Development of pollinium in two epidendroid orchids

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Development of pollinium has been studied in Cymbidium aloifolium (L.) Sw. (tribe Cymbidieae) and Smitinandia micrantha (Lindl.) Holtt. (tribe Vandeae) of the family Orchidaceae. The anther primordium develops two lobes oriented towards the labellum. Each lobe has a group of archesporial cells, which divide mitotically so as to form a spherical mass of sporogenous cells, in which there is differentiation of a dorsiventral plate of elongated sterile cells. In C. aloifolium, the sterile plate extends into about three-fourths of the fertile tissue, whereas in S. micrantha it is complete but asymmetrically oriented. Synchronous meiosis in the microsporocytes is followed by simultaneous cytokinesis so as to form microspore tetrads which organize into pollinia. Centripetal disintegration of the sterile plate during development of the male gametophyte forms two perforate (hollow) pollinia in C. aloifolium, but four unequal pollinia in S. micrantha. The anther wall is composed of an epidermis, an endothecium, two to three middle layers and the tapetum. The tapetum disorganizes during development of the male gametophyte, while the endothecium and adjacent middle layers develop fibrous thickenings.

Keywords: Anther, Cymbidium, Epidendroideae, Orchidaceae, pollinium, Smitinandia.

The Orchidaceae with nearly 800 genera and 25,000 species represents one of the most highly evolved families of flowering plants. These are monocotyledonous perennial herbs with incredible range of diversity in size, shape, colour, orientation and longevity of the flowers. All these traits contribute towards a multimillion-dollar industry of cut-flowers and potted plants in countries like Thailand, Malaysia and USA.

A number of features of the stamens in orchids, e.g. their number, relative position on the column, number of pollinia, whether they are collateral or superposed within the anther, structure of pollinia and the associated caudicle, stipe and viscidium play a significant role in the classification of the family Orchidaceae. Although the embryology of a number of orchids has been studied, detailed information regarding the early development of anther and differentiation of pollinia is lacking. Studies on some spirantheid, orchidoid and epidendroid taxa have demonstrated that the anther initiates as a bisporangiate structure and each anther lobe with a single sporogenous mass forms two to four pollinia by insertion of a partial or complete septum of sterile cells. The present study deals with development of the anther and male gametophyte in two epidendroid orchids Cymbidium aloifolium (L.) Sw. (tribe Cymbidieae) and Smitinandia micrantha (Lindl.) Holtt. (tribe Vandeae), with a view to adding to our knowledge regarding the development and variation in number and structure of pollinia in the family Orchidaceae.

C. aloifolium is an epiphytic herb distributed in Eastern Himalayas, Darjeeling and Khasia Hills at an elevation of 1500–2000 m. S. micrantha is also an epiphytic species widely distributed in the Himalayas from Garhwal to Arunachal, at an altitude between 700 and 1200 m. For the present study, inflorescences of the above species at different stages of development were collected from plants grown in the Orchidarium, Botany Department, Panjab University, Chandigarh (India) and fixed in formalin-acetic acid–alcohol (90 ml of 50% ethyl alcohol: 5 ml acetic acid: 5 ml formalin). After usual procedure of dehydration in the ethyl alcohol–t et r–butyl alcohol series and paraffin wax-embedding, sections cut from 8 to 10 µm were stained in Safranin-fast green combination.

The anther is incumbent in both the species studied here (Figure 1a). The anther primordium initiates as an ovoid mass of parenchyma cells surrounded by a protoderm (Figures 1b and 2a). It develops two lobes oriented towards the labellum, each with a group of archesporial cells of uniform size. The archesporial cells undergo mitotic divisions so as to form six to eight concentric layers of sporogenous cells, which develop a dorsiventral strip of sterile cells (Figures 1c, d and 2b, c). This sterile septum (three to four cells wide) extends from the connective side in the form of an arc into three-fourths of the sporogenous mass in C. aloifolium (Figure 2d). It, however, is oriented asymmetrically and runs through the entire width of the fertile tissue in S. micrantha (Figure 1e). A similar mode of early anther development has been reported in some of the recently studied orchid taxa. Whereas the dorsiventral partition of sterile cells is complete in many species, it is partial in Eulophia hormusjii and Rhynchostylis retusa.

