

# Pollen limitation failing reproductive success in selected animal pollinated trees of tropical moist deciduous forest of north-eastern hill region, India

Vinod Prasad Khanduri<sup>1</sup> 

**Key words:** pollination, pollen limitation, pollen quantity, bird pollination, pollination success.

**Ključne besede:** oprашevanje, pelodna omejitev, količina peloda, oprășevanje s ptiči, uspeh oprășitve.

## Abstract

The aim of this study was to estimate reproductive effort and success in tropical trees and to examine the effect of pollen limitation on reproductive success. Pollen limitation was assessed through pollen supplementation experiment to contrast the open pollination treatment. The taxa selected were *Bombax ceiba*, *Erythrina stricta* (ornithophilous trees), *Lagerstroemia speciosa*, *Mesua ferrea* and *Schima wallichii* (entomophilous trees). Index of pollen limitation was highest in *Bombax ceiba* and *Erythrina stricta* (both self-incompatible species). The remaining three species were partially self-incompatible with favouring selfing in *Lagerstroemia speciosa* and *Mesua ferrea* and supporting outcrossing in *Schima wallichii*. Therefore, the high index of pollen limitation in *Bombax ceiba* and *Erythrina stricta* might be due to the effect of either lacking quality pollen or lacking bird pollinators. All five species produce a large amount of pollen at individual tree level in the order of  $10^8$  (*Erythrina stricta*) to  $10^{10}$  (*Mesua ferrea*). Fruit and seed set following pollen supplementation were higher than the open pollination (as control) in all studied species. Pollen limitation in this study is likely associated with the effectiveness of pollinator and their frequency, as all the studied species had produced ample pollen at tree crown level which ultimately leads to pollinator resource limitation in tropical trees.

## Izveleček

V raziskavi smo ocenili razmnoževalni napor (proizvodnja peloda) in uspeh tropskih dreves in preučili vpliv omejitve peloda na razmnoževalni uspeh. Omejitev peloda smo ugotavljali z odprto oprășitvijo, ki smo jo primerjali s poskusom dodatnega oprășevanja. Izbrali smo vrste *Bombax ceiba*, *Erythrina stricta* (ornitofilna drevesa), *Lagerstroemia speciosa*, *Mesua ferrea* in *Schima wallichii* (entomofilna drevesa). Indeks omejitve peloda je bil najvišji pri vrstah *Bombax ceiba* in *Erythrina stricta* (obe vrsti sta samoneoplodni). Preostale tri vrste so bile delno samoneoplodne; večjo naklonjenost samooploditvi imata vrsti *Lagerstroemia speciosa* in *Mesua ferrea*, medtem ko je pri vrsti *Schima wallichii* spodbujana navzkrižna oploditev. Do visokega indeksa pelodne omejitve pri vrstah *Bombax ceiba* in *Erythrina stricta* lahko pride zaradi pomanjkanja kvalitetnega peloda ali odsotnosti oprășevalcev. Vseh pet vrst proizvaja velike količine peloda na posameznem drevesu, od  $10^8$  (*Erythrina stricta*) do  $10^{10}$  (*Mesua ferrea*). Količina plodov in semen, ki so posledica dodatnega peloda, je bila večja kot pri odprti oprășitvi (kontroli) pri vseh vrstah. Pelodna omejitev je bila rezultat omejene količine in kvalitete peloda. Ker so vse preučevane vrste proizvedle dovolj peloda v krošnjah, je verjetno razlog za pelodno omejitev pri tropskih drevesih povezana z uspešnostjo oprășevalcev in njihovo pogostostjo.

Corresponding author:  
Vinod Prasad Khanduri  
E-mails: khandurivp@yahoo.com

Received: 10. 10. 2020

Accepted: 23. 9. 2022

## Introduction

Tropical plants have remarkably varied sexual systems, breeding systems, flower morphology, and phenology (Bawa, 1990). Plant reproductive system forms ecosystem vitality through regeneration, speciation and ecology of organisms (Ollerton et al., 2011; Lever et al., 2014). Mating system (selfed, mixed and outcrossed) has been recognized as a best tool to decipher genetic diversity or population genomics of plants (Glemin et al., 2006; Wright et al., 2008; Jadwiszczak, 2017). The population genetic structure of a species is influenced by self-compatibility, self-incompatibility and inbreeding depression (Charlesworth & Charlesworth, 1987; Holsinger, 2000; Duminil et al., 2007; Portillo Lemus et al., 2022). However, inbreeding depression is the key factor that control evolution of plant breeding system (Hufford & Hamrick 2003; Devaux et al., 2019). Such studies on long lived tree species are lacking (Ferriol et al., 2011; Rodger & Johnson, 2013, Cristóbal-Pérez, 2020; Ahlinder et al., 2021; Khanduri et al., 2022). Tree flowering, pollination and reproductive systems have been recognized as important parts of tree improvement, breeding and management of silvicultural practices (Nikanishi et al., 2015; Khanduri & Sukumaran, 2019).

The complete development of floral characters along with the rate of pollen production in tree species is helpful in understanding the detailed pollination mechanism and barriers in pollination biology (Etcheverry et al., 2008; Khanduri et al., 2019). Furthermore, the quantity of pollen production is important to (i) determine the amount of genes a population can spread (Liu et al., 2015), (ii) establish an airborne pollen forecasting (Voukantsis et al., 2010; Csépe, 2020), (iii) assess the relative density of atmospheric pollen grains of every species in a forest ecosystem (Charalampopoulos et al., 2013), (iv) predict production of seeds in a reproductive season (Allison, 1991), (v) determine the availability of pollen for pollinators to achieve pollination success (Mu et al., 2017; Khanduri et al., 2015; Sukumaran et al., 2020), and (vi) assess genetic homogeneity of a population through ample pollen exchange among individuals (Dufayé et al., 2008; Ramos et al., 2016).

