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Investigating the pollen transport network between moths (Lepidoptera) and the economically important plants in central and eastern Himalaya

Rajesh Lenka[1,3,4], Dipayan Mitra[1,2,5], Pallab Chatterjee[1,3,6], Avishek Talukdar[1,3,8,*] and Navneet Singh[1,7]

[1]*Zoological survey of India, New Alipore, M-Block, Kolkata-700053, West Bengal, India*
[2]*Department of Botany, University of North Bengal, Raja Rammohunpur- 734014, West Bengal, India*
[3]*Department of Zoology, University of Calcutta, Kolkata- 700019, West Bengal, India*
[4]*E-mail: untamedwild85@gmail.com, ORCID: http://orcid.org/0000-0002-0164-5754*
[5]*E-mail: dip.the.nocturnal@gmail.com, ORCID: http://orcid.org/0000-0002-7216-3920*
[6]*E-mail: cpallab6@gmail.com, ORCID: http://orcid.org/0000-0001-7756-032X*
[7] *E-mail: nsgill007@gmail.com, ORCID: http://orcid.org/0000-0002-6657-7983*
[8]*E-mail: askdar94@gmail.com, ORCID: http://orcid.org/0000-0002-4808-2901*

Abstract

The current study was planned to understand the pattern of pollen transportation in commercially significant plants of the central and eastern Himalayas via non-papilionoid Lepidoptera. For data collection, 14 locations of Arunachal Pradesh, Sikkim, and North Bengal were surveyed between September 2018 to August 2019. Our investigation revealed that 37 species in seven moth families contribute significantly in pollen transportation of eleven economically significant plant species belonging to nine angiosperm families. The analysis is based on a bipartite model between the identified moths and plant taxa to measure pollen carrying capacity, selectivity, connectance, Shannon's diversity, linkage diversity, and link per species. We examined generalist and specialist moth species. The outcome of the current research work offers a basic and fundamental understanding of the ecological role of moths in pollen transportation of economically significant plants in the central and eastern Himalaya, and will thus open up a new doorway in pollination ecology of moths.

Keywords: Angiosperms, Bipartite network, Moth-plant interaction, Non-papilionoid Lepidoptera, Pollen transportation, Potential pollinators

Introduction

Pollen transportation plays a crucial role in the process of pollination, which is vital for the survival of majority of the floral components. This process is essential for the fertilization of the ovules, which leads to the development of seeds and the continuation of the floral generation. Pollen transportation can occur through various means, including wind, water, and animals (Faegri and Van Der Pijl 2014). However, transportation via animal is the most common form and involves the transfer of pollen from flower to flower by animals such as insects, birds, and bats. Pollen is a vital food source for these animals, and as they feed on the nectar within the flower, they inadvertently collect and transport pollen grains on their bodies (Macgregor and Scott-Brown 2020). The transportation of pollen is essential for pollination to occur because it enables the pollen to reach the female reproductive organs of the flower, where it can fertilize the ovules. Without the help of animals, many plant species would not be able to reproduce, and their populations would decline (Macgregor *et al.* 2019). Furthermore, the

*Author for correspondence : askdar94@gmail.com
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genetic diversity of plant populations would be reduced, which could make them more susceptible to diseases and environmental changes (Atwater 2013). In conclusion, pollen transportation is critical for pollination and the survival of many plant species (Brittain *et al.* 2013; Rader *et al.* 2016) Animal pollination, in particular, is an essential ecological process that benefits both plants and animals, and its importance should not be overlooked (Walton *et al.* 2020; Macgregor and Scott-Brown 2020; Singh *et al.* 2022)"

