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THE REPRODUCTIVE SYSTEM AND GERMINATION IN ORCHIDS

TATYANA B. BATYGINA*, ELENA A. BRAGINA, AND VALENTINA E. VASILYEVA

Department of Embryology and Reproductive Biology, Komarov Botanical Institute, Prof. Popov Str., 2, 197376 St. Petersburg, Russia

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The peculiarities of seminal and vegetative multiplication in orchids are discussed. The main pathways of morphogenesis during the formation of the whole organism of orchids in natural conditions and in vitro culture are reviewed. Peculiarities of development and the role of the protocorm in the life cycle are pointed out. Special attention is paid to the phenomenon of polyembryony of orchids and their germination.

Key words: Orchids, polyembryony, germination, protocorm, brood bud, reproductive strategy.

INTRODUCTION

There are three morphogenetic pathways to the formation of a new plant: embryogenesis, embryoidogenesis and gemmorhizogenesis (Batygina, 1984). During the evolution of the orchid reproductive system, as in some other plants, a definite program for changing morphogenesis was produced. The transition from one pathway to another (e.g., embryogeny →cleavage embryoidogeny) is observed to occur at different stages of ontogenesis, occasionally even at the earliest ones (Batygina, 1998). The formation of the protocorm is considered to be a peculiarity of postseminal development in orchids. The term "protocorm" was first introduced by Treub (1890) in lieu of the previously used "embryonic tuber" (*tubercule embryonaire*) to describe a tuberidiform structure with hairs on the lower part, which is formed during germination of club moss embryos. This term was used by Balfour (1905; in: OXFORD ENGLISH DICTIONARY, 1989) to designate the seedling of orchids.

Apart from designating the seedling in orchids and in club mosses, the term is used in a wider sense. For example, Jacques-Félix (1982) uses it to designate the embryo proper in all angiosperms. Most Russian researchers consider the protocorm of orchids to be a seedling (Batygina and Vasilyeva, 1980, 1983a,b; Batygina and Shevtsova, 1985; Batygina and Andronova, 1991; Vakhra-meeva et al., 1991; Vinogradova and Filin, 1993; Kulikov, 1995; Vinogradova, 1996, 1999; Tatarenko, 1996; Batalov, 1998). One also encounters different interpretations of the structure, which is formed during seed germination in orchids. Some researchers view it as an embryo (Teryokhin, 1977; Möller, 1989). Thus the designation of the orchid protocorm – seedling or embryo – is still debatable. Viewpoints on the morphogenetic nature of protocorm organs are likewise divergent. The boundaries of the protocorm stage in the life cycle are also variously defined.

Vegetative propagation in orchids is represented by different forms: monozygotic cleavage, nucellar and integumentary embryoidogeny, apogamety, cloning of protocorms, bud and brood bud. The basic peculiarities of reproduction in orchids are polyembryony, genetic heterogeneity of seeds, and protocorm formation on generative and vegetative organs. The genetic program can switch over from heterophasic to homophasic reproduction at different stages of ontogenesis (Batygina, 1998).

* e-mail: batygina@TB1390.spb.edu

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SEED REPRODUCTION

Intensification of seed productivity has accompanied major modifications in the organization of the orchid gynoecium. Teryokhin (1977) distinguishes two principal tends in gynoecium evolution: enlargement of the ovary placental surface and reduction of the ovule. The ovule of orchids is anatropous and tenuinucellate, with one or two integuments (Savina and Poddubnaya-Arnoldi, 1990). The vascular bundle does not enter the funiculus (Swamy, 1947; Poddubnaja-Arnoldi, 1982). In most orchids the nucellus is represented only by the epidermis enveloping the megaspore mother cell. In certain species (*Gymnadenia conopsea*, *Hetaeria nitida*, *Galeola septentrionalis*), apart from the epidermal layer the apical cells of the axial row (postament) also constitute the nucellus (Shamrov and Nikiticheva, 1992). The character and longevity of the nucellus varies with the taxon. In *Paphiopedium insigne* (Afzelius, 1916) and *G. septentrionalis* (Kimura, 1968) the nucellus in the chalazal portion of the ovule remains until fertilization, while in *Epipogium aphyllum* the nucellus persists completely at the stage of the mature female gametophyte (Afzelius, 1954). In some species the nucellus cells begin to degenerate during early embryogenesis, for example in *Gastrodia elata*, *Cypripedium parviflorum* and *Bulbophyllum neilgherrense* (Kusano, 1915; Carlson, 1940; Swamy, 1949a,b), or they continue functioning in this period, as in *Gymnadenia conopsea* and *Listera ovata* (Shamrov and Nikiticheva, 1992). The hypostase has been mentioned for just a few species of orchids such as *Calanthe veitchii*, *Cypripedium insigne*, *Dendrobium nobile*, *G. conopsea* and *L. ovata* (Zinger and Poddubnaya-Arnoldi, 1966; Shamrov, 2001).

The archesporium is usually uni- to multicellular, and parietal cells are absent (Johri et al., 1992). Megaspore tetrads are linear or T-shaped; sometimes megaspore triads are formed instead of tetrads (Swamy, 1949a; Savina, 1979; Andronova, 1988; Lagutova and Chebotaru, 1992). In Cypripedioideae the female gametophyte develops according to the Allium type. In Orchidoideae, development follows the Polygonum type or Adoxa type (Carlson, 1940; Savina and Poddubnaya-Arnoldi, 1990; Johri et al., 1992). Cases of the development of bisporic female gametophyte are noted in primitive taxa of unistamenal orchids as well, as in species from *Neottiae* and *Epipogiae* (Hagerup, 1947; Arekal and Karanth, 1981). In certain orchid species, both mono- and bisporic female gametophytes occur even in the same ovary (Andronova, 1988; Law and Yeung, 1993). The orchids are characterized by reduction of the number of nuclei in the monosporic female gametophyte from 8 to 4. Synergids are pear-shaped, with a filiform apparatus. Antipodal cells are represented by nuclei or cells (1–3). Antipodal and polar nuclei fuse in some orchids (*Calanthe*), creating a polar-antipodal group.

It has been demonstrated that fertilization proceeds normally in certain orchid species (Savina and Poddubnaya-Arnoldi, 1990). Double fertilization is normal in Diandrae, whereas usually only syngamy occurs in Monandrae (Johri et al., 1992). Different abnormalities, for example abnormal fusion of the second sperm with the nucleus or nuclei of the central cell or the complete absence of this fusion, are not infrequent. Triple fusion occurs sporadically in certain species investigated (e.g., in *Listera ovata* and *Ophrys insectifera*; Savina, 1979).