The pollination mechanism in tree species is highly significant for biological studies in ecology, co-evolution, variation and speciation, classical and applied genetics and plant breeding (Van der Niet et al., 2014). The success and failure of pollination is a prerequisite in plant breeding and for obtaining better crop yields (Wilcock & Neiland, 2002). Variability in forest tree crops is controlled by the breeding system, of which pollination

mechanism forms an integral component (Hilje et al., 2015). Knowledge of the breeding system is also important for managing breeding populations and for designing efficient genetic conservation strategies (Rao & Hodgkin, 2002).

Ecologically, plant – animal interactions is the most important phenomena without which reproductive success and reproduction cannot be achieved. Similarly without plants, the animals that dependent on flower resources, viz. pollen, nectar and other rewards, may lose their population (Kearns et al., 1998). Therefore, the plant – animal interactions are considered as the most significant classes of ecological interactions (Ollerton, 2017). Animal pollination has been reported to increase fruit and seed set for about 75% of the world’s leading food crops (Klein et al., 2007). Majority of animals have the potential to be successful pollinators when they visit the flowers. However, the effectiveness of pollinators depends on the abundance of animal in a community; the predilection for that animal to contact anthers, bear pollen, and touch stigmas; and whether or not the animal travels to visit other flower of the same species (Rodríguez-Rodríguez et al., 2013). About 94% of tropical plants rely on animal pollination (Ollerton et al., 2011). Tropical forest ecosystem has high species richness which implies that the animal pollinators may be specific for interactions with other taxa resulting to species evolution (Pauw & Stanway, 2015; Moles & Ollerton, 2016). A high number of tropical trees are animal pollinated and majority of them are of insect pollinated particularly bees pollinated (Bawa, 1990). Comparatively a small number of plants are pollinated by birds or mammals (Devy & Davidar, 2003, Fleming & Muchhala, 2008).

The shortfall in seed production that plants experience due to inadequate pollen receipt (where pollen is either of inadequate quantity or quality) compared to a situation where ideal pollination has been represented by pollen limitation (reproductive success limited by pollen). Pollen limitation arises if there are not enough visitors, or if there is not sufficient production of pollen, or if there is a lack of conspecifics to mate with. Study of pollen limitation is important to see (i) if there is potential to improve seed production, and (ii) if abundance of pollinators may be low and plant reproduction in the wild may be threatened. Pollen limitation has been examined from various perspectives viz. (i) pollen limitation of female reproduction (Bierzychudek, 1981; Burd, 1994); (ii) ‘pollination crisis’ (Kearns et al., 1998); (iii) the role of pollen limitation in plant populations (Ashman et al., 2004; Knight et al., 2005; 2006); (iv) methodology for pollen limitation (Wesselingh, 2007); (v) pollinator

dependence and self-incompatibility on pollen limitation (Rodger & Ellis, 2016); and (vi) insufficient delivery of outcross pollen and self-incompatibility on pollen limitation (Khanduri et al., 2019). Pollen limitation is best assessed by pollen supplemental hand pollination experiments in which fruit and seed set using hand pollination is compared with that of naturally pollinated flowers (Burd, 1994). When reproductive success is significantly high in supplemental pollination as compared to that of open pollination, pollen limitation is confirmed (Chen & Zuo, 2018; Khanduri et al., 2019). Most of the pollen limitation studies are restricted to the herb and shrub species (Ashman et al., 2004; Cosacov et al., 2008; Wolowski et al., 2013; Chen & Zhao, 2017). However, pollen limitation experimentally in tree species is sparse (González-Varo et al., 2009; Van Etten et al., 2015; Khanduri et al., 2019) because the crown height in trees is difficult to approach for conducting the hand pollination experiments.

This study aims to 1) estimate the reproductive effort and success of five tropical tree species, 2) examine whether the reproductive success is influenced by pollen limitation, and 3) assess inbreeding depression for each analysed species for which the data was used on the basis of experimental work done by the author (Khanduri, 2016), and the average value calculated for fruit set, seed-set and seed germination was taken. The study species were (i) *Bombax ceiba* (an economically important on account of the presence of floss surrounding the seeds, which is used for making pillows, cushions, etc.; (Khanduri & Kumar, 2017a), (ii) *Erythrina stricta* (medicinally useful; Kirthikar & Basu, 1987; Khanduri et al., 2021), (iii) *Lagerstroemia speciosa* (medicinally important; Khanduri, 2014), (iv) *Mesua ferrea* (high medicinal potential; Khanduri & Kumar, 2017b) and (v) *Schima wallichii* (commercial timber tree; Khanduri et al., 2013). The results of the study will be of immense value for understanding the behaviour of pollen grains in nature and, subsequently for various tree improvement and breeding programmes to forest geneticists, silviculturists and evolutionary biologists because of their adaptive and practical significance. The extent of variation in reproductive success on all the analyzed five tropical tree species within individual stand is highly valuable for tree improvement programme and has also the potential to conserve the genetic composition of a species. The results of the study would further be utilized for provenance research and development of seed orchards, seed production areas and genetic testing of the selected species.

## Materials and methods

### Study site

The study was conducted during the flowering season in the years 2010 and 2011 in a tropical moist deciduous forest of Bethlehem Vengthlang, situated in the district Aizawl of Mizoram State in the north-eastern hill region (NEH) of India. The study area was located at 23° 43' 47.5" N Latitude and 92° 43' 53.5" E Longitude at an elevation of 900 m above sea level (asl). Average rainfall in the study area was 1900– 2100 mm and mean annual temperature was 19.0° C. There is a dry season of six months, from October to March. The studied tropical forest was mature, mixed, uneven-aged/irregular with the associations of 18 tree species.

### Species description

Five flowering tree species were selected for study, representing a variety of floral characters, pollination syndromes and breeding systems. These included the *Bombax ceiba* (large-sized flowers, self-incompatible and commonly accepted as bird pollinated, Khanduri & Kumar 2017a), *Erythrina stricta* (cross pollinated and characteristically ornithophilous flowers, Etcheverry & Alemán 2005; Khanduri et al., 2021), *Lagerstroemia speciosa* (relatively large-sized flowers and entomophilous tree), *Mesua ferrea* (medium sized flower and entomophilous tree) and *Schima wallichii* (relatively smaller size flowers and entomophilous tree).

