Pollination in *Habenaria foliosa* var. *foetida* (Orchidaceae)[a]

Bhaurav Tukaram Dangat[1] & Rajaram Vitthoba Gurav[1]

Keywords/Mots-clés: *Habenaria*, moth/papillon nocturne, Orchidaceae, pollination biology/biologie de la pollinisation.

Abstract

The highly specialized mechanisms of orchid pollination have been the subject of many studies from Darwin to recent researchers. For the first time pollination of the terrestrial orchid *Habenaria foliosa* var. *foetida* by blue tiger butterfly (*Triumala limniace*) during day time and by a moth of the genus *Dysgonia* during the night is reported from India. The emission of a fetid odour during the daytime is a peculiar characteristic of this terrestrial orchid flower which attracts a butterfly offering nectar as a reward. During the night moths are attracted by the whitish green colour of the flower again for nectar. More flowers are effectively pollinated during day as compared to the number pollinated during the night.

Résumé

Pollinisation chez *Habenaria foliosa* var. *foetida* (Orchidaceae) – Les mécanismes de pollinisation hautement spécialisés chez les orchidées ont fait l'objet de nombreuses études depuis Darwin jusqu'aux chercheurs actuels. Cet article rapporte, pour la première fois, la pollinisation de l'orchidée terrestre *Habenaria foliosa* var. *foetida* par le papillon *Triumala limniace*, dans la journée, et par un papillon nocturne du genre *Dygsonia*, la nuit. L'émission d'une odeur fétide au cours de la journée, caractère particulier de cette fleur d'orchidée, attire les papillons. De nuit, les papillons nocturnes sont attirés par la couleur vert blanchâtre de la fleur. Dans les deux cas, la fleur offre son nectar en récompense.

[a] : manuscrit reçu le 18 avril 2014, accepté le 17 juin 2014
article mis en ligne sur www.richardiana.com le 19/06/2014 – pp. 219 -228 © Tropicalia
ISSN 1626-3596 (imp.) - 2262-9017 (élect.)

Introduction

Orchidaceae is one of the largest families of flowering plants (up to 35,000 species), making up nearly 10% of all flowering plant species in the world and exhibiting a wide range of distribution as well as pollination mechanisms (Dressler, 1993). The highly specialised mechanisms by which orchids are pollinated have been the subject of many studies, with perhaps the most well known being the book "The Various Contrivances by which Orchids are Fertilized by Insects" by Charles Darwin (1862). Most orchids are dependent on insects to effect cross-pollination as their pollen is borne in sticky masses and therefore cannot be dispersed by the wind. Self pollination is also prevented in most species by the protruding rostellum which separates the pollinia from the stigma. Orchid pollination mechanisms, which are highly dependent on specialised adaptation to their insect pollinators, are regulated by the mode of pollinator attraction, the form of the pollen and the geographic location of the plant (Paling, 2007). The floral structure of orchids is generally specialized to avoid spontaneous self-fertilization and promote insect mediated outcrossing (Dressler, 1993; Paling, 2007). Darwin famously argued that the long spurs (ca. 32 cm) of *Angraecum sesquipedale* Thouars, a Madagascan orchid species, represents an example of floral specialization for pollination by a long-tongued hawkmoth. From various reports it is confirmed that the settling moth and hawkmoth pollination in the Habenariinae is probably widespread (Arditti, 1992; Johnson & Liltved, 1997; Nilsson & Jonsson, 1985; Nilsson *et al*., 1992; Peter *et al*., 2009; Singer, 2001; Singer & Cocucci, 1997; Singer *et al*., 2007) although mosquitoes (Diptera: Culicidae) and Tipulidcrane flies (Diptera: Tipulidae) may contribute to the pollination of a few species (Peter *et al*., 2009; Singer, 2001; Singer & Cocucci, 1997; Singer *et al*., 2007; Thien & Utech, 1970) and the unusual *Herminium monorchis* R.Brown is pollinated by a wide range of minute flies and hymenoptera (Nilsson, 1979). In most of the moth-pollinated species in this sub-tribe, pollinaria are attached to the eyes of moths (Alexandersson & Johnson, 2002; Arditti, 1992; Johnson & Liltved, 1997; Nilsson & Rabakonandrianina, 1988; Nilsson & Jonsson, 1985; Nilsson *et al*., 1992; Peter *et al*., 2009; Singer, 2001; Singer & Cocucci, 1997) while in one species the pollinaria are attached to the proboscis of the pollinating insects (Singer & Cocucci, 1997). In India the genus *Habenaria* Willdenow is represented by 72 species of which 38 are endemic (Kumar & Manilal, 1994). Typically the *Habenaria*