Vertebrate and invertebrate animal pollinators are contributing towards the pollination of approximate 87.5% angiosperm plants and 25-30% pollination services are provided by non-bee insects (Rader *et al.* 2016). Insects are major pollen transporters especially the nocturnal lepidopterans which are extremely species rich and constitute major portion of nocturnal flower pollinating insects (Faegri and Van Der Pijl 2014; Macgregor *et al.* 2017, 2019; Macgregor and Scott-Brown 2020; Singh *et al.* 2022) 2019; But, moths are less studied for their role as pollen transporters but these nocturnal pollinators plays a vital role as pollen transporters of wild flowers in agricultural landscape (Walton *et al.* 2020, 2021)". In agricultural landscape, macro moths provide an unique and complex pollen transport links which makes them a vital player in overall plant–pollinator network (Walton *et al.* 2020) However, in Indian scenario, no paper could be reviewed with information on pollen transportation of, particularly, economically important plants of Himalayan ecosystem, a home to various agricultural/horticultural plants like, Apple (*Malus domestica*; Rosaceae), Kiwi (*Actinidia deliciosa*; Actinidiaceae), Orange (*Citrus sinensis*; Rutaceae), Plum (*Prunus domestica*; Rosaceae), Walnut (*Juglans rigia*; Betulaceae), Grapes (*Vitis vinifera*; Vitaceae), Pomegranate (*Punica granatum*; Punicaceae), Potato (*Solanum tuberosum*; Solanaceae), Brinjal (*Solanum melongena*; Solanaceae), Tomato (*Solanum lycopersicum*; Solanaceae), Cabbage (*Brassica oleracea*; Brassicaceae), Cucurbits (Cucurbitaceae), oilseeds like soybean (*Glycine max*; Fabaceae), Mustard (*Brassica juncea, Brassica campestris*; Brassicaceae), spices like Large Cardamom (*Elettaria cardamomum*; Zingiberaceae), Ginger (*Zingiber officinale*; Zingiberaceae), Turmeric (*Curcuma longa*; Zingiberaceae) (Xu *et al.* 2009; Basannagari and Kala 2013).

Though, Singh *et al.* (2022) published an important study establishing 140 settling moth species as pollen transporter moth species responsible for transporting pollen of 21 plant families. In another study by Chakraborty *et al.* (2021), the seasonal dynamics of plant-pollinator networks based upon pollen transportation in agricultural landscapes have been analysed and the importance of moths as connector species in transporting pollen has been highlighted.

The present study aims (1) to find out the role of moths in pollen transportation of economically important plants of central and eastern Himalaya, and (2) to find out moth species which may be potential pollinator of various economically important plant species prevailing in Himalayan ecosystem of North-East India.

Material and Methods

Collection surveys and Study Area

Survey cum collection tours were conducted from September 2018 to August 2019 in 14 sites of Arunachal Pradesh, Sikkim and North Bengal (Figure 1). The details of the study sites are given in Table 1.

Collection of Moths and isolation of proboscis

Moth specimens were collected using vertical sheet light trap with mercury vapor lamp (160 W) and killed using individual killing jars fumigated with Ethyl acetate to eliminate any chances of pollen contamination. After that proboscides were isolated from each moth using sterilized forceps for slide preparation and the moths were kept in separate envelop with proboscis code number. Finally, moths were relaxed, stretched and preserved for identification according standard techniques of Lepidopterology (Holloway 2001).

Light microscopy of collected pollen from proboscides of moths

Individual proboscides of moth sample was taken on a glass slide and incubated with few drops of phenol-glycerin solution (1:1) for 1–2 minutes for relaxation. After that, one or two drops of basic Fuchsine dye was added with it and the slides were mounted with cover slips and sealed with nail varnish. Photographs have been taken with Nikon 50i compound light microscope fitted with DP–25 digital camera under 40× magnification (Atwater 2013).

Identification of moths and pollen

Moths were identified using available literatures and compared with the samples housed as the samples with the national collection in Zoological Survey of India. For pollen identification, various websites and available literatures were (Agashe and Caulton 2009; Bhattacharya *et al.* 2014;

Halbritter *et al.* 2018; Global Pollen Project 2022; PalDat 2022).