Certain authors consider the breakdown of double fertilization in orchids to be a consequence of female gametophyte reduction, which may be caused by insufficient nutrient supply, since the ovule in this family is known to have minimal vascular supply almost always. This feature is probably connected with depression of respiration, the sluggishness of nitrogen exchange as indicated by a lower quantity of amino acids, and the impoverishment of such substances as heteroauxin, ascorbic acid, etc. (Zinger et al., 1964).

Both premitotic and postmitotic types of sperm nucleus fusion with the egg cell nucleus have been found in orchids (Savina, 1979).

The endosperm of orchids is nuclear. In certain cases double fertilization does not occur and the endosperm does not form. The degree of endosperm development and degeneration differs between taxa. The data of some authors indicate that endosperm reduction in orchids is directly dependent on the degree of embryo reduction and/or on the degree of suspensor development (Teryokhin and Kamelina, 1969, 1972; Teryokhin, 1977).

Embryo development in orchids proceeds by the Asterad, Onagrad and Cymbidium types (Swamy, 1949). In the Cymbidium type, the divisions in the terminal and basal cells of the two-celled proembryo are irregular. Piperad type of orchid embryogenesis was described later (Savina and Poddubnaya-Arnoldi, 1990).

In the majority of species the embryo in the mature seed shows histological differentiation, displaying three tissues – epidermal, meristematic (small-cellular) and parenchymal (large-cellular).

Cell size is heterogeneous in the protoderm layer. The ratio of dimensions of these zones varies by species and by embryo development stage. Differentiation of vascular elements is absent (Veyret, 1965, 1974; Batygina and Vasilyeva, 1983a,b; Batygina and Shevtsova, 1985; Shamrov and Nikiticheva, 1992; Andronova, 1997).

The embryo of *Epipogium aphyllum*, consisting of only a few cells, is the least developed. In *Spiranthes autumnalis* and *Manniella reich* embryos the cells of the basal portion reach exceptionally great size. The embryo is largest in Laelieae and Polystachyeae subtribes. In *Epidendrum radicans* (subtribe *Laelieae*) the embryo has a suspensor and is largest. The wide small-cellular region in the zone of the future shoot apex is distinguishable and the protoderm is very conspicuous. In some genera of orchids (*Arundina graminifolia*, *Bletilla striata*, *Epidendrum vitellinum*, *Polystachya microbambusa*, *Sobralia macrantha*, *Thunia alba*) the apical part of the embryo forms a "tubercle," referred to as a "cotyledon" (Veyret, 1965, 1974; Teryokhin and Nikiticheva, 1968; Nishimura, 1991).

Polyembryony is typical of the representatives of Orchidaceae (Hagerup, 1944; Swamy, 1949b; Poddubnaya-Arnoldi, 1960). The presence of several embryos in a single seed has been mentioned in certain tropical species; this feature does occur rarely in species growing in the temperate zone, such as *Dactylorhiza baltica* (Andronova, 1988) and *Hammarbya paludosa* (Bragina, 2001). Additional embryos can be created as a result of nucellar, integumentary and monozygotic cleavage embryoidogeny, as well as gametophytic apomixis (Batygina, 1998). Additional embryos of different origin occasionally are observed in the same species (e.g., *Calanthe veitchii*).

Cleavage embryoidogeny (Fig. 1) is widespread among the orchids and can be observed at different stages of daughter sporophyte development (*Calanthe veitchii*, *Habenaria platyphylla*, *Eulophia epidendraea*, *E. nuda*, *Geodorum densiflorum*) (Swamy, 1949b; Poddubnaya-Arnoldi, 1960). Swamy (1943) described two variations of the development of monozygotic twins in *Eulophea epidendraea* seed: (1) the zygote divides with abnormalities, forming a great number of cells; the cells located closer to the chalazal end grow simultaneously and give rise to numerous somatic embryos (embryoids); and (2) somatic embryos form from suspensor cells or from the body of an embryo itself; this is observed at late stages of daughter sporophyte development.

In the case of nucellar and integumental embryoidogeny (Fig. 1), additional somatic embryos in the orchid seed can be formed from cells located beyond the female gametophyte, from cells of the nucellus or integument (*Zeuxine sulcata*, *Zygopetalum mackayi*, *Spiranthes cernua*, *Gastrodia elata*) (Poddubnaya-Arnoldi, 1960). An interesting type of somatic embryo development has been described in *Nigritella nigra* (Afzelius, 1928, 1932). As in other representatives of this family, the nucellus is reduced to one layer of cells which surrounds the megaspore mother cell. Then the chalazal megaspore, which remains viable, produces a female gametophyte. Its development, however, stops at the nuclear stage. Simultaneously with the development of the female gametophyte, one of two cells of the nucellar epidermis grows in size, divides and give rise to somatic embryos. The latter are located at the micropylar end of the female gametophyte.

In one of the races of *Spiranthes australis*, Swamy (1948) reported the development of somatic embryos out of integumentary tissues. Fertilization is absent, and cells of the inner layer of inner integument give rise to somatic embryos, some of which (2–6) can reach maturity. Cleavage, nucellar and integumental embryoidogeny, as well as gametophytic apomixis (agamospermy) are generally typical for tropical orchids.

Gametophytic apomixis was discovered in a number of orchid species (*Phalaenopsis schilleriana*, *Stanhopea tigrina*, *Neottia nidus-avis*, *Goodyera repens*, *Cymbidium*, *Zygopetalum mackayi*, *Cypripedium insigne*) (Fig. 1). In the seed of *Calanthe veitshii* two embryos are formed, one from the egg cell and one from a synergid (Poddubnaya-Arnoldi, 1960). The same has been described in *Ophrys militaris* (Savina and Poddubnaya-Arnoldi, 1990).

Polyembryony follows two patterns of development: (1) sexual and asexual processes take place side by side in one seed; and (2) cloning of either maternal or daughter organisms occurs. Embryos and seedlings formed by nucellar or integumentary embryoidogeny or by gametophytic apomixis (except in androgenesis and hemigamy) are genetically different from those produced by monozygotic embryoidogeny occurring in the same seed. The seeds are therefore genetically heterogenous.

Genetic heterogeneity of seeds is clearly manifested in orchids (Fig. 1) (Batygina, 1999). In nucellar and integumental embryoidogeny and in gametophytic apomixis, cloning of the mother sporophyte occurs, but in the case of monozygotic embryoidogeny a new daughter sporophyte is formed.