A meristicematic activity in the wall layers forms an anther beak towards the rostell surface (Figure 1g). Epidendroid orchids frequently show the formation of a terminal beak.

The connective region comprises large, vacuolated cells with a procambium strand in the centre (Figures 1e, f and 2b, c). The hypodermal layer of cells undergoes periclinial and anticlinial divisions so as to form an anther wall consisting of two (S. micrantha) or three middle (C. aloifolium) layers and tapetum (Figures 1f and 2e, f). The anther wall is usually four-layered in the spirantheid taxa, as there is only one middle layer. It is five or six-layered in cyperpized orchids (two to three middle layers) while it may be four, five or even six-layered in epidendroid taxa depending upon the number of middle layers.

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In *C. aloifolium*, the epidermal cells become papillate during development of the male gametophyte (Figure 2 h), while in *S. micrantha* they are stretched and flattened (Figure 1 g, h). The epidermal cells usually become stretched in other Orchidaceae. Some notable exceptions, however, include *Zeuxine longifolia* and *Epipactis gigantea*, where these cells become hypertrophied and densely cytoplasmic. Thus the epidermal cells may be concerned with nutrition besides their usual function of protection.

In both the orchids studied here, the endothecial cells in mature anthers become radially elongated and along with the adjacent middle layers develop oval, arc-shaped or simple rod-like thickenings (Figures 1 h, i and 3 e). Two to three endothecial layers have been reported in *Limodorum* and *Epipactis gigantea*, whereas in *Cleistostoma racemifera* (= *Sarcanthine pallidus*), *E. horminum*, *Rhinchoystis retusa* and *Cyperipedium cordigerum*, only two are present. This feature is more frequent in epiphytic taxa compared to terrestrial ones.

The tapetal cells remain uninucleate but they do not form a conspicuous layer as the cell size is not large compared with other cells of the anther wall (Figures 1 e–g...
and 2f, g). The tapetum is of glandular type, as the constituent cells disorganize in situ during development of the male gametophyte (Figure 3b).

After several cycles of mitotic division, the sporogenous cells differentiate into microsporocytes (Figures 1e and 2f). Meiosis is synchronous and accompanied by simultaneous cytokinesis resulting in the formation of tetrahedral, isobilateral, decussate or rarely linear and T-shaped tetrads of microspores (Figure 3a, b). Kanta and Tsuji15 classified tetrads into six general types: tetrahedral, isobilateral, decussate, rhomboidal, T-shaped and linear. All six types of pollen tetrads were reported in 30 out of 36 species studied. Decussate tetrads, however, were found to be most common. Microspore tetrads do not separate out of the tetrad configuration and the tetrads in turn form a coherent and compact mass commonly designated as the pollinium (Figures 1h, i and 3d). There are two pollinia in C. aloifolium which become hollow due to centripetal disintegration of the sterile plate of cells running obliquely from the connective side into about three-fourths of the microsporocytes (Figure 3d). The sterile plate of cells in S. micrantha is laid in such a manner that there are four
pollinia in an anther, all of which are of different size (Figure 1 g) and oriented at an angle of 45° to the longitudinal axis of the column.

In Orchidaceae, the shedding units may be single grains, e.g. *Cyperipedium cordigerum*, *Paphiopedilum druryi*, compound grains, i.e. tetrads as in *Aa achalensis*, *Epipactis helleborine* & *E. veratrifolia* and *E. latifolia*. These may be septic pollinia, e.g. *Zeuxine stratumaticata*, species of *Habenaria* or may be liberated as pollinia – most of the Epidendroideae. A good deal of variation occurs in the number of pollinia produced and their structural details in each anther in the epipendroid orchids studied thus far, e.g. there may be two hollow pollinia (*Eulophia streptopetala*, *E. hormusijii* and *R. retusa*), four supersed pollinia (*Pholidota imbri cata*, *Corallorrhiza maculata*), four pollinia oriented at an angle of 45° to the long axis of the column (*Coelogynne lactea*, *Polystachya laxiflora*), four pollinia: two large and two small (*Pelatanthera etenoglossum*), four curved pollinia (*Sobralia micrantha*), eight pollinia in four pairs (*Eria javonica*, *Thelasis pygmaea*) or eight clavate pollinia (*Calanthe rubens*, *C. masuca*).

