

DECEPTIVE POLLINATION IN AN ENDANGERED ORCHID, *VANDA COERULEA* GRIFF. EX LINDL. (ORCHIDACEAE)

Bhaskar Buragohain and S K Chaturvedi¹

Department of Botany, Mariani College, Mariani, Assam-785 634, India

¹Department of Botany, Nagaland University, Lumami, Nagaland-798 627, India

Abstract

The flowers of *Vanda coerulea* are non-rewarding and follow the deceptive pollination system. Blooming takes place from October and continues till the end of December. Flowers are large, showy, long lasting and visited by the Hymenopterans, *i.e.*, *Andrena parvula*, *Lasioglossum* sp. and *Xylocopa (Biluna) nasalis* (carpenter bees). However, the carpenter bee was identified as the legitimate pollinator of *V. coerulea*, in Nagaland. The co-occurring melliferous *Dolichos lablab* flowers attracted bees of *X. nasalis* and functioned as the magnet plants. As the visits of pollinators were very rare, the rate of success of natural pollination was low. However, the smaller isolated bees *Andrena parvula* and *Lasioglossum* sp. were unable to pollinate the flowers due to their smaller size but rarely they helped in removing the anther cap so as to initiate the autogamy in this endangered species as an alternate last resort for pollination. The flowers of *V. coerulea* were self-compatible and exhibited 100% reproductive success through hand pollination (Geitonogamy and Xenogamy). The unpollinated flowers remained fresh for 25-40 days but senescence was initiated within 48 hrs after pollination. Anthecological studies showed attachment of pollinaria on the dorsal surface of hind part of the head and thus the mode of pollination was *Nototribic*.

Introduction

THE ORCHID flowers exhibit both biotic and abiotic modes of pollination (Chaturvedi, 2011). However, according to Johnson and Steiner (2000), Trembly (1992) and Trembly *et al.* (2005), the orchid flowers exhibit highest pollinator specificity. This specialization maximizes the pollination efficiency of orchids (Nilsson, 1992), but the reproduction rate of orchids is often being strongly limited or dependent on pollinators (Trembly *et al.*, 2005). Although, various groups of pollinators of orchids have so far been reported, yet 60% of these orchids are pollinated by the insects belonging to order Hymenoptera (bees, wasps, and ants) alone and rest of the 40% of these orchid flowers are pollinated by the other groups of biotic pollinators like birds, beetles, flies butterflies, moths, mouse, crickets *etc.* (Trembly, 1992; Van der Pijl and Dodson, 1966).

Present paper deals with the studies on pollination biology of *Vanda coerulea* as it shows poor fruiting in the natural conditions. Although much work has been done on the micropropagation of this taxon, yet there is no report on the pollination biology of this endangered orchid species with beautiful and long lasting flowers. Therefore, the present studies on pollination of *V. coerulea* were undertaken with a view to identifying the pollinators and developing the alternate strategies for pollination so as to conserve these in its natural localities.

Materials and Methods

The studies of pollination biology of *V. coerulea* were carried out at two sites of Nagaland, *i.e.*, Arkong Ward Mokokchung (26° 19' 24.9½ N and 94° 30' 55.2½ E, Alt. 1270 m; site A), Nagaland, India and Sumi Settsu (26° 16' 12.7½ N and 94° 28' 36.4½ E, Alt. 1156 m; site B), Zunheboto district, Nagaland, India; for three consecutive years (2012 – 2014). *V. coerulea* is an epiphytic perennial orchid with an axillary racemose inflorescence comprising of 6-14 flowers. Flowers are thinly textured, widely open, 7-9 cm in diameter. Sepals and Petals sky blue tessellated, the colour is more prominent in shaded condition than direct exposure of sunlight (Fig. 1A). Labellum deep blue in colour, fleshy, spurred and attached to the base of column (Fig. 1E). Spur conic, 7 × 3 mm, without nectar. Column 9 mm long, anther cap white, Pollinia two with short stipe and broad viscidium. Rostellum medium sized, stigma represented by a concave cavity.