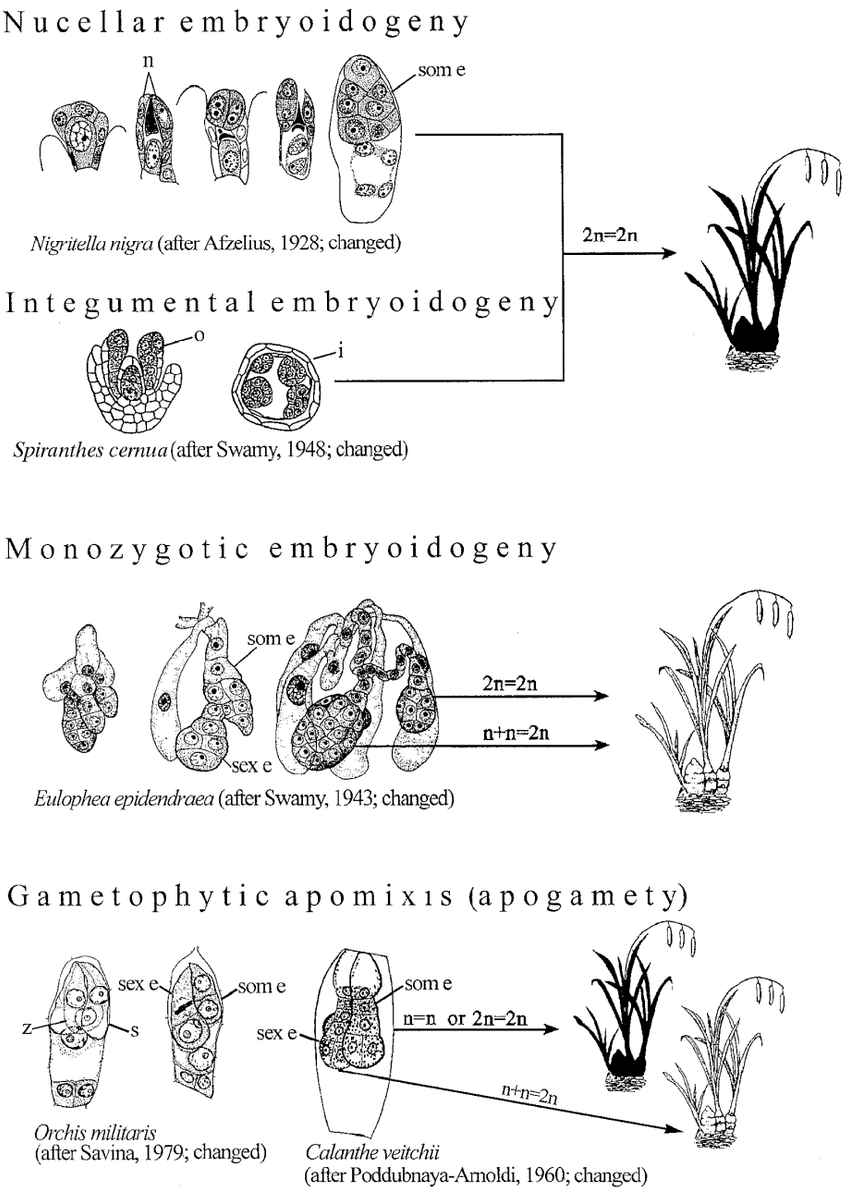
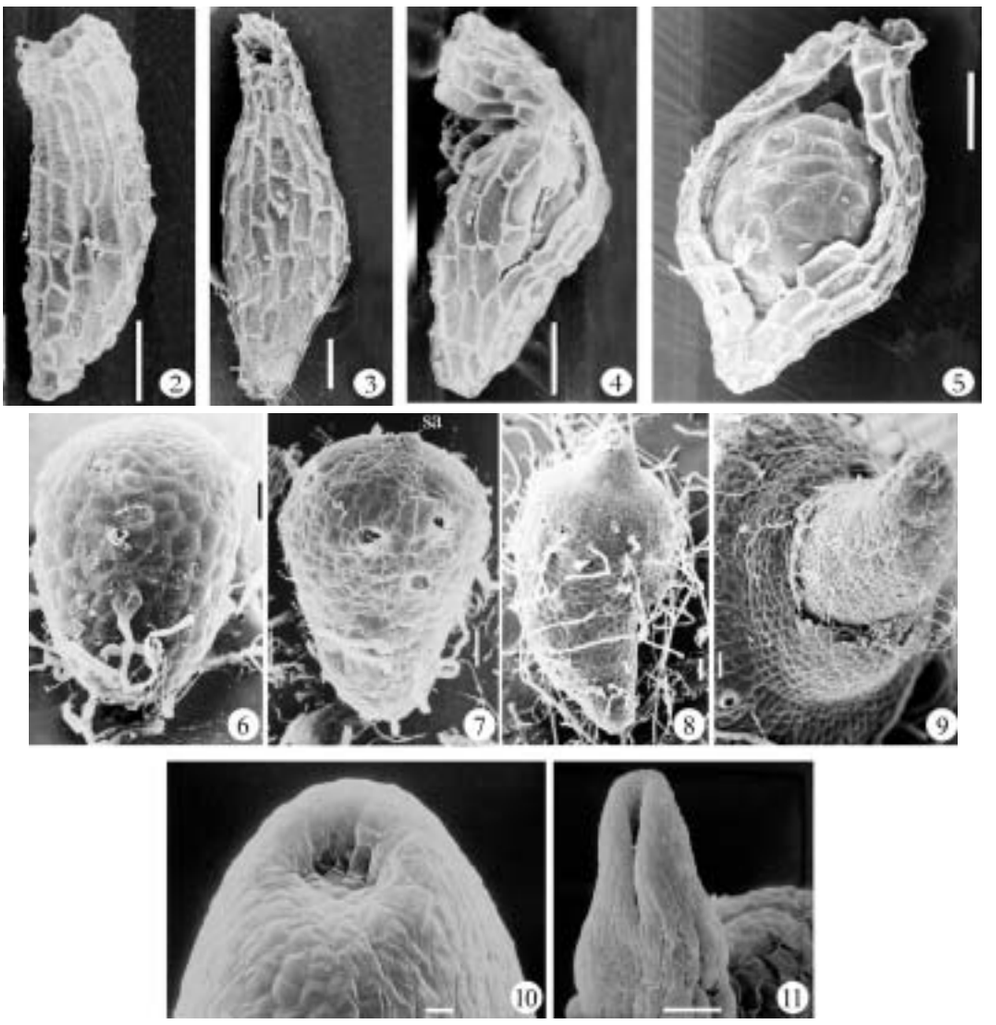


Fig. 1. Pathways of embryo formation resulting in polyembryony in orchids. Plant with uniparental heredity is shown black, plant with biparental heredity is shown white. i – integument; n – nucellus; o – ovule; s – synergid; sex e – sexual embryo; som e – somatic embryo; z – zygote.