flowers have a long nectariferous spur ranging from 1 to 16 cm, white or greenish white colour and are foetid or sweet scented. These characteristics are the result of morphological adaptations to attract specific pollinators. The structure of flowers is designed in such a way that it permits access to nectar in long, narrow tubes called as spurs projected away from the lip. The nectar in the spur is a reward for pollinators whose proboscis length matches spur length and which are able to pore it through the narrow opening to suck the nectar. Generally insects capable of reaching into such long, narrow tubular spurs of length more than 10 cm are hawkmoths having long proboscis. The size and the flight patterns – i.e. hovering over flowers with uncoiled proboscis and spread wings – make them ideal agents for carrying pollen or pollinaria. During the present study we focused on the pollination of *Habenaria foliosa* A.Richard var. *foetida* (Blatter & McCann) Bennet which has a nectariferous spur that is almost 4 cm long. (Fig. 1, c & d).

Material and Methods

Study site: a study was conducted on a population of 22 individuals of *Habenaria foliosa* var. *foetida* growing in Botanical Garden of Shivaji University, Kolhapur. Inflorescence heights for this population range from 35-50 cm (Fig. 1, a & b). Observations were recorded continuously for 7 days from 8.00 am to 8.00 am. This time schedule was adopted since previous observations indicated that (1) the flowers of *Habenaria foliosa* var. *foetida* emit a foetid smell during the day time, and (2) efficient activity of moth was observed during night at 9 pm. Photographs were taken with a Nikon D90 digital SLR camera. Digital images were edited and assembled on figures using Adobe Photoshop 7.0 (Adobe Inc., San Jose, CA, USA).

Pollinator behaviour: pollinator behaviour at inflorescence was recorded with the help of a Sony digital handy cam. A flashlight with low power torch was used to locate pollinators during the night. During the day, pollinators were observed for their activity, efficiency to pollinate, hovering and landing pattern, removal of pollinaria and attachment site of pollinaria. During the night all available inflorescences were illuminated looking for the pollinators every 10-15 minutes, since continuous illumination distracts them and after recognizing light they flew away high in the sky and return after 10-15 min. Moth pollinators were captured with the help of a butterfly net and subsequently observed for number of polli-

