

## POLLINATION BIOLOGY OF ORCHIDS: AN UNEXPLORED AREA OF RESEARCH IN INDIA

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### Abstract

Orchidaceae is one of the largest families of flowering plants in the world. It consists of about 25,000 to 35,000 species, distributed everywhere except the poles. Orchids employ different types of strategies to accomplish sexual reproduction. India has rich biodiversity of orchids and inhabits every agro-climatic regions of the country. It is estimated that over 1300 species occur in India, and the pollination biology has been studied in only a few species. Hence, considering the diversity and endemism in orchids, research on this area is almost lacking. For sustainable conservation of orchid genetic resources, it is necessary to understand orchid and pollinator relationship, breeding system, pollination biology and many other aspects that limit reproductive success both pre- and post-pollination. Similarly, the impact of human activities on the pollination system needs to be documented for sustainable conservation of orchid genetic resources.

### Introduction

ORCHIDACEAE IS one of the highly evolved families of flowering plants in the world. It comprises about 25,000 to 35,000 species; inhabited everywhere except the poles (Dressler, 1981). The flowers of this family have a unique trait where the male part of the flower comprises only one stamen and is fused with female parts of the flowers to form a structure called column. Pollens lay at distal end of the column as discrete masses (pollinia). Pollinia attach with stipe or caudicle to a sticky viscidium to form a pollinarium. This pollinarium sticks to the body of pollinator and precisely delivered on to the stigma so as to effect cross-pollination. Pollinators play a significant role in evolving new novel forms and in maintaining orchid species diversity (Buragohain and Chaturvedi, 2016; Chaturvedi, 2010a, 2011; Xu *et al.*, 2012).

### History of Orchid Pollination

Orchidaceae is one of the largest families of flowering plants with different reproductive mechanisms, ideally suited for pollination studies. In the paper, *On the various contrivances by which British and foreign orchids are fertilized by insects, and on the good effect of intercrossing*, Darwin (1862) described the unique relationship between orchids and its pollinators. He supported his evolutionary theory by mutualism between flowers and pollinators. Since Darwin's innovative discoveries, orchids became a focal point for pollination and evolutionary studies. In early years, adaptation and co-evolution of flowers and pollinators were explained by researchers relating orchid floral structure with specific insect morphologies. Pseudocopulation, a

phenomenon of pollination of orchids was not accepted by the biologists despite its early discovery. Dodson (1966) compiled and reviewed the researches on orchid-pollinator interaction and published their second classical book on orchid pollination. The book put forth the hypothesis of orchid pollination syndromes where pollinators were considered as the axis of the evolution of the orchid family. Present researches on orchid pollination also seems to get influenced by this hypothesis. Advancement in ecology, instrumentation, newer concepts in evolutionary biology have renewed the interest of researchers working on orchid pollination.

### Flower Morphology and Pollination

Orchid flower has a column at its centre; the male and female parts of the flower lie on the distal end of the column. It exercises control over removal and deposition of pollinia in orchids. The pollina (fused pollens) are located on the distal end of the column and separated from stigma by rostellum that prevents them from self-pollination. The stigma also lies just near the pollinia but on the underside of the column. Just beneath the column, lies labellum or lip which is the landing platform for most of the pollinators. In some orchids, nectar-producing glands (nectaries) are located at the base of the lip. Some orchids also have an extended portion of the lip called spur where the nectar is secreted and stored for the pollinator as an award for pollination. Pollinator lands on the labellum or lip, comes into contact with distal end of the column where the pollens lay under the pollen cap in the form of pollinia. Pollinia are attached with stipe or caudicle to a sticky viscidium forming pollinarium. While retreating from the flower, the pollen cap gets loosened, and pollinarium gets stuck with the body of pollinator. On the body of an insect, the pollinarium

undergoes differential drying, and with bending or twisting of stipe or caudicle, pollinarium gets re-oriented in such a way that it strikes the stigma when the insect visits the next flower. On successful pollination, the flower shows signs of withering and the symptoms of withering vary with the species. In some orchids like *Cymbidium*, labellum become red due to anthocyanin secretion and in others like *Vanda*, it may lose colour. The ovary starts swelling and eventually thousands of ovules and subsequently, the seeds are formed in the capsule. On maturity, the capsule dehisces and the microscopic seeds get blown into the air and finally come into the contact of the substrate where they germinate in association with suitable mycorrhizal fungi, in nature. Orchid seeds are devoid of food reserves. Hence, their initial stage relies on mycorrhiza which makes the nutrients and other growth factors required for carrying out life processes available to the germinating seeds.