### Flower, pollen and seed production

Flower and pollen production along with ovule number per flower was assessed by choosing ten trees each of five investigated tree species as per the method of Khanduri et al. (2015). The ovule number per tree for each analysed species was calculated by multiplying the ovule number per flower with number of flowers per tree. The number of seeds per fruit was estimated at the time of fruit maturity of each analysed species. The time of fruit maturity was different for different species, i.e. 1.5 months for *B. ceiba* and *E. stricta*, 6 months for *M. ferrea* and 9 months for *L. speciosa* and *S. wallichii*. Fruit development was monitored in 15 days interval for *B. ceiba* and *E. stricta* and after every one month intervals for remaining three species. 50 mature fruits of each species which have been matured through open pollination were harvested to estimate number of seeds produced per fruit/pod. Number of seeds per fruit and subsequently for 50 fruits was counted manually and an average value (seeds

per fruit) for each species was calculated. The number of fruits/pods per tree was calculated by multiplying number of fruits per sub-branch (in case of *B. ceiba*, *M. ferrea* and *S. wallichii*) and per inflorescence (for *E. stricta* and *L. speciosa*) by the number of sub-branches /inflorescences per branch, which was multiplied by the number of fruit bearing branches per tree. Number of seeds per tree was calculated by multiplying the average number of seeds produced per fruit by the number of fruits produced per tree following open pollination.

## Flower visitation

Flowers visitors were recognized by observing 10 individuals of each of the five tree species, for two-hour periods, at peak stages of flowering. Bird visitors to flowers were identified using 8×40 binoculars at a distance of 8 to 10 meter to avoid disturbance to the visitors, and the number of visits by each species was recorded. Visitation frequency was recorded as visit per small branch for *B. ceiba* and visit per inflorescence for *E. stricta*. Photographs of the birds foraging nectar from the flower was also taken. The standard method used by Dawson & Bull (1975) was followed to measure the relative abundance of bird species.

The insect visitors to flowers were identified by taking a sample of several inflorescences enveloped in a pillowcase and tapping the branch to dislodge insects. The insects were captured in a collecting jar (Oldroyd, 1958), and killed by inserting cottonwool soaked in ethyl acetate. Later, the specimens were examined and identified upto genus and species level. Sampling was replicated on ten randomly selected individuals of all insect-visited tree species.

## Pollen supplementation experiment and pollen limitation

Pollen supplementation experiment was performed to assess pollen limitation. The selected ten trees of each analysed species were used to carry out the experiment. Pollen from each study species was collected from the neighbouring trees growing at a distance of 50 to 80 m from the experimental trees. Collected pollen grains from different trees of each species were mixed and kept in air-tight vials and stored at 4 °C in the refrigerator prior to use. Flowers of each tree species was emasculated before anthesis and isolated with fine net. Collected pollen grains were applied to the stigmas of isolated flowers during peak receptivity using brush and isolated again till the completion of flowering in the population. Acetocarmine test (Zheljzakov et al., 2021) was done to check the viability of pollen grains before application. For open

pollination, flowers in different inflorescences and sub-branches were tagged and left to natural pollination by insects and birds. Each pollination treatment (pollen supplementation and open pollination) was applied on 20 flowers per individual tree distributed on 4–5 branches in case of *B. ceiba*, *M. ferrea* and *S. wallichii* because of the solitary flowers on the branches. While 10 inflorescences distributed on 5 branches per individual tree for *E. stricta* and *L. speciosa* were selected for pollination treatment. A total of 1200 flowers were marked and monitored for fruit set and brood size, 600 and 600 as open pollination and supplemental pollination, respectively for *B. ceiba*, *M. ferrea* and *S. wallichii*. Whereas a total of 400 inflorescences, 200 for supplemental pollination and 200 for open pollination for *E. stricta* and *L. speciosa*, were selected for fruit set. Branches within a tree were pooled for each treatment for data analyses. Percent conversion of flowers to fruits was calculated by dividing the average number of converted fruits produced following open pollination and supplemental pollination by the number of flowers selected/manipulated at the time of pollination per tree for each species. A comparison of fruit set following open pollination and supplemented pollination was made and presented in a bar diagram to differentiate between two treatments.

The values obtained for fruit set following open pollination and supplemented pollination in each species was averaged. First the fruit set was obtained for each individual tree and then averaged across trees for each species. A factorial analysis of variance was done to test the effects of species, tree individuals, pollen level and interaction of trees and pollen level on fruit set using JMP statistical software pro 14.

For each analyzed tree species, fruit set obtained for both treatments were used to calculate a pollen limitation index expressed as:  $PL = 1 - OP/SP$ , where OP represents fruit set of open pollination and SP represents the fruit set of supplemental pollination treatments following Larson & Barrett (2000). Fruit set percentage was used to estimate the pollen limitation index which is a widely used measure of fertility for pollen limitation (Larson & Barrett, 2000). Index of pollen limitation varies from zero to one, with zero indicating no pollen limitation and one showing full pollen limitation (e.g., Larson & Barrett, 2000; Tamura & Kudo, 2000; Lázaro & Traveset, 2006).

## Seed germination

Seed germination test of each studied species was done under laboratory conditions. Mature seeds of each species were taken and placed in the Petri-dishes with Whatman blotting paper. Thirty seeds with three replications in the

Petri-dishes were used in each species. The germination tests were conducted under room temperature and the Whatman blotting paper was used as a germination media, which was moistened with the running tap water so that the result of seed germination would be similar to that of the natural conditions.