Statistical Analysis

Bipartite network was constructed for showing the interaction between the identified moth species and angiosperms using 'bipartite' package in Rstudio software (R core development team 2021); Dormann *et al.* 2021). In the metadata used for constructing the bipartite network (Figure 2 and 3), the moths are taken along the columns and the angiosperms are taken along the row. Number of pollen grains per proboscis was considered for analyzing network-level indices and the species-level indices of the bipartite network. For showing the characteristics of the bipartite network, various species network-level indices viz. Nestedness, Selectivity (H'_2), Shannon's diversity index, connectance, links per species, linkage density, interaction evenness, interaction strength asymmetry, are calculated. PDI score or species-level paired difference index: value ranges from 0 to 1; 0 shows complete generalism and 1 indicate the total specialism in both trophic level of a bipartite network. It is used to find out the generalist and the specialist in both trophic level of a bipartite network.

Abbreviation used

PTMS- Pollen Transporter Moth Species (A moth species which carries pollen grains)

PPMS- Potential Pollinator Moth Species (A moth species which carries at least five or more than five pollen grains of a particular plant taxa)

Results and Discussion

A total of 37 species in seven families of moths are identified as the pollen transporter of 11 economically important angiosperm species in eight families. Of the 37 PTMS, maximum species came from Geometridae (12 species; 32.43% of total PTMS), followed by Erebidae (10 species; 27.30%), Sphingidae (nine species; 24.32%), and Crambidae (three species; 8.11%). Whereas, three families i.e., Drepanidae, Nolidae and Noctuidae, each are represented by single PTMS. Among 37 PTMS, 35 (94.59% of all PTMS) are identified as PPMS. The highest number of PPMS came from Geometridae (12 species; 34.28% of all PPMS), followed by Erebidae (10 species; 28.57%), Sphingidae (eight species; 22.85%), Crambidae (two species; 5.71%) and the least PPMS are found from Drepanidae, Nolidae and Noctuidae (one species each). The highest numbers of pollen grains are found to be carried by *Cechetra minor* (Sphingidae) i.e. 246 pollen grains of *Alnus* sp. (Betulaceae), followed by *Sarcinodes aequilineata* (Geometridae) with 200 pollen grains of *Malus*. The least number of pollen grains are found to be carried by *Eudocima falonia* (Erebidae) with only one pollen grain of *Butea* sp. (Fabaceae) and *Simplicia brevicosta* (Erebidae) with only two pollen grains of *Rhododendron* sp. Among these, pollen of *Malus* sp. (Rosaceae) are found to be carried by maximum number of moth species (14 species), followed by Rhododendron sp. (Ericaceae) (six species), Betula sp. (Betulaceae) (five species), Olea sp. (Oleaceae) (two species), Salix sp. (Salicaceae) (one species), Brassica sp. (Brassicaceae) (one species). (one species).

The bipartite network has shown high value of selectivity i.e. $H'_2 = 0.94$ as compared to other networks constructed in moth-plant bipartite network related studies (Macgregor *et al.* 2019; Walton *et al.* 2021; Singh *et al.* 2022). Furthermore, the network also shows high value of nestedness, connectance, linkage density and Shannon's diversity (Table 2), and thus, showing the importance of this network in terms of maintaining the ecosystem function and pollination service provided by moths. Moth-plant bipartite network and module web shows that pollens of *Malus* sp. (Rosaceae), *Rhododendron* sp. (Ericaceae) and *Zea mays* (Poaceae) has the highest interactions with different moth species (Figure 3). The high selectivity value of our bipartite network (H'_2 = 0.94) indicates that more specialized species are interacting against generalized species (Rodríguez-Gironés and Santamaria 2006; Blüthgen *et al.* 2006).

Only three moth species e.g. *Rhagastis olivacea* (Sphingidae), *Achaea* sp. (Erebidae) and *Acosmeryx naga* (Sphingidae) show lesser value of PDI score, which means they are not specialist species (those species which have unique interaction in a bipartite network) rather than they are found to be generalists species (having more number of common interactions in a bipartite network).