Seed germination begins with utilization of reserve substances and embryo swelling (Figs. 2, 3). The seed coat cracks and the protocorm emerges from it (Figs. 4–9). Early in germination, chlorophyll appears in the protocorm cells of certain orchids (*Bletilla*, *Thunia*, *Cymbidium* etc.) as described by Yakovlev and Zhukova (1973). In a number of cases, embryos turn green at the moment of dissemination, as in *Microelia hirshbergii* (Savina and Poddubnaya-Arnoldi, 1990). However, most terrestrial orchids do not develop chlorophyll for several months (Dressler, 1990).

The form of the orchid protocorm is taxon-specific: oboviform, oval, elongated, disk-shaped,

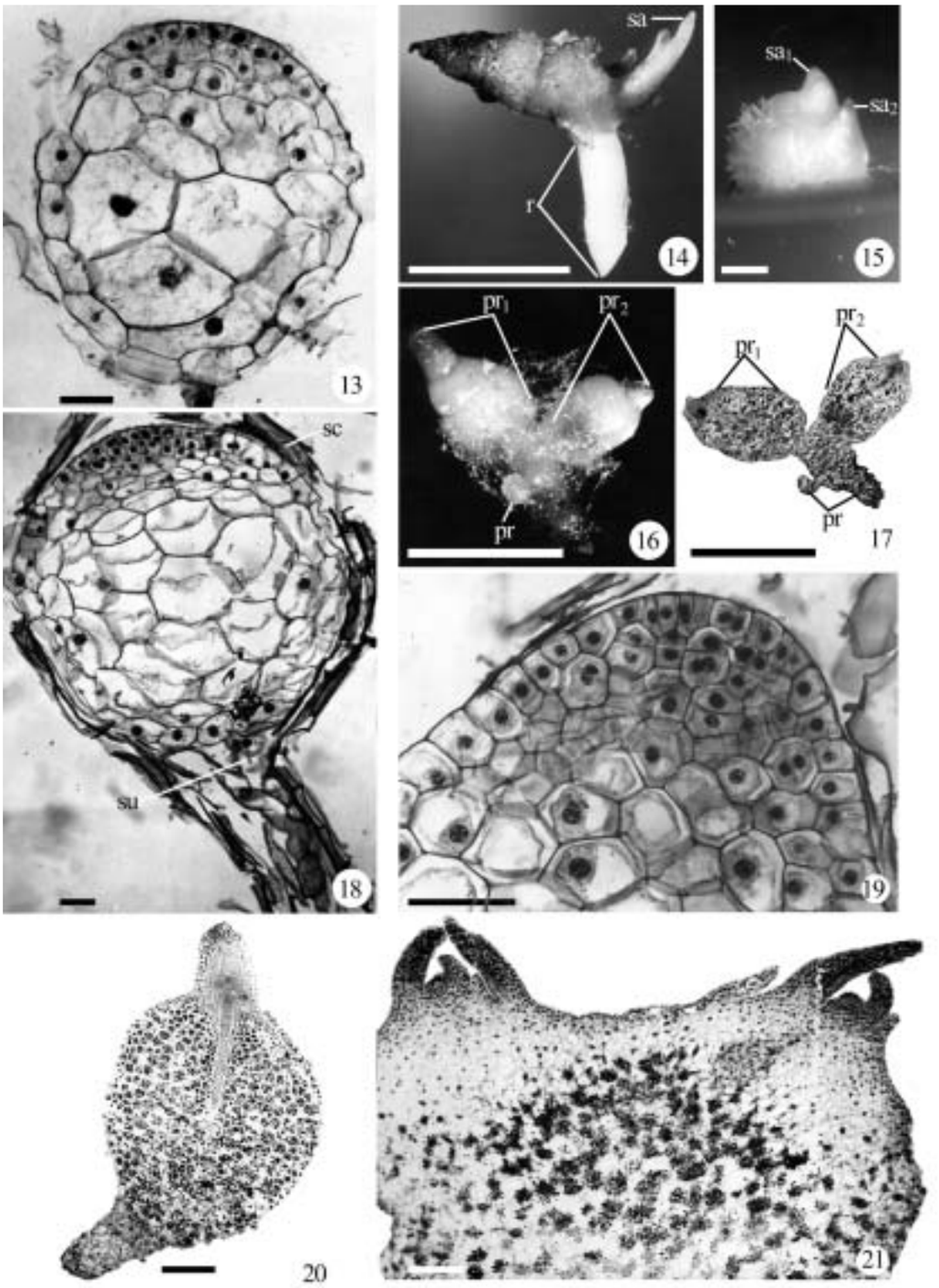


Figs. 2–9. *Dactylorhiza maculata* (L.) Soó. SEM photographs of protocorms growing under natural conditions. **Fig. 2.** Seed before germination. **Fig. 3.** Swollen seed. **Figs. 4–5.** Stage of testa breaking. **Fig. 6.** Protocorm before organ differentiation. **Figs. 7–8.** Protocorms with leaf primordium. **Fig. 9.** Shoot apex with leaf primordium. sa – shoot apex. Bars in all figures = 100 µm. **Figs. 10–11.** Successive stages of first leaf development in *Thunia alba* Rchb. Bar = 10 µm (after Batygina and Andronova, 1988, 2000).

branched, thorn-shaped, spherical or spindle-shaped (Veyret, 1965, 1974; Batygina and Shevtsova, 1985; Clements, 1995, in: Cribb, 1999; Vinogradova, 1999). In its early development, usually the protocorm is radially symmetrical in the genera examined. As the shoot organs are formed it can either retain its symmetrical structure (*Bletilla, Dactylorhyza*) or become asymmetrical (*Calipso bulbosa*). In some genera the protocorm becomes a dorsoventral structure (*Phalaenopsis*).

The apical part of the protocorm, consisting of smaller cells, is the shoot apex. The basal part, functioning as a "storage organ," consists of larger

Fig. 12. Different age groups of *Dactylorhiza maculata* (L.) Soó plants at the beginning of the vegetative season (end of April).