### Inbreeding depression

The inbreeding depression was calculated by using the formula;  $\delta = 1 - W_s/W_o$ ; Where  $\delta$  = inbreeding depression,  $W_s$  = the result of progeny from self-pollination and  $W_o$  = the outcome of progeny from cross-pollination (Lande & Schemske, 1985). A delineation inbreeding depression value of  $\delta = 0.5$ , signified that the selfing is preferred below the value and beyond which outcrossing is favoured (Lande & Schemske, 1985).

**Table 1:** Flowering traits of five selected tropical trees.

**Tabela 1:** Značilnosti cvetov petih izbranih tropskih dreves.

Study species	Flower phenology	Flower morphology	Number of anthers and their arrangements in the flower	Secretion of nectar from nectaries
<i>Bombax ceiba</i>	Flowering: second week of January to first week of March; Anthesis: 05:00 to 11:00 hr of the day Anther dehiscence: half an hour after anthesis	Bisexual, large cup shape flower red to orange colour. Calyx green and leathery, corolla consists of five petals, tubular at the base	Infinite; Occurs in five bundles	Nectar production starts at anthesis in the morning
<i>Erythrina stricta</i>	Flowering: second week of February to third week of March. Anthesis: 06:00 to 11:00 hr of the day Anther dehiscence: half an hour after anthesis	Bisexual, one well-developed bright red standard petal, two greenish-red keel petals, two poorly developed very light-red wing petals. Keel petals form a carinal-like structure that holds nectar inside.	10; Monadelphous, usually bend upwards facing the standard petal	Nectar secretion starts from anthesis onwards.
<i>Lagerstroemia speciosa</i>	Flowering: Mid April to mid July Anthesis: 06:00 to 10:00 hr of the day Anther dehiscence: half an hour after anthesis	12–15mm long calyx, funnel- or bell shaped, six-lobed, fleshy, brownish in colour; petals often six, inserted near the mouth of the calyx tube, pink to pinkish white or mauve-purple, clawed, wrinkled.	Infinite; arranged in several rows	Flowers do not produce nectar.
<i>Mesua ferrea</i>	Flowering: mid March to first week of May Anthesis: 05:00 to 10:00 hr of the day. Anther dehiscence: one hour after anthesis.	White flowers with four large corolla free, fleshy, retuse, and spreading; calyx four green.	Infinite; free and connate at the base.	Nectar secreted in traces at the beginning of anthesis.
<i>Schima wallichii</i>	Flowering: Third week of March to first week of May. Anthesis: 06:00 to 10:00 hr of the day Anther dehiscence: one hour after anthesis.	Flowers are conspicuous, with five connate white, dialypetalous and actinomorphic corolla	Infinite; inserted around the style head	Nectar secretion starts at the beginning of anthesis

## Results

### Flower, pollen and ovule production

Among the 5 study species, 3 are deciduous and 2 are evergreen in nature. The deciduous species, viz. *Bombax ceiba*, and *Erythrina stricta* blooms when they are devoid of foliage. The flowers of analysed species are large in size, showy with a striking colour, full of nectar (*Bombax ceiba*, *Erythrina stricta*), and abundant pollen that can be visible from a long distance to attracts the pollinators, both birds and insects. The flower phenology, morphology, time of the day when anthesis starts, number of anthers and their arrangements in the flower, and secretion of nectar from nectaries is presented in Table 1. There was variation in the amount of pollen production per anther in different species. The maximum number of pollen grains per anther was recorded in *B. ceiba*, followed by *M. ferrea* and

**Table 2:** Reproductive effort, pollen limitation and fecundity in five tropical tree species ( $\pm$  SE).

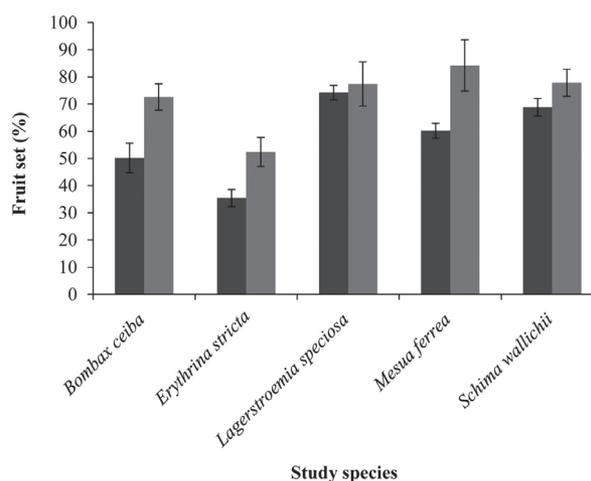
**Tabela 2:** Razmnoževalni napor, pelodna omejitev in plodnost pri petih vrstah tropskih dreves ( $\pm$  SE).

Studied variables	<i>Bombax ceiba</i> (2011)	<i>Erythrina stricta</i> (2011)	<i>Lagerstroemia speciosa</i> (2010)	<i>Mesua ferrea</i> (2010)	<i>Schima wallichii</i> (2011)
Pollen grains/ anther	14160 $\pm$ 1005	1022 $\pm$ 78.64	956 $\pm$ 60.62	3564 $\pm$ 202.4	1932 $\pm$ 110.42
Pollen grains per Tree	1.20 $\pm$ 0.11 $\times 10^9$	1.26 $\pm$ 0.32 $\times 10^8$	9.19 $\pm$ 1.14 $\times 10^8$	6.12 $\pm$ 0.20 $\times 10^{10}$	1.60 $\pm$ 0.40 $\times 10^9$
Flowers per Tree	1232 $\pm$ 132	13426 $\pm$ 3416	4542 $\pm$ 254	14986 $\pm$ 1235	6274 $\pm$ 1306
Fruit set (%) open pollination	50.18 $\pm$ 5.42	35.46 $\pm$ 3.16	74.2 $\pm$ 2.64	60.20 $\pm$ 2.74	68.84 $\pm$ 3.24
Ovule numbers per flower	372 $\pm$ 20.60 307.3	08 $\pm$ 0.76 5.65	154 $\pm$ 10.86 133	1–4	22 $\pm$ 1.84 15.4
Ovule numbers per tree	446204 $\pm$ 2850	108562 $\pm$ 1986	698894 $\pm$ 5786	30250 $\pm$ 1042	141254 $\pm$ 2190
Seed numbers per tree (open pollination)	168845 $\pm$ 1680	31378 $\pm$ 876	389018 $\pm$ 3986	24200 $\pm$ 986	55197 $\pm$ 1288
Pollen limitation index (fruit set)	0.3088	0.3233	0.0413	0.2850	0.1152
Pollen limitation index (seed set)	0.3833	0.0334	0.1368	0.1342	0.2522
Seed Germination (%)	63 $\pm$ 2.46	87 $\pm$ 4.56	53 $\pm$ 2.56	60 $\pm$ 2.16	67 $\pm$ 4.42
Inbreeding depression ( $\delta$ )	1.0	0.82	0.39	0.37	0.52

