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ANT FACILITATED POLLINATION OF *HERMINIUM LANCEUM* (THUNB. EX SW.) VUIJK (ORCHIDACEAE)- AN ENDANGERED TERRESTRIAL ORCHID OF NORTHWESTERN HIMALAYAS

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Abstract

A species of Black Carpenter Ant (*Camponotus pennsylvanicus*) was observed to be a significant pollinator of *Herminium lanceum* (Thunb. ex Sw.) Vuijk. The present paper describes the pollination mechanism of a terrestrial and endangered orchid species, *H. lanceum*. The flowers were observed as self-compatible but mainly cross-pollinated. As the flowers are nectarous, these were frequently visited by a variety of insects. The presence of a large number of flowers in *H. lanceum* enhanced the attraction of pollinators towards them. The pollinators visited the flowers many times for foraging. Four species of insects (1 species of black carpenter ant loaded with pollinia, 2 species of unknown fly, and 1 species of crab spider) were found visiting the flowers. Since, the transfer of pollinarium, from anther to stigma, took place through the dorsal surface of the head (occiput) of the pollinator ant, the mode of pollination is *Nototribic.* As a single individual of ant was observed to pollinate a large number of flowers in its single visit to the inflorescence, the rate of pollination and fruit set was very high in *H. lanceum.* Interestingly, it was observed that the frequency of insect visit decreased as the day passed by.

Introduction

THE FAMILY Orchidaceae is one of the most advanced and largest families of angiosperms (Willis, 2017), comprising of about 28,484 species (Govaerts *et al*., 2017) and has always been interesting to evolutionary biologists because of their remarkable floral forms and their diversity in pollination systems (Darwin, 1877). The Eastern Himalayas (including the NorthEast India), the Western Ghats, and the Western Himalayas are the three major centers of orchid diversity in India with a total strength of about 1,256 species of orchids in 155 genera (Singh *et al*., 2019). The great geographic expanse of the country encompassing a variety of bioclimatic zones and the enormous diversity of ecosystems account for the bewildering array of orchid species in India (Kumar *et al.,* 2017; Kumar *et al.,* 2018; Pathak *et al.,* 2010; Prakash and Pathak, 2019; Prakash *et al.,* 2018). The extraordinary taxonomic and morphological diversity of orchids is accompanied by a remarkable range of pollinators and pollination systems (Gaskett, 2011; Pal *et al*., 2019). Most orchid flowers have the same basic reproductive structures. A central structure known as the column is a unique adaptation of orchids that houses both the male (anther) and female (stigma) parts of the flower (Roberts and Dixon, 2008). The anther is located at the distal end of the column. The stigma is located near the distal end and just on the underside of the column. Directly below the column is the labellum or lip, a modified petal that acts as a landing area for pollinators and sometimes also to attract the pollinators. When present, the floral nectaries (nectar producing tissue on the plant) are typically located at the base of the labellum or as a nectar spur behind the flower. As the pollinator moves on the labellum, it comes into contact with the pollen. Unlike the loose- and often wind-dispersed pollen grains of most flowering plant families, orchid pollen grains are fused together into compact structures called pollinia. The pollinia are located under the male anther cap and contain a sticky structure called the viscidium which helps the pollinia adhere to a pollinator as it feeds or seeks to mate with the column (Roberts and Dixon, 2008). When a pollinator visits another flower, the pollinia are likely transferred to the stigma.

The orchid flowers exhibit both biotic and abiotic modes of pollination (Buragohain and Chaturvedi, 2016; Chaturvedi, 2011; Pal *et al*., 2019), and they show high specificity for their pollinators (Johnson and Steiner, 2000; Trembly, 1992; Trembly *et al.,* 2005). Although, this specialization greatly enhances the pollination efficiency of orchids (Nilsson, 1992), but it often results in a very low rate of pollination. 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 (Brodmann *et al.,* 2008; Peakall and Beattie, 1989, 1991; Sugiura, 2013), and rest 40% of these are pollinated by the other groups of biotic pollinators in the orders Lepidoptera, Diptera, Coleoptera, Orthoptera *etc.* like beetles, flies, butterflies, moths, crickets *etc.* including birds and even mouse (Jersakova and Johnson, 2007; Johnson,