To observe the occurrence of autogamy, the inflorescences were bagged by nylon net bags before anthesis. The experiments for testing the self-compatibility were performed by placing the pollinaria of a flower on its own stigma. Cross pollination experiments were performed between the flowers of the same plant to observe the result of geitonogamy as well as between the flowers of plants growing at a distance of about 10 meters so as to see the results of xenogamy (Buragohain *et al.*, 2016). After the experiments, the flowers were tagged and the

inflorescences were covered by nylon net bags. However, a few inflorescences were left open as control to observe the results of natural/open pollination. The experiments were performed during 2012 to 2014 in the months of October to November. The insect visitors/pollinators were captured with the help of wide mouthed glass test tubes and a watch glass so as to avoid the removal of pollinaria from the body of pollinators (Buragohain *et al.*, 2015). The captured pollinators were sent to ZSI, Kolkata and Jai Narayan Vyas University, Jodhpur, Rajasthan for authentic identification. The herbarium of plants have been deposited in the department of Botany, Mariani College, Mariani, Assam, India. Field photographs were taken with the help of Pentax Digital camera (model K 10 D) with 50 mm and 100 mm macro lens and Sony Digital Camera (Model DSC-HX 7V). Anthecological studies were conducted under Leica Streo Zoom Microscope. Repeated observation were made during the periods of flowering, so as, to note the visiting behaviour of insects and to identify the pollinators as well as the mechanism of pollination in the open populations of *V. coerulea*.

Results

As the flowers of *V. coerulea* are non-rewarding and non-odoriferous, these were rarely visited by pollinators/visitors. The flowers followed a generalized food deceptive system (Jersakova *et al.*, 2006). During the study of three years, it has been observed that the flowers of *V. coerulea* were visited by insects of order Hymenoptera, *i.e.*, *Andrena parvula*, *Lasioglossum* sp. and *Xylocopa (Biluna) nasalis* (carpenter bees) only, but out of these, the giant carpenter bee *Xylocopa (Biluna) nasalis* has been identified as the only legitimate pollinator. A total of 7 visits of the pollinator bee were recorded during the study period. The morphological study of flowers revealed that the melliferous flowers of *Dolichos lablab*, growing in the vicinity of *V. coerulea* populations, helped in attracting these hymenopteran insects and functioned as *Magnet plants* as suggested by Johnson *et al.* (2003) for this orchid. The pollinators were misled by the floral structures and colouration of the flowers of *V. coerulea*, for which the pollinators were unable to distinguish the flowers of rewarding and deceptive plants and forage the flowers of *V. coerulea*. The presence of large number of flowers in *V. coerulea* enhanced the attraction of pollinators towards the deceptive species.

Mechanism of Pollination

About 20 individuals of the giant carpenter bees *Xylocopa (Biluna) nasalis* were observed to forage the

flowers of *Dolichos* sp., which were growing at the close vicinity of *V. coerulea* populations, at a regular interval throughout the day. The pollinators hovered the flowers of *V. coerulea* for several times but forage the flowers occasionally. The pollinator bees *X. (Biluna) nasalis* perched over the labellum of the flower and hold it firmly (Fig. 1B) and soon after perching over the labellum, these foragers inserted their proboscis deep into the spur and pushed their head in between column and labellum so as to lick up the reward (Fig. 1C). Thus, the pollinator bees exerted a pressure on the labellum and in turn on the column of the flower. While the pollinator withdrew its proboscis from the spur and retract itself out of the flower, the protruding portion of the column came in contact with the dorsal portion of the occiput of the head of these visitor bees. As the bee moved backwards, a pressure was created over the column pushing the rostellum upwards so that the anther cap became slightly separated and the viscidium of the pollinarium stucked on the occiput of the head. As these bees left the flowers, the pollinaria were removed out of the anther and remain attached on the occiput region of the head of these insects (Fig. 1D). Reconfiguration of pollinarium took place immediately and the pollinia moved backwards (Fig. 1D). It has been observed that immediately after the attachment of pollinia over the head, the bees fly away without further visiting any other flower of the same population. When the pollinator bees with pollinaria attached on their occiput forage other flowers of *V. coerulea* and repeat the same process of foraging, the pollinaria carried by the pollinator become inserted into the sub-apically situated stigmatic cavity and get stucked to it. During the retraction, out of the flower, the pollinaria became separated from the occiput of the pollinator and the process of pollination was completed.

Indirect Autogamy

The flowers *V. coerulea* were also visited by the small sized hymenopteran bees, *i.e.*, *Andrena parvula*, and *Lasioglossum* sp. which were unable to operate the mechanism of pollination in this orchid due to their smaller size. As these bees moved around the apical region of the column, the anther cap became slightly displaced. In such flowers, the pollen grains germinated inside the anther cap sometimes and the pollen tubes grew towards the stigmatic surface bypassing the rostellum and finally lead to successful fertilization and fruit set (Fig. 1G).