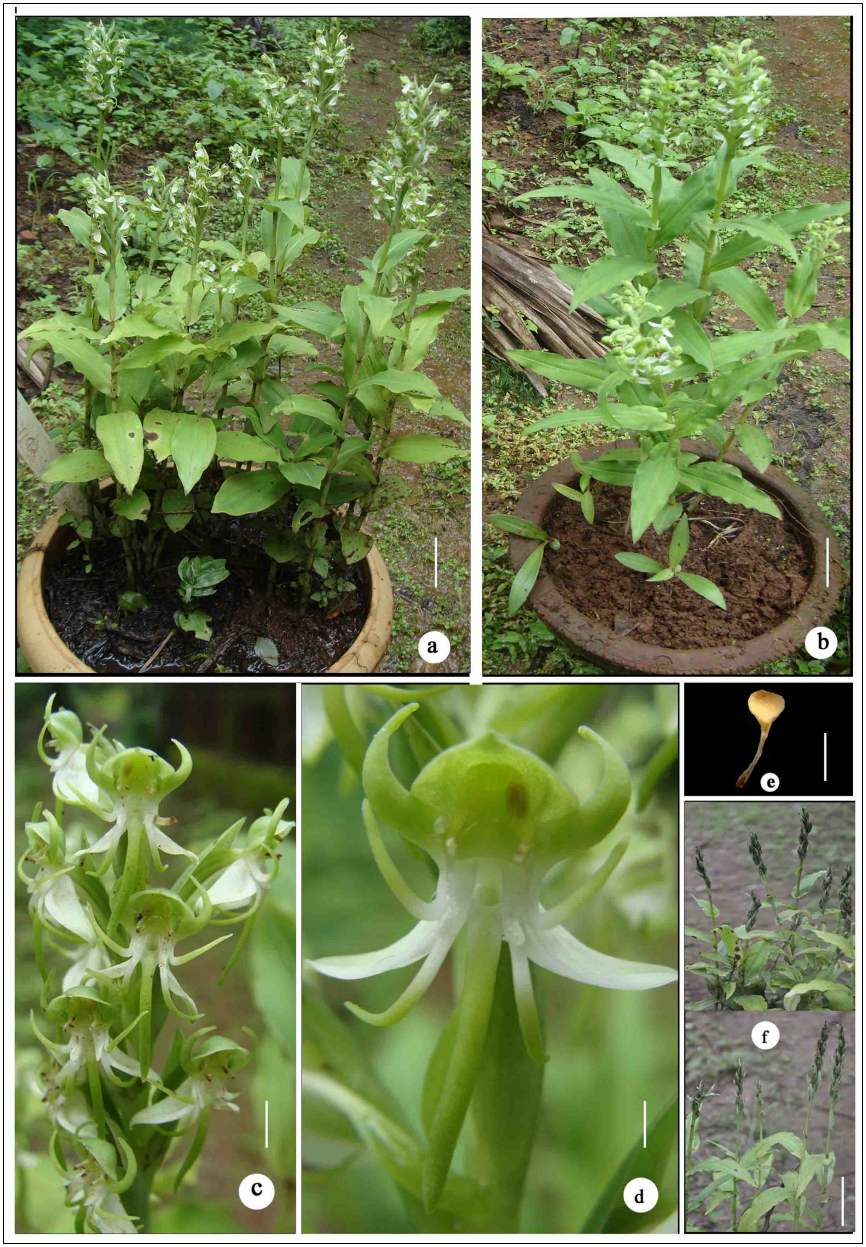

Fig. 1: Details of morphology of *Habenaria foliosa var. foetida*

a. & b. habit, c. inflorescence, d. entire flower, e. single removed pollinarium, f. capsules formation after pollination - scale bars: a&b: 2.4 cm, c: 1.2 cm, d: 0.4 cm, e: 0.5 cm, f: 6.6 cm

naria attached, site of attachment and proboscis length. These observations were recorded in the form of field notes and photographs. Number of visited flowers and time of visits were recorded, along with surrounding environmental conditions (local weather and cloud cover). Proboscis of the captured moth was unrolled using a blunt pin and the length was measured using a ruler and finally whole moth was mounted on thermacol sheet for identification.

Odour: the smell of inflorescence was determined by the human nose at an interval of 10-15 min. during the study time. All 22 individuals were sampled for this purpose.

Results

Floral morphology of the studied species: *Habenaria foliosa* var. *foetida* is a tuberous, perennial, robust herb (Fig. 1) widespread throughout the Western Ghats. Stem erect, stout, ternate, up to 50 cm high, leaves scattered along the stem, inflorescence lax, flowers white with green tinge, subsessile, with a foetid smell whose intensity is highest around 11 am. This is the only taxon within the genus *Habenaria* having strong foetid smell during the day. Within the population studied, the 4 cm long spur was frequently observed containing nectar. The population shows a mean inflorescence height of about 40 cm, each inflorescence bearing a maximum of 12 flowers. Flowers are characterized by unequal, 3-nerved green sepals, petals white, 2-partite, 3-nerved, lip greenish white, 3-partite to the base, spurred, up to 4 cm (Fig. 1, d). Pollinia 2, obliquely ovate in outline, caudicle slender dilated towards base and attached to small yellow sticky viscidium (Fig. 1, e); stigma sub-cylindrical, blunt, recurved, entrance to the spur lying at its base.

Individual flower remains open for a period of 12-15 days until it is either pollinated or eventually wilt, then dropping down from the inflorescence stalk. The number of flowers open on a single plant at a time is 10 (Fig. 1). This flowering pattern is inconsistent at different localities due to the differences in rainfall. Plants start to grow in late July while blooming starts at the end of August and may continue until mid September according to various climatic conditions.