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1996; Micheneau *et al.,* 2010; Peter and Johnson, 2014; Sun *et al*., 2014; Trembly, 1992; van der Pijl and Dodson, 1966; van der Niet *et al.,* 2011; Wang *et al.,* 2008). There exists different kinds of orchid-pollinator relationships. While some orchid species are pollinated by numerous taxa, the others are specialized and are pollinated by only one pollinator species. Specialization allows direct transfer of pollen to the same orchid species and reduces the chance that the pollinia will be dropped or transferred to the wrong orchid species (Scopece *et al*., 2010). Also, specialization may involve the attachment of pollen to one specific location on the pollinator, ensuring that even if the pollinator visits another species, the pollinia likely will not be transferred until the pollinator revisits an orchid of the same species that donated the pollen (Scopece *et al*., 2010).

The present paper deals with the studies on pollination biology of *Herminium lanceum* (Thunb. ex Sw.) Vuijk, a terrestrial and endangered orchid of NorthWestern Himalayas. Although there are a few reports available on the micropropagation of this taxon (Mitra, 1971; Singh and Babbar, 2016; Verma, 2016), there is no report on the pollination biology of this endangered orchid species. Since, this species is medicinally very important (Kumar *et al.,* 2018; Pathak *et al.,* 2010; Prakash *et al.,* 2018), its natural populations are decreasing at an alarming rate due to ruthless collections and over-exploitation. Hence, conservation measures need to be taken so as to protect its survival in natural habitats. Therefore, the present studies on pollination biology of *H. lanceum* were undertaken with a view to identifying the pollinators and developing the alternate strategies for its propagation so as to conserve it in its natural localities.

Material and Methods

The Study Site

The field observations on pollination biology of *Herminium lanceum* were carried out during June to August, 2019 at Taradevi hill station of Shimla district, Himachal Pradesh, India. Taradevi hill station is located at 77°8´21.59" N and 31°4´26.82" E, at an average elevation of 1,500-2,000 m amsl. The field observations of the pollinators were made mainly between 9 am to 3 pm; at other times, either very rare or no insect activity was recorded. To determine whether the flowers are autogamous or not, the inflorescences were bagged by nylon net bags before anthesis so as to exclude any insect visits. To determine whether the flowers are self-compatible or not, the pollinia of a flower was inserted into its own stigma with the help of a fine needle. However, a few inflorescences were left open as control to observe the results of natural/open pollination. Field photographs and videos were taken with the help of Nikon digital camera (COOLPIX P900; 16MP, 83× optical zoom). Frequent visits and repeated observations were made during the period 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 natural populations of *H. lanceum.*

Observations and Results

Plant Distribution

Herminium lanceum, a terrestrial and endangered species of orchids is found at an altitude of 1,500-4,000 m amsl throughout the NorthWestern and NorthEastern India. In India, it is distributed in the Himalayan region (Kashmir to Arunachal Pradesh), and in the entire seven sister states of NorthEastern India. It is also found in Bhutan, China, Burma as well as the entire SouthEast Asia (Chowdhery, 1998; Deva and Naithani, 1986; King and Pantling, 1898; Singh *et al.,* 2019).

Flowering

July-October

Fruiting

October-September

Biological Status

Endangered (EN)

Flower Morphology

The *plants* are terrestrial, 40-70 cm in height (Fig. 1A). *Tubers* small, ovoid. *Stem* erect glabrous, 2-4 leaved. *Leaves* sessile, distantly arranged on the stem, linear-lanceolate, acuminate, sheathing at the base. *Inflorescence* densely flowered. *Flowers* whitish-green, up to 1 cm long. *Sepals*- dorsal sepal ovate, concave, forming a hood with petals, obtuse; lateral sepals ovate, obtuse. *Petals* linear-lanceolate, somewhat acute. *Lip* having a semicircular, narrow clawed base, 3-lobed; lateral lobes filiform, obtuse; apical lobe small or often only reduced to a knob like.

Habitat Ecology

Herminium lanceum is a terrestrial orchid found in humus rich soils intermixed with litter and bark, present singly or in groups of 2-5 plants. The distribution pattern is of both random and patchy type. It is rare in distribution and presently collected from Taradevi hill station at an altitude of 1,500-2,000 m amsl (Distt. Shimla) of Himachal Pradesh. It shares the habitat with grasses, mosses, and other herbaceous plants.