Our study revealed high potentiality of moths in trans portation of pollen grains as well as their potentiality in pollination of economically important angiosperms. Our results are based on the pollen attached exclusively on the proboscides and thus provide robust inferences for the reasons, 1) pollens on proboscides are less contaminated, and 2) proboscis is the main interacting organ of moth which interacts with flower. Though it is a preliminary study but different network-level indices indicate that this interaction network has high diversity of species and high interaction between specialized and generalized species, particularly in Indian Himalaya, which is a sensitive and mega diverse zone. Our study is providing the baseline information regarding pollen transportation by moths and their importance in pollination. We believe that in the conservation related policies and long-term monitoring of the ecosystem health, moths must be part of management strategies. Decline in moth population could be a potential threat to survivability of economically important angiosperms of this region.

Tables and Figures

Table 1. Details of the sampling localities

Sl. No.	Locality Name	District	State	Latitude (in °)	Longitude (in °)	Elevation (in m)
1	Sitong	West Bengal	Darjeeling	26.9378	88.3714	712.4
2	Eche gaon	West Bengal	Kalimpong	27.1336	88.5744	1773.9
3	Jhendi	West Bengal	Kalimpong	27.0189	88.6472	1742.2
4	Lataguri	West Bengal	Jalpaiguri	26.7475	88.7653	104.8
5	Rishop	West Bengal	Kalimpong	27.1086	88.6486	215.7
6	Jayanti	West Bengal	Alipurduar	26.7039	88.6094	712.6
7	Panthyang	Sikkim	Gangtok	27.3639	88.5686	2022
8	Dikchu	Sikkim	Gangtok	27.3990	88.5233	794
9	Bermiak	Sikkim	Gyalshing	27.2532	88.2332	1453
10	Ravangla	Sikkim	Namchi	27.2857	88.3541	2013
11	Tenga Valley	Arunachal Pradesh	West Kameng	27.2128	92.4642	1339
12	Salari	Arunachal Pradesh	West Kameng	27.3075	92.4096	1285
13	Shergaon	Arunachal Pradesh	West Kameng	27.1342	92.2739	2044
14	Eaglenest WLS	Arunachal Pradesh	West Kameng	27.1575	92.4608	2330

Table 2. Network-level indices of the moth-plant bipartite network

Network-level indices	**Value**
connectance	0.101299
links per species	0.847826
nestedness	17.73053
interaction strength asymmetry	0.608633
linkage density	2.766793
Shannon diversity	2.837667
interaction evenness	0.476659
H'_2	0.948107