*S. wallichii*. The least was estimated in *L. speciosa*. An anther of *B. ceiba* produces 4 times more pollen than the anther of *M. ferrea*. The production of pollen grains per tree was maximum for *M. ferrea* ( $6.12 \pm 0.20 \times 10^{10}$ ), followed by *S. wallichii* ( $1.60 \pm 0.40 \times 10^9$ ) and *B. ceiba* ( $1.20 \pm 1.1 \times 10^9$ ). This pattern of variation in different species is due to variation in the production of anthers per flower and flowers per tree. The production of ovule numbers per tree was estimated maximum for *L. speciosa*, which was 1.5, 5.0, 6.4 and 23.0 times more than the ovule number per tree for *B. ceiba*, *S. wallichii*, *E. stricta* and *M. ferrea*, respectively (Table 2). Seed germination was recorded between 53 and 87% in the study species. The maximum was recorded for *E. stricta* and minimum for *L. speciosa*. The results of inbreeding depression suggests that *B. ceiba*, and *E. stricta* are outcrossed species whereas *L. speciosa* and *M. ferrea* inclining towards selfing. *S. wallichii* is persuading towards outcrossing (Table 2).

### Pollen limitation

All the studied species have shown that maximum reproductive success is not achievable due to pollen limitation. Pollen limitation index was weaker for *L. speciosa* and *S. wallichii* (Table 2). The lowest pollen limitation index was recorded for *L. speciosa* (0.0413). The supplementation of pollen grains had significantly augmented the fruit set in all the five tropical tree species (Figure 1). There was a significant effect of pollen addition on fruit set in respect of different studied species ( $p < 0.0054$ ) and among individuals of a species ( $p < 0.0026$ ). The pollination treatments (pollen level) and the interaction of trees and pollination treatments had also shown a significant ( $p < 0.0001$ ) effect to fruit set (Table 3).



**Figure 1:** The mean fruit set of five tropical tree species under pollen limitation treatments, i.e. open pollination vs supplemental pollination. Vertical bars represent standard error.

**Slika 1:** Povprečna količina plodov za pet dreves tropskega gozda v različnih obravnavah pelodne omejitve, t.j. odprta opraišitev v primerjavi z dodatno opraišitvijo. Navpične črte predstavljajo standardno napako.

**Table 3:** ANOVA statistics for the effects of species, trees and pollination treatments on fruit set.

**Tabela 3:** Analiza ANOVA vpliva vrst, dreves in opraiševalcev na količino plodov.

Response variable	Df	F	P
Species	4	24.34	0.0054
Trees	9	18.64	0.0026
Pollen level (Pollination treatments)	1	30.38	0.0001
Trees $\times$ Pollen level	9	26.82	0.0001

Df = degrees of freedom, F = F-ratio, p = probability

## Flower pollinators

Varieties of birds were recorded visiting flowers of *B. ceiba* and *E. stricta* (Table 4 and Figure 2). The highest frequency of bird visitation was recorded in the morning hours to both species. *Pycnonotus cafer* was recorded visiting the flowers of *B. ceiba* and *E. stricta* throughout the day except between 12:00 to 15:00 h. Bimodal pattern of visitation to the flowers of *B. ceiba* was recorded for *Dicrurus adsimilis aillbiritus* and *Turdoides caudata*. Similar pattern to the flowers of *E. stricta* was observed for *D. leucophaeus* and *Zosterops palpebrosus*. Fourteen species of birds were observed visiting to the flowers of both tree species. Nine species were common to both *Bombax* and *Erythrina* species. *P. cafer* and *D. macrocercus* was the most prevalent flower visiting bird species for *B. ceiba* (Table 3), which in case of *E. stricta* was *P. cafer* and *D. leucophaeus* (Table 4).

A strong correlation between the visitation rate and abundance of each bird species was observed for both tree species (spearman's test for correlation: *B. ceiba*:  $rs = 0.9$ ; *E. stricta*:  $rs = 1.0$ ). *D. a. aillbiritus* and *P. cafer* were observed showing close contact with the stamen and pistil of the *B. ceiba* flower while foraging the nectar that has been shown in Figure 2B and 2A, respectively. Similarly, the bird species i.e. *P. cafer* (Figures 2E), *Chloropsis aurifrons* (Figures 2G. i) and *D. eucophaeus* (Figures 2H. ii) were recorded as effective bird pollinators for *E. stricta*, as all are indicating close contact to the reproductive parts of the flowers of *Erythrina* while foraging the nectar.

Visitation of insects was recorded in all the five studied tree species (Table 4). Hymenoptera, Lepidoptera, Diptera and Coleoptera were the recorded flower-visiting insect orders. Highest diversity of insects was observed for *S. wallichii*, followed by *L. speciosa* and *M. ferrea* (Table 5).