In India, orchids are represented by 1141 species and 166 genera. They are distributed in the Himalayan, northeastern and peninsular regions of the country. However, embryological data are available in only 68 species, primarily based on the development of the ovule and female gametophyte. Considering the importance of the features of the anther in taxonomy and phylogeny, little is known about the development of the anther and male gametophyte in Indian orchids. Therefore, a comparative study of anther and male gametophyte development in orchids of different habitats and taxonomic categories is likely to give deeper insight into the evolutionary trends in the anther wall layers, organization of pollen grains and the associated caudicle, stipe and viscidium.

Psychrophilic fungi from Schirmacher Oasis, East Antarctica

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This communication presents results of a preliminary study on the fungal biodiversity of soils of Schirmacher Oasis, East Antarctica. Using 2% malt extract agar medium, serial dilution method was followed to recover the fungi in culture from the soil samples. Fungal colonies were visible in culture plates only when maintained at 2–5°C for up to 45 days. Several taxa of fungi were recovered.

Keywords: Psychrophilic fungi, Schirmacher Oasis, soils, serial dilution method.

Psychrophiles thrive at very low temperatures. These include organisms living in deep sea (~1 to 4°C), Arctic and Antarctic (~1 to ~35°C during wintertime), and glacial ice habitats (~5°C). Little is known on the biodiversity of such habitats, especially microfungal diversity. A few reported organisms from these habitats are gaining popularity in recent years with the advent of genomics and proteonomics. Further, some of these, especially fungi and bacteria, are now known to produce unique enzymes and secondary metabolites of immense biotechnological potential.

The physiological and ecological mechanisms that help fungi to overcome and survive cold environmental conditions are well explained by Robinson. He indicated that there is a predominance of sterile mycelia in the Antarctic soils and this could be a physiological adaptation to overcome the harshness of sub-zero temperatures. He also attributed the production of melanin by these fungi as a protective mechanism for survival under extreme temperatures.

Antarctica is a continent located at the South Pole. Barring 2% of the area, thick sheets of ice cover the remaining parts. Only a few species of fungi and bacteria have been described from the region in the recent past, most of them being from the marine environment, i.e. sea water and sea ice. Little investigation has been carried out on soil microorganisms of ice-free areas. Studies of Nichols et al. in the recovery of 769 strains of Actinobacteria from the Antarctica. They suggested that the terrestrial environments of the region are a rich source of novel and rare genera. They also studied at molecular level, the total microbial diversity of the polar and deep-sea environment.

India has established a permanent research station, Maitri at Schirmacher Oasis, East Antarctica and launched a series of scientific expeditions since 1981. Earlier studies suggested that life at Schirmacher Oasis is dominated by li-chens, mosses and algae. Studies on bacteria and yeast were conducted by Shivaji. Effect of temperature on bacterial populations was observed by Matondkar. Microfaunal studies of the region were carried out by Ingle and Parulekar.

Sharma reported nine species of fungi from the Antarctica region. These include Arthrobotrys ferox on moss, Torulopsis psychrophila and Phoma herbarum on bird excreta, P. herbarum on skeletal remains, Acremonium antarcticum and A. psychrophilum on lichens and species of Torulopsis, Psychrophila and Cryptococcus on orthogenic soils. Besides, a few alien species of fungi, viz. Hormoconis resinae on oil spills and species of Dacrymyces and Exidia on wooden debris have also been reported by Sharma. Some of the tropical saprophytic fungi, viz. Chaetomium globosum, Stemphylium sp., Curvularia lunata, Memnoniella echinata, Aureobasidium pullulans, Aspergillus niger, Paecilomyces variotii, Penicillium funiculosum and Cladosporium sp., were exposed to Antarctic environment for a period of 14 months by Dayal et al., in order to study their viability, growth rate and virulence, but no major variation in activity was observed. In subsequent years, steady increase in summer temperatures and concomitant glaciological changes resulted in further exposure of soils in Antarctica and warranted continued studies on the life of the region.

The authors had an opportunity to examine the fungal diversity of soils of Schirmacher Oasis based on samples collected during a recent expedition by one of the authors.

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