Discussion

The flowers in the presently studied *V. coerulea* were observed as self-compatible and non autogamous.



Fig 1. A-H. *Vanda coerulea* showing pollination biology: A, *V. coerulea* blossoms; B, *Xylocopa (Biluna) nasalis* foraging a flower; C, Pollinator's head touching the column; D, Magnified view of the pollinator showing the attachment of pollinaria on the occiput; E-F, Measurement of floral dimension and head of pollinator; G, Magnified view of column showing growth of pollen tubes towards stigma; H, Pollinator of *V. coerulea* foraging the flower of *Dolichos lablab*. (POLL, Pollinator; COLM, Column; LBM, Labellum; REC, POLA, Pollinaria after reconfiguration; OCCP, Occiput; PROS, Proboscis; POLN TS, Pollen tubes; STIG, Stigma).

Table 1. Size of *Vanda coerulea* flowers and the size of its pollinator *Xylocopa (Biluna) nasalis*.

Floral Measurement	Size/Length (Mean)	<i>X. (Biluna) nasalis</i> body measurement	Size/Length (Mean)
Length of Labellum	1.8 cm	Total body length	2.5 cm
Length of the spur	1 cm	Length of proboscis	1 cm
Distance between spur and anther cap	1.9 cm	Distance between occiput and end part of proboscis	2 cm
Depth between viscidium and floor of labellum	7 mm	Height of thorax	7 mm

Table 2. Pollination system and fruit set in *Vanda coerulea* at site A.

Experiments	Number of inflorescence used			Number of flowers treated			Number of flowers set fruits			Pollination success (%)			Mean pollination success (%)
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014	
Agamospermy	4	3	4	16	12	16	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Autogamy	4	4	4	48	48	50	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Self pollination	6	5	3	18	20	15	18	20	15	100	100	100	100
Geitonogamy	5	4	4	20	16	16	20	16	16	100	100	100	100
Xenogamy	4	4	6	16	6	2	16	16	24	100	100	100	100
Open Pollination	9	10	12	108	120	148	5	5	4	2.77	4.16	2.70	3.21

However, indirect autogamy, with the help of *Andrena parvula*, and *Lasioglossum* sp. in these was possible. Although the flowers of *V. coerulea* show self-compatibility, these are dependent upon the biotic agents for successful pollination. The insects of the order Hymenoptera have been recorded as the visitors and pollinators of *V. coerulea*. Since, the transfer of pollinaria, from anther to stigma, takes place through the dorsal surface of head (occiput) of the pollinator bee, the mode of pollination in *V. coerulea* is Nototribic. However, similar mode of pollination through the

agency of bumble bees and carpenter bees has earlier been described by Chaturvedi (2010) in *Aerides odorata* as *Frontotribic*, as the pollinaria get attached on the fore-head. Due to the specificity of pollinators as well as the pollination system, the natural rate of open pollination was significantly low. Even in some of the populations of *V. coerulea*, no fruit set was observed through the natural pollination. Analyzing the reasons of low rate of pollination success, the specificity of pollinators and the pollination system seems to be the major reason for low percentage of fruit set in this

Table 3. Pollination system and fruit set in *Vanda coerulea* at site B.

Experiments	Number of inflorescence used			Number of flowers treated			Number of flowers set fruits			Pollination success (%)			Mean pollination success (%)
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014	
Agamospermy	5	5	6	20	20	24	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Autogamy	5	5	5	20	20	20	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Self pollination	6	6	6	18	18	18	18	18	18	100	100	100	100
Geitonogamy	5	5	5	20	20	20	20	20	20	100	100	100	100
Xenogamy	5	5	6	15	20	24	15	20	24	100	100	100	100
Open Pollination	12	8	14	140	96	168	7	5	8	5	5.20	4.76	4.98

species. However as suggested by Li *et al.* (2010), the learning and avoidance process of the pollinators in case of non-rewarding flowers also influence the lowest rate of natural pollination success.

The hand pollination and bagging experiments exhibited complete absence of autogamy, however indirect autogamy was observed due to the inefficient and smaller sized pollinators. The self pollinated flowers showed 100% fruit set. The geitonogamous and xenogamous experiments also produced 100% fruit set at both the study sites. The pollination success of natural/open pollination showed 3.21% and 4.98% fruit set in the study sites A and B respectively (Table 1, Table 2 and Table 3).

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