**Table 4:** Bird visitors/pollinators abundance in *Bombax ceiba* and *Erythrina stricta*.

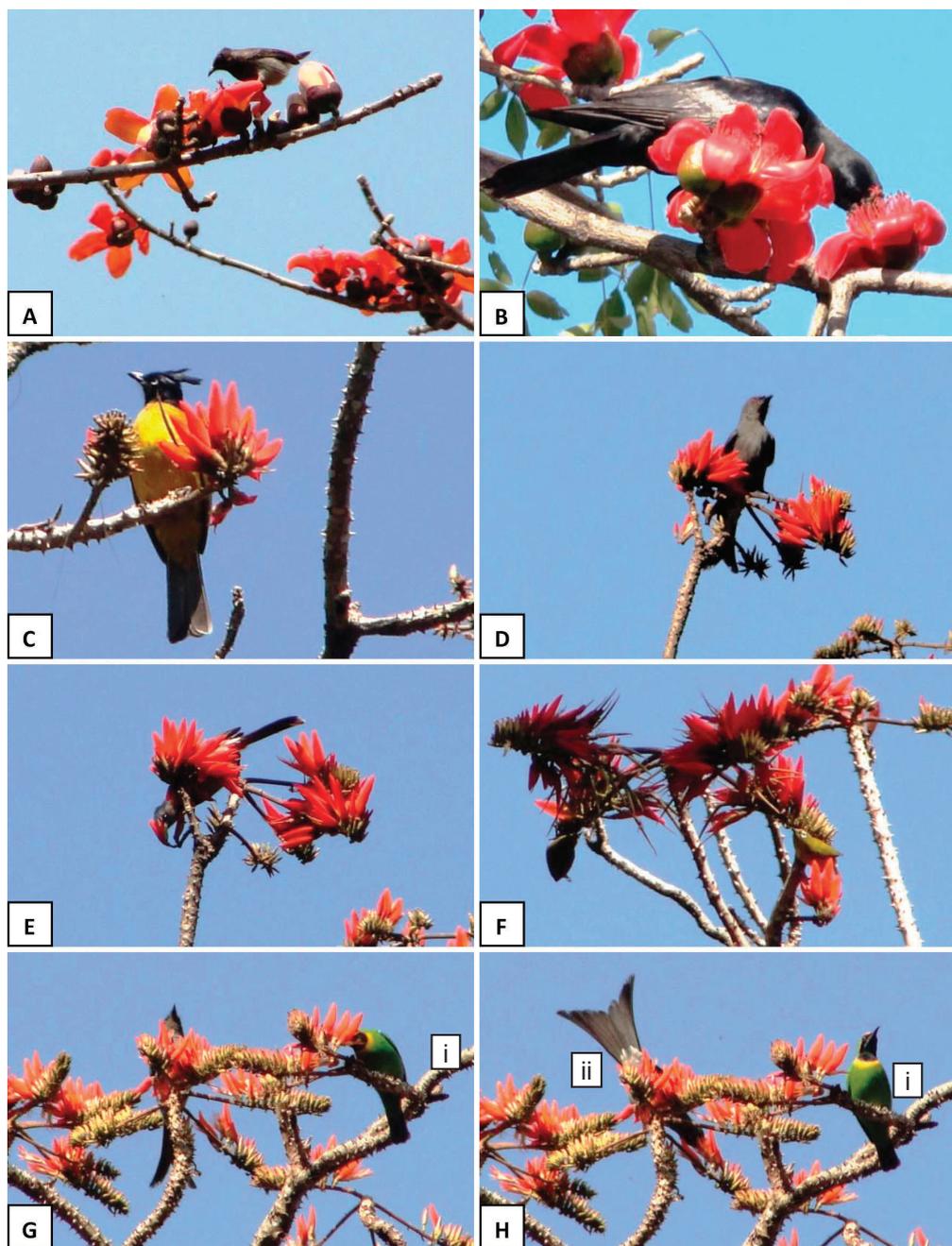
**Tabela 4:** Abundanca ptic obiskovalk/opraševalk na vrstah *Bombax ceiba* in *Erythrina stricta*.

Scientific Name of Bird	Bird Visit per branch at different hour <i>Bombax ceiba</i>				Bird Visit per branch at different hour <i>Erythrina stricta</i>			
	06:00–08:00	0800–1000	1000–1200	1500–1700	0600–0800	0800–1000	1000–1200	1500–1700
<i>Pycnonotus cafer</i>	8	12	4	8	6	10	04	5
<i>Pycnonotus melanicterus flaviventris</i>	-	-	4	2	-	6	7	3
<i>Pycnonotus jocosus</i>	-	8	7	5	-	-	-	-
<i>Dicrurus adsimilis aillbiritus</i>	18	-	-	8	-	-	-	-
<i>Dicrurus hottentottus</i>	6	6	-	-	-	-	-	-
<i>Dicrurus macrocercus</i>	9	7	-	-	-	-	-	-
<i>Dicrurus leucophaeus</i>	-	-	-	-	8	10	-	8
<i>Dicrurus paradiseus</i>	-	12	-	-	-	8	-	-
<i>Macronous gularis</i>	-	14	-	-	-	12	-	-
<i>Stachyridopsis ruficeps</i>	14	-	-	-	-	8	-	-
<i>Heterophasia gracilis</i>	-	6	-	-	-	4	-	-
<i>Turdoides caudata</i>	4	-	-	3	-	-	-	-
<i>Zosterops palpebrosus</i>	-	-	-	-	6	-	-	4
<i>Falco severus</i>	2	-	-	-	2	-	-	-
<i>Cuculus micropterus</i>	6	-	-	-	4	-	-	-
<i>Chloropsis aurifrons</i>	-	-	-	-	-	4	-	-