Pollinator behaviour: during the period of observation, twelve (12) blue tiger butterfly and one (1) moth visited the flowers. All of them were seen probing the flowers (Fig. 2). Duration of the visits of both pollinators

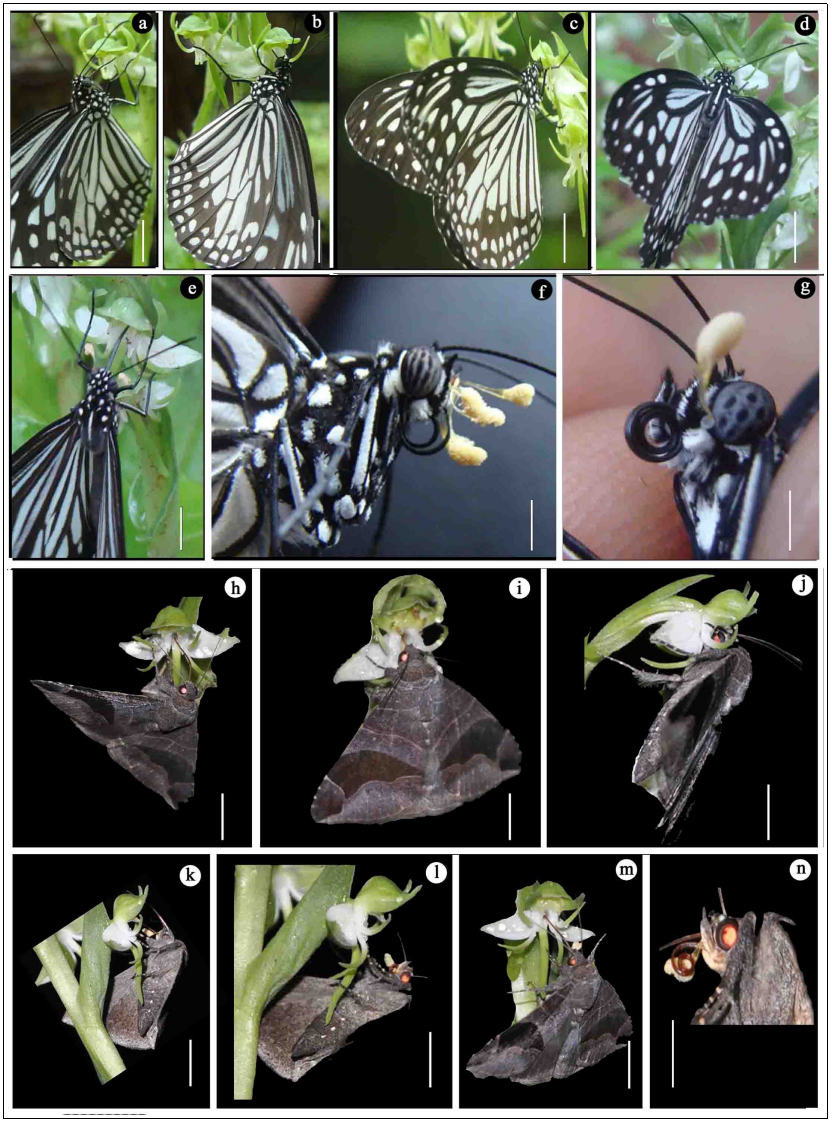

Fig. 2: Activity of Butterfly and Moth during pollination of *Habenaria foliosa* var. *foetida* and comparative account

a-g: *Triumala limniace.* a. - d. probing flower, e. removal of pollinaria, f. an individual bearing pollinaria on its eyes, g. eye attachment of single pollinarium. h-n: *Dysgonia sp.* h.-j. probing flower, k.-l. pollinarium removed from the flower, m. transfer of pollinarium to another flower, n. pollinarium attached to head – scale bars: a-d: 1cm, e: 1.2 cm, f-g: 2.0 mm, h-m:1 cm, n: 2 mm