### Orchid Pollinators

The flowers of orchids are pollinated by various vertebrate and invertebrate taxa. Their pollinators range from insects, birds, and rodents. However, Hymenoptera (bees and wasps) are common pollinators of orchids. Pollination rewards include nectar (insects and birds), floral fragrance (male euglossine bees), resins (used by bees for nest building), and oils (used by anthropoid bees to feed larvae). Deceptive pollination also occurs in orchids (Buragohain and Chaturvedi, 2016; Fantinato *et al.*, 2017; Thalwitzer *et al.*, 2018). Orchids deceive pollinators in several ways like sexual deception, food or brood site deception, nectar deceit, and oil deceit. In flowers of *Ophrys apifera*, the lip of the flowers is structured in such a way that it looks like a female of the pollinating insect (*Eucera* spp.). While the male insect tries to copulate with the deceptive flower labellum, the pollinia gets attached to its body. The insects make similar attempts with other flowers and pollinia get deposited on the stigma of different flowers. Pollination by this mechanism is also referred to as pseudocopulation. Some orchid species are pollinated by various pollinators, but some are very specific and are pollinated by particular pollinators. The specialization reduces wastage of pollinia and allows transferring the pollinia to the specific flowers. The specialty is favoured by body size of insect, flower morphology, and the position of pollen attachment on pollinator's body. The highest numbers of orchids are pollinated by bees followed by wasps, flies, birds, setting moths, hawkmoths, butterflies, and beetles.

#### Bee-Pollinated Orchids

Bees or wasps pollinate about 60% of the orchids. These flowers have well-developed landing platform and have nectar glands or marks of contrasting colour pointing the way to nectar. The nectar may be present in minimal

quantity and more or less concealed. Bee-pollinated flowers emit a fresh and sweet odour and are usually horizontally placed (D'Auria *et al.*, 2019). The flowers are typically coloured with violet, blue, green, and yellow. Some species of orchids produce pseudopollen which is used as a substitute reward by pollinating bees. Bees also collect an award from orchids as oil, food, and resin for nest building. Euglossine bees collect scent compounds for the use of courtship display. The flower morphology also deceives bees. The two non-rewarding species of orchids, *Dendrobium infundibulum* and *Cymbidium insigne* mimic in flower morphology of *Rhododendron lyl*, a nectar-rewarding species. The flowering season of these species overlaps and hence, get pollinated by *Bombus eximius* (Kjellsson *et al.*, 1985). Some species of *Eria* (*Eria monostachya* and *E. paniculata*) and *Maxillaria* (Beck, 1914) and most of the species in genus *Polystachya* show mimicry in the production of pseudopollen. Some orchids use both physical appearance and chemical cues to deceive the pollinators. The flowers of *Drakaea* spp. are similar to the female wasp in the genus *Zaspilothymus*; it also releases a chemical that mimics a mating pheromone of this female wasp. The pollens get transferred in the process of mating with deceptive flowers. Orchids also use traps, triggers or false nectar rewards to attract pollinators. *Rhynchostylis retusa* is pollinated by *Xylocopa violacea* and *X. aestuans* (Buragohain *et al.*, 2015), *Phaius tankervilleae* is pollinated by *X. violacea* (Buragohain *et al.*, 2016), and *Cymbidium pendulum* by *Apis mellifera* (Attri and Kant, 2011).

#### Moth-Pollinated Orchids

For pollination, moths either land on the flowers or hover in front of the orchid flower. The flowers usually open during the night. The flowers are placed either horizontally or hanging; the colour of flower ranges from white to green. Long-tongued moths pollinate the long spur flowers whereas short tongue moths pollinate species bearing moderate spur flowers. They produce abundant nectar placed down below the spurs. Some orchids have a very specialised relationship with the pollinators. Darwin's orchid (*Angraecum sesquipedale*) and Ghost orchid (*Dendrophylax lindenii*) require specific Lepidopteran pollinator whose proboscis can reach long nectar spur.