**Table 5:** Insects visiting the flowers of analyzed tropical forest trees.

**Tabela 5:** Vrste žuželk, ki so obiskovale cvetove preučevanih dreves tropskega gozda.

Order	Family	Scientific Name	Visited tree species
Hymenoptera	Apidae	<i>Apis cerena</i>	<i>Erythrina stricta</i> , <i>Mesua ferrea</i> <i>Lagerstroemia speciosa</i> , <i>Schima wallichii</i>
		<i>Apis florea</i>	<i>Mesua ferrea</i> , <i>Lagerstroemia speciosa</i>
		<i>Xylocopa</i> spp	<i>Bombax ceiba</i> , <i>Erythrina stricta</i> , <i>Mesua ferrea</i> <i>Lagerstroemia speciosa</i> , <i>Schima wallichii</i>
Diptera	Calliphoridae	<i>Chrysomya megacephala</i>	<i>Lagerstroemia speciosa</i>
Lepidoptera	Lycanidae	<i>Arhopala eumolphus</i>	<i>Mesua ferrea</i> , <i>Schima wallichii</i>
Lepidoptera	Hesperiidae	<i>Matapa aria</i>	<i>Schima wallichii</i>
Lepidoptera	Geometridae	<i>Cabera pusaria</i>	<i>Schima wallichii</i>
Lepidoptera	Geometridae	<i>Dysphania militaris</i>	<i>Schima wallichii</i>
Lepidoptera	Zygaenidae	<i>Callamesia midama</i>	<i>Schima wallichii</i>
Lepidoptera	Zygaenidae	<i>Eterusia aedeia</i>	<i>Schima wallichii</i>
Lepidoptera	Noctuidae	<i>Ahypia</i> spp	<i>Schima wallichii</i>
Coleoptera	Scarabaeidae	<i>Maladera castanea</i>	<i>Schima wallichii</i>



**Figure 2:** A. Redvented Bulbul (*Pycnonotus cafer*) making contact with the stigma of the *Bombax* flower during nectar foraging, B. *Dicrurus adsimilis albiriltus* touching reproductive parts of *Bombax* flower effectively while taking nectar, C. Black-crested Bulbul (*Pycnonotus melanicterus*) sitting to the *Erythrina* flower to probe the nectar, D. Ashy Drongo (*Dicrurus leucophaeus*) resting in the *Erythrina* flower bough; E. Red-vented Bulbul (*Pycnonotus cafer*) showing full contact with the floral reproductive parts of *Erythrina*; F. Indian white-eye (*Zosterops palpebrosa*) ready to go to another flower of *Erythrina* after taking nectar from the sitting flower; G(i). Golden-fronted Leafbird (*Chloropsis aurifrons*) showing valuable contact with essential reproductive parts of the *Erythrina* flower at the time of foraging nectar; H. (i) Golden-fronted Leafbird taking rest just after probing the nectar and (ii) Ashy Drongo (*Dicrurus leucophaeus*) during foraging of the *Erythrina* flower.

**Slika 2:** A. *Pycnonotus cafer* v stiku z brazdo cveta vrste *Bombax* med hranjenjem z medičino, B. *Dicrurus adsimilis albiriltus* se dotika reproduktivnih delov cveta vrste *Bombax* med odvzemanjem medičine, C. *Pycnonotus melanicterus flaviventris* sedi na cvetu vrste *Erythrina* in išče medičino, D. *Dicrurus leucophaeus* počiva na veji s cvetom vrste *Erythrina*; E. *Pycnonotus cafer* v polnem stiku z razmnoževalnimi deli vrste *Erythrina*; F. *Zosterops palpebrosa* se pripravlja na let na drug cvet vrste *Erythrina* po tem, ko je popil medičino; G(i). *Chloropsis aurifrons* v stiku z razmnoževalnimi deli cveta vrste *Erythrina* v času pitja medičine; H. (i) *Chloropsis aurifrons* počiva po pitju medičine in (ii) *Dicrurus leucophaeus* med prehranjevanjem na cvetu vrste *Erythrina*.

## Discussion

The amount of pollen produced by a tree is to recompense for low pollination efficiency and warrant fertilisation. Pollen quantity and quality provide in measuring and forecasting several factors, e.g. crop yield in trees (Allison, 1990; Hirayama et al., 2005; Iwaizumi & Takahashi, 2012) and yield in agriculture crops (Westgate et al., 2003), fruit size and production of seeds (Faegri & Iversen, 1989; McKone, 1990; Baskin & Baskin, 2018), ancient and current vegetation (Moore et al., 1991; Rogers, 1993; Obigba, 2021) and respiratory allergy symptoms from specific taxa to human beings (Filon et al., 2000; Gioulekas et al., 2004; Damialis et al., 2011; Sousa-Silva et al., 2021). In the present study the amount of pollen produced by individuals of selected five species was considered as pollen quantity which can be consumed by the animals and can be lost to the environment (through abiotic factors). However, while taking the pollen and nectar, the animals carry dislodged pollen on their body to the receptive part of pistil of the other flowers that achieves pollination.

Among the five studied tropical tree species, two tree species, viz. *B. ceiba* and *E. stricta*, are bird pollinated (Khanduri, 2016). Despite a high production of pollen grains at tree crown level in the order of  $10^9$  in case of *B. ceiba* and  $10^8$  for *E. stricta*, the index of pollen limitation in both species is highest as compared to other studied three species. This clearly is indicating that the pollination failure in both species is due to unavailability of quality pollen from neighbouring trees in the absence of sufficient pollinator resources (i.e. bird). However, presence of high number of bird pollinators in both tree species indicating that the pollinator resources are sufficient. Nevertheless, the birds were observed foraging several flowers simultaneously within a tree during their visit which leads to geitonogamy (inter-flower selfing) pollination and both tree species are self-incompatible indicating pollination failure due to pollen quality. The other three species, i.e. *S. wallichii*, *L. speciosa* and *M. ferrea* are insect pollinator dependent and partially self-incompatible, which have shown lower pollen limitation. *Xylocopa* bee was the key pollinator for *L. speciosa* and *M. ferrea* due to proper physical fitness of *Xylocopa* bee to the flowers of both species (Khanduri, 2014; Khanduri & Kumar, 2017b). *Xylocopa* bee makes frequent visit to the several flowers within a crown of a tree and other trees in a population with a very high foraging speed (Khanduri, 2014; Khanduri & Kumar, 2017b), indicating ample pollination success to both tree species ultimately leading to weaker pollen limitation.