Figs. 13–21. Development of orchid protocorms in culture in vitro. **Figs. 13–17.** *Dactylorhiza maculata* (L.) Soó. **Fig. 13.** Embryo 1.5–2 months after cultivation. **Fig. 14.** Germinating protocorm. **Fig. 15.** Protocorm with two shoot apices. Figs. **16–17.** "Budding" of protocorms originating from one embryo. **Figs. 18–21** *Cymbidium hybridum* Hort. **Fig. 18.** Embryo in swollen seed (195 days after pollination). **Fig. 19.** Apical part of embryo (3 weeks after cultivation). **Fig. 20.** Protocorm with leaf primordium. **Fig. 21.** Protocorm with two shoot apices. sa, sa_1, sa_2 – shoot apices; sc – seed coat; su - suspensor; r – root; pr, pr_1, pr_2 – protocorms. Bar in Figs. 13, 18, 19 = 0.01 cm, in Figs. 14, 16, 17 = 0.5 cm, and in Figs. 15, 20, 21 = 0.1 cm.

parenchymatous cells (Figs. 6–8, 13, 18, 20). In its early development, usually the leaf primordium looks like a closed ridge (*Listera ovata*) or open ridge (*Dactylorhiza baltica*) (Batygina and Andronova, 1991). As the primordium keeps growing, the opening formed by the edges of the ridge gradually moves to a lateral position. The primordium becomes cone-shaped. In a number of cases the edges of the space close completely.

Interpretations of the first foliar organ of orchids differ. It is referred to as either a cotyledon (Burgeff, 1936; Teryokhin, 1977) or as a leaf proper (Batygina and Vasilyeva, 1983a,b). Teryokhin and Nikiticheva (1968) state that not only the first but also the second appendicular organs formed on the protocorm are cotyledons.

The term "cotyledon" is still debatable. The majority of researchers consider cotyledons to be modified leaves initiated at the embryo stage of sporophyte development in flowering plants. In view of the fact that the foliar organs in the majority of orchids appear during postseminal development, that is, at the protocorm stage, it is inexpedient to refer to them as cotyledons or leaf-like organs (Batygina and Vasilyeva, 1983a,b; Batygina and Andronova, 1991). These are the shoot leaves proper, whereas the embryo itself has no cotyledons.

Research by Andronova and Batygina (1992) and Batygina et al. (1994) using scanning electron microscopy of the embryo and the protocorm demonstrated that the structures produced in the apical part of the embryo differ completely in structure and form between two taxa: in *Bletilla striata* an appendicular organ resembling a crescent-shaped roll forms, whereas the embryo in *Thunia alba* (Figs. 10, 11) is devoid of any rudiment of this organ. During embryo germination the apical part of the protocorm forms a "tubercle" which consists of a number of cells. Such a structure is identifiable only at an early stage of protocorm development; it gradually disappears during the formation of apical domain cells. Formation of a foliar organ in *Thunia alba* proceeds at the protocorm stage in the same roll-like fashion as in non-cotyledonous genera (Batygina and Andronova, 1991, 2000; Andronova, 1997). Studies of the architecture of the surface layer and internal embryo tissues of the mature seed demonstrated the presence of large pyramidal cells in the axial zone of the apical part of the embryo, which retain their form and size during germination. These cells enter the mitotic period later than do other cells in the apical and the middle portions of the embryo.

The above account suggests that embryo form, as identified in longitudinal sections, is not a sufficient criterion but does establish whether or not there is a cotyledon, that is, the rudiment of a foliar organ. The data on embryo and seedling development in *Thunia alba* indicate the absence of an appendicular organ. Therefore this genus most likely should be excluded from the "cotyledonous" type.

The embryos and seedlings in *Bletilla striata* and *Sobralia macrantha* can also be classified as non-cotyledonous because their appendicular organs are not modified.

No radicular meristem is formed in the basal part of the protocorm. The roots of orchids are adventitious and appear after differentiation of the shoots (Figs. 12, 20) according to Veyret (1965), Batygina and Vasilyeva (1983a,b) and Barabé et al. (1993).

The seedling develops at the expense of the nutrients accumulated in the basal organ of the protocorm (Ricardo and Alvarez, 1971; Batygina and Vasilyeva, 1983a,b). As a rule this organ functions until adventitious roots appear on the protocorm. Its life span varies between genera and also within a single genus. Data obtained in vitro demonstrate that the storage organ of the protocorm degenerates immediately after the initial adventitious root forms (*Angrecum maculatum*, *Dactylorhiza maculata*). In the case of *D. baltica*, for a number of protocorms the basal part dies off immediately following initial root formation, whereas in others it persists for a considerably longer time and is discernible even at the stage of the seedling with several developed roots (Andronova, 1997).

There are two viewpoints on the morphogenetic nature of the basal part of the orchid protocorm. Some researchers hold it to be a hypocotyl (Nishimura, 1991; Teryokhin, 1977; Clements, 1995, quoted from Cribb, 1999). According to others it is root-like, as indicated by the presence of epidermal hairs (Veyret, 1965, 1974).

In embryos of flowering plants, the boundary between the hypocotyl and root is difficult to identify. In a seedling it is easily located by the presence of epidermal hairs, which usually belong to the root and are missing in the hypocotyl. This is connected with particular characteristics of epidermis differentiation in the two organs in the seedlings of most flowering plants studied (Duckett et al., 1994). In contrast, during protocorm formation in orchids, the epidermis in practically all parts of the structure (except the shoot apex) produces epidermal hairs. Thus the basal and middle parts of the protocorm of

orchids are covered with hairs and constitute a single organ in terms of both external and internal structure (parenchymatous tissue). Apparently the basal part of the embryo and the protocorm of orchids is by nature not a root but is homologous to the hypocotyl of the embryo and the seedling in other flowering plants (Teryokhin, 1977; Clements, 1995, quoted from Cribb, 1999).

For further development of the protocorm, including the shoot apex, contact with fungal hyphae is required (Figs. 6–9). Fungal hyphae penetrate the protocorm through epidermal hair, as a rule (Kulikov, 1995; Rasmussen, 1995). Orchids differ interspecifically not only in the degree of embryo and endosperm differentiation but also in protocorm shape and dimensions. Probably in orchid evolution a certain species-specific critical mass of protocorm and corresponding critical mass of meristematic center has been elaborated, from which a bud of definite size is created (Batygina and Vasilyeva, 1980).