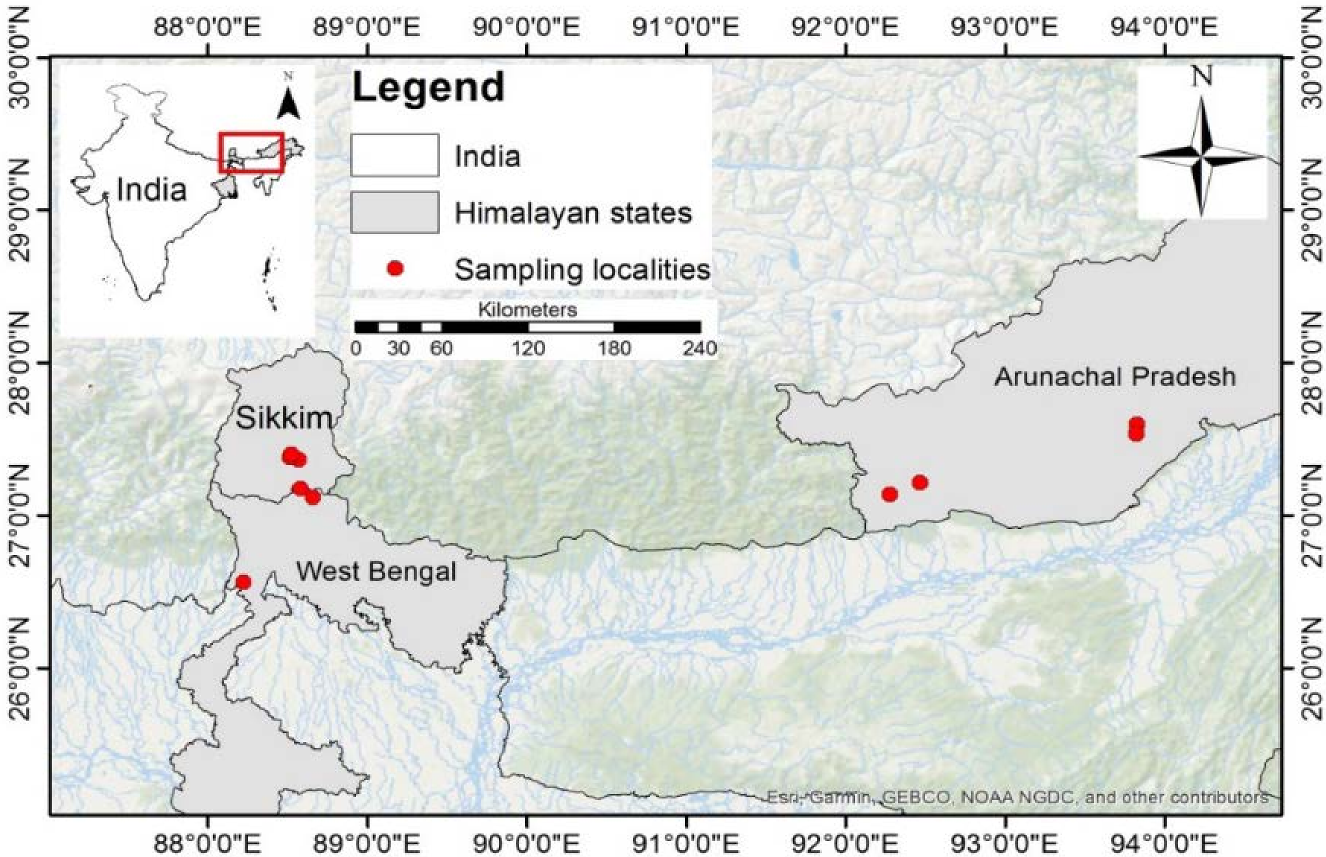

Figure 1. Different sampling localities for the study (ArcGIS V.10.5)