Fig. 1. A-F. Pollination of *Herminium lanceum*: A, Plant in its natural habitat; B-C, Black Carpenter Ant (*Camponotus pennsylvanicus*) foraging the flower; D, *C. pennsylvanicus* loaded with pollinia; E-F, Other insects (flies and crab spider) visiting and foraging the flowers.

Amongst orchids, *Epipactis helleborine, Goodyera biflora, Habenaria edgeworthii, H. intermedia, Crepidium acuminatum*, and *Satyrium nepalense* were found to grow in the vicinity.

Pollination Biology

The flowers in the presently studied *H. lanceum* were observed as self-compatible but mainly cross-pollinated. As the flowers are nectarous, these are frequently visited by a variety of insects. The presence of large number of flowers in *H. lanceum* enhanced the attraction of pollinators towards them. The pollinators visited the flowers many times for foraging. Four species of insects [1 species of Black Carpenter Ant (*Camponotus pennsylvanicus* De Geer, Order: Hymenoptera; Family: Formicidae) loaded with pollinia; 2 species of unknown fly; and 1 species of crab spider] were found visiting the flowers (Fig. 1B-F). *C. pennsylvanicus* made the maximum visits to flowers. The ant visited the flower randomly in the spiral manner. While visiting, the pollinator ant inserted its head into the flower so as to search for the nectar (Fig. 1B-C). In the process, the pollinarium gets attached to the occiput region of its head. While the pollinator ant withdrew itself from the flower and moved backwards, the pollinarium was removed out of the anther and remained attached to the occiput region of the head (Fig. 1D). When the pollinator ant with pollinarium attached to its occiput visits other flower of the same inflorescence and repeats the same process of foraging, the pollinarium gets deposited onto the stigmatic cavity of the other flower and gets sticked to it. During the retraction of ant from the flower, the pollinarium became separated from the occiput of the pollinator and the process of pollination occurred. As a single individual of ant was observed to pollinate a

large number of flowers in its single visit to the inflorescence, the rate of pollination and fruit set was very high in *H. lanceum*. Interestingly, it was observed that the frequency of insect visit decreased as the day passed by.

Discussion

There are no reports so far, in the literature, concerning the pollination mechanism of *Herminium lanceum*. Also, there is an extreme rarity of ant pollination in orchids (Claessens and Seifert, 2017). However, there are a few earlier reports concerning the ant pollination in orchids. Ants were the exclusive pollinators in the two tropical orchids *Leporella fimbriata* and *Microtis parviflora* (Jones, 1975; Peakall and Beattie, 1989, 1991; Peakall *et al*., 1987, 1990; Siegel, 2014). In some Palaearctic species, ants are facultative pollinators, *e.g*., in *Epipactis palustris* (Brantjes, 1981) and *E. thunbergii* (Sugiura *et al*., 2006).

H. lanceum is an allogamous species, which means it relies on insects for pollination. Although, the flowers of *H. lanceum* showed self-compatibility, but these are dependent upon the biotic agents for successful pollination as well. Since the transfer of pollinarium, from anther to stigma, took place through the dorsal surface of the head (occiput) of the pollinator ant, the mode of pollination is *Nototribic*; same mode of pollination has earlier been described by Buragohain and Chaturvedi (2016) in *Vanda coerulea*. Another mode of pollination has earlier been described as *Frontotribic* in *Aerides odorata* (Chaturvedi, 2010), as the pollinaria get attached on the fore-head of the pollinator. As single individual of ant was presently observed to pollinate a large number of flowers in its single visit to the inflorescence, the rate of pollination and fruit set was very high in *H. lanceum.* To conclude, the present studies were successfully made on pollination biology of *H. lanceum*, thereby leading to identification of its pollinators, mechanism of pollination, and per cent fruit set.

Such studies would help in assessment of the biological status of presently selected species, *H. lanceum* and the other related species, which will further help us in giving direction to develop the suitable strategies for their *in vitro* mass propagation so as to restore and conserve these ever declining populations in their natural habitats.

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