The duration of the period in which the protocorm is modified into juvenile plants is taxon-specific. In *Epipactis palustris* it takes 2–3 years (Summerhayes, 1951), and in *E. helleboriae* 9 years after seed germination (Ziegenspeck, 1936). The protocorms of certain Orchideae species require no more than a year for development. In the second year they become juvenile plants having the same growth characteristics as adults, and the first flowering of a new specimen takes place after 3–5 years (Möller, 1989). These data agree with the results from the study of *Dactylorchiza maculata* germination in natural conditions (Batygina, unpublished data) and in vitro culture (Batygina and Vasilyeva, 1983b). In *D. maculata*, by autumn one year after dissemination the protocorm possesses a fully formed apical bud, from which the shoot develops in the next year (Vinogradova, 1999). Protocorms of European orchids develop hypogealy for several years, and the first flowering of a specimen sets in from 5–7 years (most Orchideae) up to 8–17 years (*Cypripedium*) according to Ziegenspeck (1936), Curtis (1943), and Vakhrameeva and Denisova (1988). The question of the length of age stages and the life cycle in orchids is yet under discussion.

Orchid protocorms are probably secondary formations which have appeared as a result of the elimination of different stages of sexual embryo development (heart-shaped, torpedo-shaped, initiation of the shoot and main root apices) common in most flowering plants (Batygina, 1998). Similar structures, developed as a consequence of heterophase reproduction, are observed also in *Paeonia* (coenocytic-cellular structure) and in some parasitic plants (protosoma). They enable the transition from heterophase to homophase reproduction, that is, to vegetative multiplication, in different plant groups at various periods throughout their development (Batygina, 1998).

Irrespective of the type of structure (generative or vegetative) and the mode of reproduction (sexual or asexual), reproduction and propagation of orchids both under natural conditions and in vitro are related to protocorms or protocorm-like structures (Figs. 13–21), as noted by Shevtsova et al. (1986) and Batygina (1998).

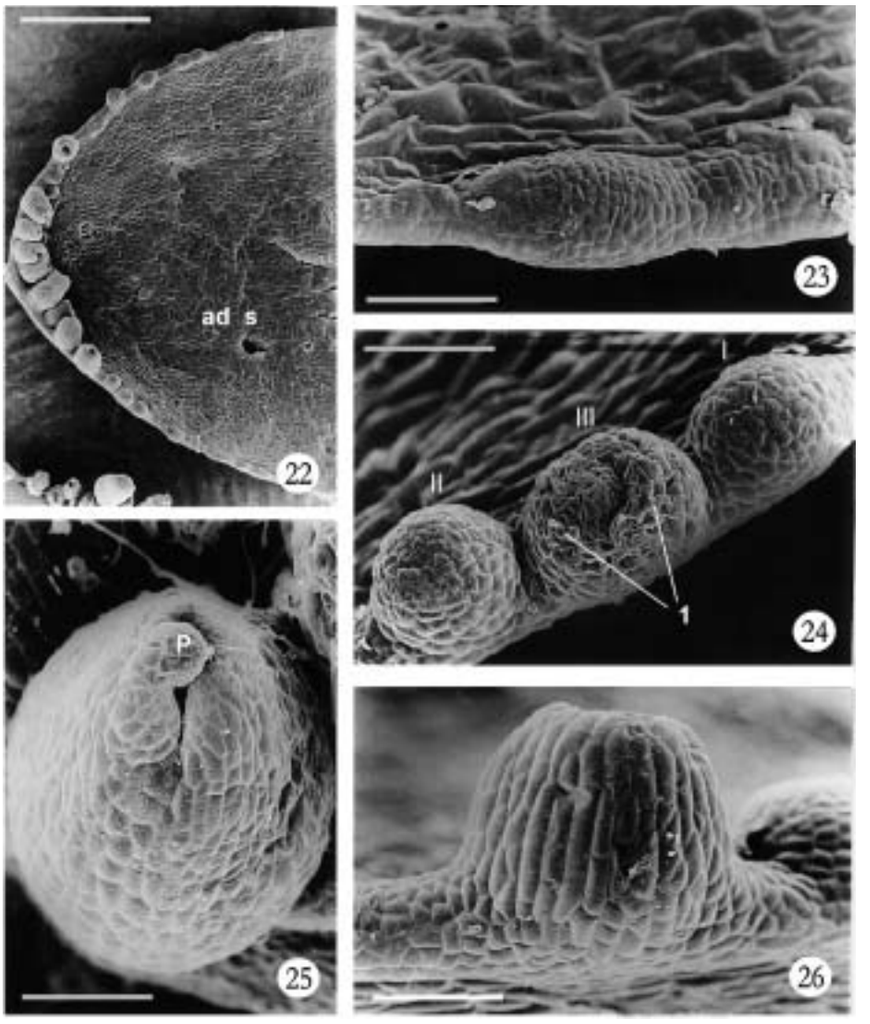
The multiplicity of modes of seminal and vegetative propagation testifies to the great reproductive reserves available at different stages of ontogenesis.

VEGETATIVE PROPAGATION

All orchid species have the capacity for vegetative propagation. It manifests itself at various stages of ontogenesis and establishes a system of reproductive reliability (Batygina and Vasilyeva, 1980; Batygina and Shevtsova, 1985; Shevtsova et al., 1986). There are several patterns of vegetative reproduction of orchids, varying between species possessing different life forms (Tatarenko and Vakhrameeva, 1998). In some groups of orchids the capacity for vegetative propagation is obvious even at the protocorm stage (Ziegenspeck, 1936; Batygina and Vasilyeva, 1983a,b; Andronova, 1988; Vinogradova, 1996). Protocorms can possess several centers of meristematic activity, but usually they develop only one shoot. Vegetative reproduction of specimens at protocorm stage is hardly ever observed in nature (Tatarenko and Vakhrameeva, 1998), whereas in culture in vitro protocorms are employed to obtain the greatest number of regenerants (Batygina and Shevtsova, 1985; Shevtsova et al., 1986).

In vitro, two pathways of protocorm cloning have been revealed: the formation of a great number of shoot apexes, with further endogenous production of adventitious roots (Figs. 15, 21, 27) and the formation of numerous secondary protocorms from epidermal cells of a single protocorm (Figs. 16, 17, 27) (Batygina and Vasilyeva, 1983a,b; Batygina and Shevtsova, 1985; Shevtsova et al., 1986; Andronova, 1988; Batygina, 1998; Andronova et al., 2000; and Batygina and Andronova, 2000).