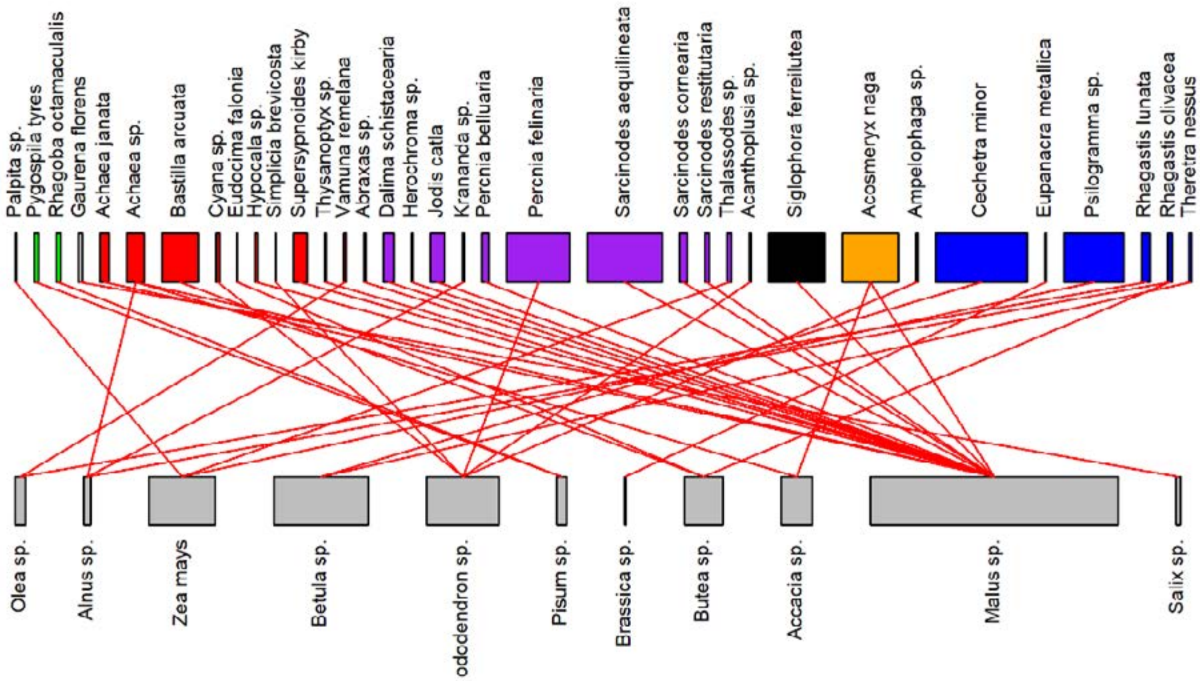

Figure 2. Bipartite network of crop plant species and moth species collected during this study

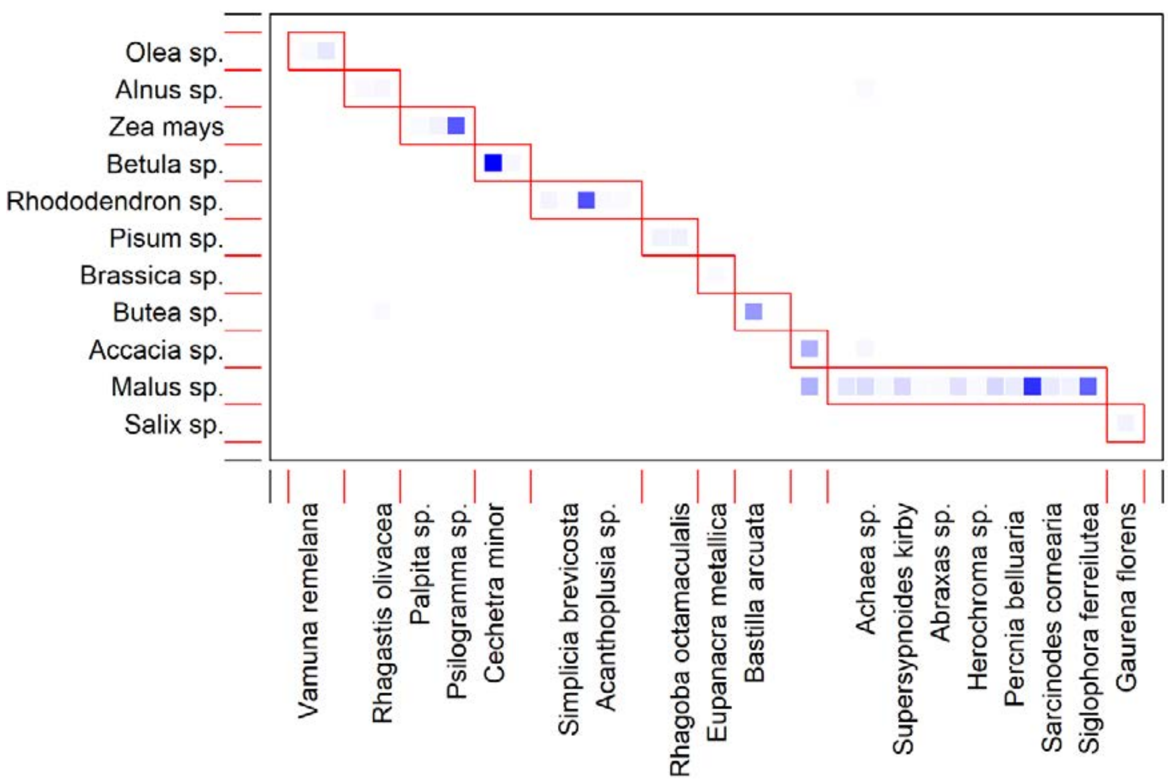

Figure 3. Module web showing only the moth species that taking part actively in their respective plant taxa in the bipartite network constructed during the study

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