varies greatly depending on the local climatic conditions. During clear sunshine, butterfly is present whereas hawkmoth is active when the conditions are overcast, cloudy and drizzly without rain and cool breeze. Besides the two insects named, other visitors such as *Danaus chrysippus*, *Euploea core*, *Neoscona rumpfi*, *Hesperotettix speciosus* were observed (Table 1). The highest activity of the blue tiger butterfly was observed from 10.30 to 11.30 am, the lowest activity being during the evening. With respect to the moth, the highest activity was observed between 8.30 and 9.30 pm with the minimal activity occurring beyond 11.00 pm onward up to 1.00 am. An individual visit to a particular flower by a blue tiger lasted between 10 and 30 seconds whereas visits by the moth lasted between 2 and 6 seconds (Fig. 2). On arriving at the flower both pollinators showed more or less similar activity such as (1) flying/hovering around the inflorescence with coiled proboscis, then (2) landing on the flower, with the legs on the labellum or on a neighbouring flower, uncoiling their proboscis and inserting it into the tubular spur. While inserting its proboscis the insect bends its head towards the flower for complete insertion. At this time the head nearly enters into the connate hood of sepals (Fig. 2, a to g). This action of foraging lasts for about 10-30 seconds. After foraging and feeding on the available nectar the blue tiger butterfly removes its proboscis and moves to another flower by walking, rarely by flying, and sits on it. In the case of the moth the insect removes its proboscis, flies off and hovers about the inflorescence again after 1-2 minutes.

Pollinator morphology and identity: upon critical examination and study the moth was identified as a species belonging to the genus *Dysgonia* Hubner. The blue tiger butterfly was identified as *Triumala limniace* Cramer. Normally both insects keep their proboscis coiled under their head and extend it when they probe flowers for feeding. The proboscis length recorded for blue tiger was around 4.0 cm while the proboscis of the moth was up to 3.6 cm. These lengths correspond to the length of the spurs of the plants studied.

Pollinaria transfer by moth and butterfly: observations were made on actual number of blue tiger butterfly and moth visitors captured on site in order to confirm whether they carried pollinaria or not (Table 1). Moth hovers while blue tiger butterfly flies and sits on flower to insert proboscis in the spur. During this movement they press the sticky viscidium with the base of their head. Due to the sticky glue on the viscidium this pressure

detaches the pollinaria by pulling them out on the attached stalks. Viscidium with pollinarium was found to be attached either to the proboscis or regions of the head (in moths) (Fig. 2, n) and to the eyes or antennae (in butterflies) (Fig. 2, f & g). When a moth carrying several pollinaria probes another flower, the head comes up and flushes against the lower inner part of the column. Here several pollens are easily transferred onto the sticky stigma (Fig. 2, l & m). The butterfly carries more pollinia than the moth. During a single visit the butterfly carries 6-8 pollinaria at a time while moth carries only two. Once 2-4 pollinia get detached from flowers and attached to the eyes of butterfly (enough to assure efficient pollination) it tries to remove them for 1-2 min., as it seems that it becomes uncomfortable for the butterfly but when it comes for probing another flower, pollinia get rubbed on stigma. As the number of insects and the number of pollinaria carried out by one insect are higher in the case of butterflies, there are more chances of transferring pollinia on stigma.

Observed visitors / Time interval	*Triumala limniace*	*Dysgonia sp.*	*Danaus chrysippus*	*Euploea core*	*Neoscona rumpfi*	*Hesperotettix speciosus*
8–12 am	✓	X	✓	✓	✓	X
12–4 pm	✓	X	X	✓	X	X
4–8 pm	✓	X	✓	✓	X	X
8–12 pm	X	✓	X	X	✓	X
12-4 am	X	X	X	X	✓	✓
4-8 am	X	X	X	X	✓	✓
Proboscis length (cm)	4	3.6	3.4	3.9	-----	----
Carrying pollinaria	✓	✓	X	✓	X	X

Table1. List of visitors and pollinators observed during study period.

Smell: flowers had foetid smell throughout the day, however strong foetid smell was recorded during clean sun shining days at 11.00 to 11.30 am and decreased from around 9-11 pm, being minimum around 3 am.

Acknowledgments

The authors are indebted to Dr. Mayur Nandikar for his help in observing activity of pollinators. We also wish to thank Dr. Sachin Gurule for the identification of the blue tiger butterfly and the moth. Furthermore we thank Professor Dr. S.R.Yadav, Head, Department of Botany, Shivaji University, Kolhapur, Maharashtra, India for providing the necessary lab facilities. We are also thankful to UGC for providing Research Fellowship under RGNF Scheme.

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1 : Department of Botany, Shivaji University, Kolhapur, Maharashtra (India).
corresponding author: schndangat@gmail.com