Juvenile and immature plants usually do not reproduce vegetatively in most orchids. Young spe-



Figs. 22-26. *Hammarbya paludosa* (L.) O. Kuntze. SEM photographs of successive stages of brood bud development on leaf. **Fig. 22.** General view of brood buds on leaf tip. **Fig. 23.** Centers of meristematic activity on leaf margin arising by means of epidermis cell proliferation. **Fig. 24.** Primordia of brood bud (I, II, III) showing initiation of first leaf (falcate ridge) at apical part (II, III). **Fig. 25.** Brood bud showing first leaf, embracing inner part. **Fig. 26.** Brood bud with primordium of secondary brood bud. ads – adaxial surface of the leaf; l – first leaf; p – primordium of secondary brood bud. Bar in Fig. 22 = 1000 μm, and in Figs. 23–26 = 100 μm. (Figs. 22–26 – after Batygina and Bragina, 1997).

cimens of *Listera cordata*, *Pogonia japonica*, *Goodyera repens*, *Gastrodia elata* (Tatarenko and Vakhrameeva, 1998) are considered to be an exception. The most widespread pattern of vegetative multiplication in orchids is the formation and germination of two or more buds, including dormant ones, on axial organs such as rhizomes, creeping shoots and shoot tubers. The daughter shoots are connected with the maternal ones for a long time in short-rhizome orchids (*Cephalanthera longifolia*, *Epipactis heleborine*, *Cypripedium calceolus*). Separation of the vegetative generation happens much quicker in long-rhizome (*Cypripedium guttatum*, *Epipactis palustris*) and short-rhizome (*Calypso bulbosa*, *Oreorchis patens*) bulbotuber orchids. The daughter shoots in orchids with shoot rhizomes (tuberoids), – *Dactylorhiza*, *Orchis*, *Ophrys*, *Platanthera*, etc. separate most rapidly, after 0.5–1 years; vegetative propagation in these orchids is extremely rare, except in *Platanthera hologlottis*, a vegetative annual with a shoot rhizome tuberoid, and *Habenaria radiata*, a vegetative annual with a spherical

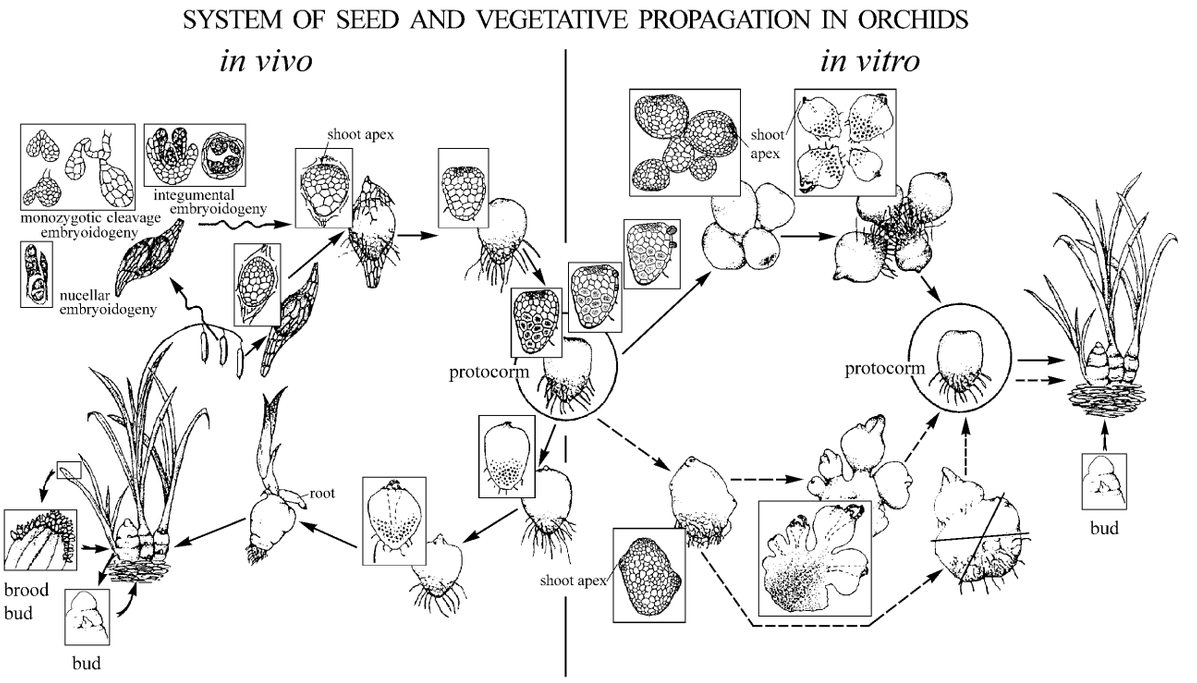


Fig. 27. System of seed and vegetative propagation in orchids (after Batygina, 1998, modified).

shoot rhizome tuberoid on a long stolon. There is a certain increase of vegetative propagation in orchids that are vegetative annuals with digitipartite shoot rhizome tuberoids (*Gymnadenia conopsea*) near the northern limit of their distribution (Tatarenko and Vakhrameeva, 1998).

The creeping-root orchids *Listera cordata* and *Pogonia japonica* can form adventitious buds on roots even in the first stages of ontogenesis (Stoutamire, 1974). *Cephalanthera rubra* and *Neottia nidus-avis* also potentially produce buds on roots (Ziegenspeck, 1936; Rasmussen, 1986; Cribb, 1999). In stress situations (drought, damage of specimen) adventitious buds arise on roots and root tips of tuberoids in *Cypripedium guttatum*, *Epipactis thunbergii*, *Tulotis ussuriensis* and *Platanthera bifolia* (Tatarenko and Vakhrameeva, 1998).

The emergence of brood buds on shoot tubers is observed in some specimens in populations of *Malaxis monophyllos* and in species of the genus *Liparis* after drought (Ziegenspeck, 1936; Tatarenko and Vakhrameeva, 1998).

Regular vegetative propagation through brood buds takes place in *Hammarbya paludosa* (Taylor, 1967; Bragina et al., 1996; Batygina and Bragina, 1997) (Figs. 22–27). A brood bud is a bipolar structure with shoot and root apexes, while a typical bud is a monopolar structure with only a shoot apex. Adventitious roots form in brood buds, but they either never appear or appear only during the regeneration process in typical buds. A brood bud is an individual, while a typical bud is part of an organism. They also function differently: a brood bud provides a means for vegetative propagation, while a typical bud participates in the growth of the vegetative body and in development of reproductive structures. The similarity of these structures lies in their genesis, location on the plant (on a leaf, stem or root) and in having a shoot apex. Brood buds of *H. paludosa* are formed exogenously from proliferation of cells in the upper epidermis of a leaf or bract. A brood bud consists of the inner part – a shoot apex with primordia of three leaves – and a leaf which completely surrounds it. Adventitious roots develop after the brood bud separates from the leaf (Batygina and Bragina, 1997; Bragina, 2001).

