnoldi (1958) believed that irregularities in the double fertilization of orchids are due to the reduction in the embryo sac. This was demonstrated by Poddubnaya-Arnoldi (1958) in *Paphiopedilum insigne*, *Dendrobium nobile* and *Calanthe veitchii*, showing various degrees of reduction. A species with maximum reductions (*Calanthe veitchii*) showed extremely irregular double fertilization, and endosperm formation did not take place. The present study confirms these findings.

The use of histochemical method (Zinger & Poddubnaya-Arnoldi, 1959; Zinger, Poddubnaya-Arnoldi & Petrovskaya-Baranova, 1964) made it possible to trace the localization of physiologically active substances and enzymes in the orchid ovule. It demonstrated that the orchid embryo sac differs greatly from the embryo sac of other angiosperms by its sharply decreased physiological activity. It was established that a peculiar feature of orchids is suppression of oxidative process, and the loss of some physiologically active substances. There is a correlation between the morphological peculiarities, and their physiological activity. The investigators mentioned above, concluded that the reduction of orchid embryo sac is apparently connected with the suppression of oxidative processes, inertness of nitrogen metabolism, and lack of physiologically active substances (heteroauxin, and ascorbic acid).

Recently, ultramicroscopic technique has been used for the study of orchid embryology. The research on *Epidendrum scutella* (Cocucci & Jensen, 1969 a, b) provided the data concerning the structure of a mature embryo sac, and the changes therein after fertilization. It was shown that ribosomes are characteristic for a non-fertilized egg cell, and the central cell. After fertilization

they combine into polysomes. This, as the authors note, shows that the above-mentioned cells of the embryo sac pass from the state of relative dormancy to active state. Further, in *E. scutella* the endosperm does not develop beyond the initial stage and the authors assume that the factors determining the abortion of endosperm are connected with the phenomena of nuclear fusion or division.

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