#### Butterfly-Pollinated Orchids

Butterfly-pollinated orchids are bright in colours e.g. red, orange, blue or yellow, and bear sweet fragrance. Such flowers may or may not have a nectar gland. Sometimes nectar is concealed deep below in the spurs. In India, *Habenaria foliosa* var. *foetida* is pollinated by blue tiger butterfly (*Tirumala limniace*) during day time and by

moth of the genus *Dysgonia* during the night. The fetid odour during day time attracts butterfly offering it with nectar whereas during the night, the moths are attracted by whitish green colour and for nectar (Dangat and Gurav, 2014). Orchids also attract pollinators by releasing compounds that are exacting to sex pheromones or sometimes secrete a chemical that is collected by pollinator for defence or mating attraction. *Epidendron paniculatum* releases pyrrolizidine alkaloids (PAs) which is used by lepidopterans and nymphalid butterfly as mating attraction and also for protection. In China, *Ludisia discolor* and *Calanthe argenteostriata* are pollinated by butterflies (Zhang *et al.*, 2010).

#### *Fly-Pollinated Orchids*

Orchids are pollinated by flies belonging to different families. The colour of the flies varies from yellow to brown. The flowers of fly fertilized species are horizontally placed, fringed and some have landing platform. Nectar may be present or absent, if available it is superficially accessible. The flowers may open during day or night and emit sweet to an unpleasant odour. Kumar and Rawat (2011) reported that *Epipactis veratrifolia* is pollinated by hoverfly (*Ischidon scutellaris*) while it rests in its flowers for laying the eggs.

#### *Bird-Pollinated Orchids*

Some orchids have also been reported to be pollinated by humming birds. The flowers of humming bird-pollinated orchids are tubular, placed horizontally, hanging, and brightly coloured. In China, *Coelogyne rigida* is pollinated by birds (Wang *et al.*, 2008).

#### *Rodent-Pollinated Orchids*

In China, *Cymbidium serratum* is pollinated by rodents. The wild mountain mice prefers to eat brightly coloured succulent lips of *C. serratum* because the lips are sweet, whereas lateral petals and sepals are slightly bitter in taste. The body length of the pollinator matches with the flowers height enabling the pollinator to obtain the flowers. The production of odour also synchronises with mouse activity (Wang *et al.*, 2008). It was the first report showing that orchids may also get pollinated by rodents.

#### *Beetle-Pollinated Orchids*

The beetle pollinated orchids possess readily available nectar, produces fruity smell or distinct odour. Flowers are opened during day or night. The beetle-pollinated flowers are dull white, green to dark brown, and purple to bright yellow. In China, *Holcoglossum rupestre* is the only orchid pollinated by beetles (Jin *et al.*, 2005).

#### *Auto-pollination in Orchids*

In autopollinated species, the pollinia are located just above the stigma and get fertilised in the absence of pollinators. The process may occur due to the presence of powdery pollinia and deformation of part of the column. The autopollination is most common where the pollinators are rare. The anthers of *Holcoglossum amesianum* oppose the direction of gravity by 360° and pollens get inserted into its stigma cavity (Liu *et al.*, 2006). In *Paphiopedilum parishii*, the anther is liquidated and dropped onto the stigmatic surface (Chen *et al.*, 2012). In *Epipogium roseum*, rostellum degenerates and loses its function and enables the contact between stigmatic secretions and pollinia at the time of bud development (Zhou *et al.*, 2012). Autopollination is considered as possible adaptation mechanism of orchids in insect-scarce habitats (Chen *et al.*, 2012; Liu *et al.*, 2006).

### **Future Research**

India has rich biodiversity of orchids and inhabits almost every agroclimatic regions of the country. It is estimated that over 1300 species occur in India but only a few reports are available on pollination of orchids. Considering the diversity and endemism of orchids in India, research on the pollination biology in orchids is lacking. For sustainable conservation of orchid genetic resources, it is necessary to understand orchid pollinator relationship, breeding system, biology, and many other aspects that limit reproductive success both pre- and post-pollination. So far, no studies have been conducted to study the impact of human activities on the pollination system in preserved habitat v/s fragmentation/ degradation habitat. There are reports that pollination success is disturbed and preserved habitats should be studied in detail. As amongst the different species of the same genus of the orchids the attachment of pollinaria on the body parts of the visitors/pollinators is always constant, the orchid flowers seems to be adapted for inter specific hybridization in nature (cf. Chaturvedi, 2010b). Furthermore, the advent of sophisticated instrumentation technology like chemoeconomic, electrophysiologic, genomics, and proteomic needs to be used for how the floral features are helpful in attracting the pollinators.

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