Thus, vegetative propagation in orchids takes different forms (cleavage, nucellar and integumentary embryoidogeny, apogamety and gemmorhizogeny) and is realized in two patterns of morpho- genesis – organogenesis and embryoidogenesis (Batygina, 1998). The elementary struc-

tural units of propagation are the bud, brood bud, somatic embryo and protocorm (Batygina, 1998). Three groups of orchids can be distinguished according to the occurrence of vegetative reproduction: (1) those with obligate vegetative propagation; (2) those with facultative vegetative propagation; and (3) those with vegetative propagation occurring in exceptional cases. The first group includes autotrophic long-rhizome and short-rhizome orchids, as well as saprophytic orchids. The second includes short-rhizome and most tuberoidous orchids, and the third is represented by rhizomacious species with epigeal shoot tubers (Tatarenko and Vakhrameeva, 1998).

The reproductive systems of the main orchid groups in the temperate zone differ in a number of features (the ratio of reproduction types, rates of ontogenesis, etc.) and in the character of adaptations to a seasonal climate (the occurrence of seed dormancy, seasonal periodicity of seedling growth) that determine their living strategy. These adaptations are most visible in the members of geophyte groups, which are connected phylogenetically with the temperate zone, while gemicryptophyte forms differ only slightly from tropical epiphytic orchids in the character of reproductive strategy (Kulikov and Philippov, 2000).

REPRODUCTIVE STRATEGY

Many reproductive structures of orchids possess great morphogenetic potential, conditioned by the high totipotency of their cells. This probably allows orchids to have a complicated system of reproduction, which accounts for their large area of distribution.

In evolution, the orchids have developed a system of morphogenetic pathways in which they switch over during the reproductive phase of the life cycle. The developmental program switches over from sexual to asexual (vegetative) reproduction during seminal and postseminal development and can be represented by different variations of morphogenesis. An example of the switching over of pathways in the orchid seed is monozygotic cleavage embryoidogeny, which results in monozygotic twins, triplets, etc., in the seed.

Morphogenesis, both in natural conditions and in vitro, proceeds with a peculiar metamorphosis of the embryo, leading to the formation of a protocorm. It is a peculiar initial form of seedling. The emergence of the protocorm in ontogenesis of orchids can be regarded as a necessary step in the transition from sexual to asexual reproduction characteristic of this highly specialized group of flowering plants. The particularity of the orchid protocorm lies in its tendency to vegetative propagation. There are two ways protocorms are differentiated and cloned: through the formation of a great number of shoot apices with further endogenous initiation of adventitious roots, and through the formation of a multitude of protocorms from epidermal cells of one protocorm.

Seminal multiplication and renewal of orchid populations in a biocoenosis strictly depends on the occurrence of specific pollinators and fungi. When at least one of these consorts falls out of the biocoenosis it brings to naught the reproductive effort of the plant and inevitably leads to the disappearance of the entire orchid population.

The potential seminal productivity of flowers in orchids is to a great extent under the influence of such peculiarities of the pollination system as the dimension of massulas, the area of the stigma, the number of pollinia on insect vectors, the degree of morphological and behavioral diversity of the pollinators, and the mode of their attraction by flowers (Nazarov, 1995). The ratio of seminal and vegetative propagation in a general system of orchid reproduction is taxon-specific (Batygina, 1998).

In primitive rhizome geophytes (*Cypripedioideae* and *Neottieae-Limodorinae*) the features of a stress-tolerant living strategy are most visibly displayed in resistance to unfavorable abiotic environmental factors, longevity, and lower rates of renewal. Vegetative propagation by the rhizome is the prevalent pattern of population maintenance. The seed crop in such populations can be both stably low (*Cypripedium guttatum*) or rather high (*C. macranthon, Epipactis* sp.) (Kulikov and Philippov, 2000). In the case of high seed production, seminal renewal is of low efficiency due to poor seed germination; that is compensated by high longevity of clones and their capacity for measurable growth (Kulikov and Philippov, 1998, 2000).

In root tuber-like geophytes of Orchideae the features of stress-tolerant species and species with low competitive ability are combined in the living strategy successfully. Maintenance of populations of this group of species depends completely on seminal renewal, except in *Herminium monorchis* and *Platanthera hologlottis* (Kulikov and Philippov, 2000). In species of Gymnadeniinae, adapted to rather damp climate, the seeds have no dormant period (Kulikov and Philippov, 1998). In this group it is typical for the share of juvenile specimens in the population to fluctuate considerably, probably a re-

sponse to alternating periods of favorable and unfavorable conditions for protocorm survival in the soil (Kulikov and Philippov, 2000). Rapid fluctuations of seminal renewal are characteristic of the Orchidinae subtribe as well, the seeds of which have a dormant period, shorter than in rhizome species (Möller, 1989; Kulikov and Philippov, 2000).

Populations of terrestrial gemicryptophytes of Epidendroideae employ seminal and vegetative propagation in approximately equal measure (*Calypso bulbosa*). However, in certain species (*Malaxis monophyllos*) seminal reproduction predominates.

Long-term studies (from 1978) under our department's program for preservation of rare and disappearing plant species have yielded data on the reproductive biology of some temperate zone orchids. On the basis of this data four orchid species have been successfully repatriated in the Leningrad region (Makoveychuk and Batygina, 1994). Juvenile plants of *Dactylorzhiza maculata* (10 plants), *Gymnadenia conopsea* (nearly 100) and *Epipactis palustris* (15) obtained by asymbiotic culture from seeds collected in nature in 1989 were transferred to soil in 1991, and plants of *Cypripedium calceolus* (nearly 50) in 1993 to the grounds of the Otradnoje experimental station of the Komarov Botanical Institute. It was established that plants from the culture in vitro undergo the first ontogenetic stages more rapidly and achieve generative state 5–6 years from the moment of seed germination. At present the total number (26 plants) and the area occupied by the artificial population of *D. maculata* has increased due to successful seed propagation.

Collection of further data and advancement of theoretical knowledge about the development of reproductive structures and methods of growing plant regenerants in large quantities will enhance the effectiveness of the program for repatriation and for the introduction or creation of secondary populations under natural conditions for rare orchid species.

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