In *Schima wallichii*, the important pollinators were *Apis cerena*, *Dysphania militaris* and *Xylocopa* spp. *Apis cerena* that stay in the single flower for about 3 minutes and

feed on pollen grains leading to self-pollination. Honey bees are considered to be the best and efficient pollinators among insects. They have the quality which is commonly known as floral fidelity in which bees visits the flowers of the same crop repeatedly either for pollen or nectar or both till the end of the bloom. In this way, chances of pollination are highly secured. *Dysphania militaris* and *Xylocopa* spp make frequent interflower movement within a tree and between trees in a population leading to geitonogamy and xenogamy pollination in *S. wallichii*. Pollen limitation using fruit set and seed set in *S. wallichii* was moderate among the five studied tropical tree species and the mating system of *S. wallichii* is partially self-incompatible supporting outcrossing (Khanduri, 2016), indicating that the pollination failure is due to quality pollen limitation and limitation of pollinator resources.

Pollen limitation has been reported greater for self-incompatible than self-compatible species (Knight et al., 2005; Larson & Barrett, 2000). Self-incompatible species depend on the pollen from neighbouring trees and rely on pollinators visit frequency to effect pollen transfer for pollination success whereas, self-compatible or partially self-incompatible species might be capable to produce seeds by receiving mixed pollen loads of self- and cross-pollen (Schoen & Lloyd, 1992). Self-pollen is prohibited from fertilizing ovules in self-incompatible species while self-compatible species may form seeds from self-fertilized ovules, but with lower germination success due to inbreeding depression. Therefore, pollen limitation would be minimal for self fertile species, which however, leads to low pollen-quality limitation and reduced inbreeding depression (Duminil et al., 2009). Partially self-incompatible species with mixed pollen loads seems to have higher pollen limitation than self compatible species, but lower than the self-incompatible species. The estimated pollen limitation in the present study was found at par to that of the other studies of insect/bird pollinated species. For example, a study consisting of 21 shrub and herb species of tropical communities at Itatiaia National Park, southeastern Brazil revealed pollen limitation in 11 species with the overall pollen limitation index value was 0.39 (Wolowski et al., 2013). The pollen limitation index of 0.335 was reported in *Zygophyllum xanthoxylum*, a self-compatible species from the Gansu Province of Chiana (Chen & Zhao, 2017). The reported value is slightly higher than the value observed for partially self-incompatible species in the present study. Nevertheless, a high pollen limitation index of 0.478 on fruit set basis and 0.575 for seed set data basis was recorded in self pollinated tree species i.e. *Sophora microphylla* from New Zealand (Van Etten et al., 2015). The index of pollen limitation for seed set is lower than the fruit set for *Erythrina stricta*, *Lagerstroemia*

*speciosa* and *Mesua ferrea* whereas the value was higher in case of *Bombax ceiba* and *Schima wallichii*.

The supplementation of pollen was found to be increasing the fruit and seed set significantly in all five studied species. Confirmation from earlier studies implies that supplementation of pollen enhances the fruit/seed set, size and weight of a seed in a flower (Campbell, 1993; Yang et al., 2005; Gómez et al., 2010; Bartoš et al., 2020). Pollen limitation was the resultant of both limited pollen quantity and quality (Colling et al., 2004). The pollen quantity limitation in this study is related with the effectiveness of pollinators and their frequency (Ashman et al., 2004; Chen & Zuo, 2019), as all the studied species had produced ample pollen in order of  $10^8$  to  $10^{10}$  at tree crown level ultimately leading to pollinators limit fecundity in tropical trees. However, pollen limitation here may be a matter of quality rather than quantity (Aizen & Harder, 2007; Bona et al., 2022), on the basis that visitation rates appear to be high but there is a high proportion of movements between flowers on the same tree, potentially leading to geitonogamy.

## Conclusions

Pollen limitation is influenced by pollen quantity and quality, leading to pollinators limit fecundity in selected tropical trees. The self-incompatible species of *Bombax ceiba* and *Erythrina stricta* (both bird pollinated) produce low fruit and seed set in open pollination condition thereby proclaimed greater pollen limitation as compared to that of other three species, i.e. *S. wallichii*, *L. speciosa* and *M. ferrea*, which are partially self-incompatible and insect pollinated and have revealed lower pollen limitation. In out-crossed/self-incompatible species pollination success is achieved only when the flowers with receptive stigmas receive pollen from neighbouring trees in the population and is also dependent on visit frequency of pollinators for effective pollen transfer whereas, partially self-incompatible or self-compatible species have the chances of secure pollination by accepting mixed pollen (self- and cross-pollen) leading to produce more seeds.

## Acknowledgements

This work was supported by the Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, vide its project no. 38(1186)/08/EMR-II.

### Conflict of interest

The authors declare that they have no conflict of interest.

Vinod Prasad Khanduri  <https://orcid.org/0000-0002-3858-7121>

## References

- Ahlinder, J., Giles, B. E., & García-Gil, M. R. (2021). Life stage-specific inbreeding depression in long-lived Pinaceae species depends on population connectivity. *Scientific Reports*, *11*(1), 1–15.
- Aizen, M. A., & Harder, L. D. (2007). Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, *88*(2), 271–281.
- Allison, T. D. (1990). Pollen production and plant density affect pollination and seed production in *Taxus canadensis*. *Ecology*, *71*(2), 516–522.
- Ashman, T. L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M.R., Johnston, M.O., Mazer, S.J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, *85*(9), 2408–2421.
- Bartoš, M., Janeček, Š., Janečková, P., Chmelová, E., Tropek, R., Götzenberger, L., Klomberg, Y., & Jersáková, J. (2020). Are reproductive traits related to pollen limitation in plants? A case study from a central European meadow. *Plants*, *9*(5), 640.
- Baskin, J. M., & Baskin, C. C. (2018). Pollen limitation and its effect on seed germination. *Seed Science Research*, *28*(4), 253–260.
- Bawa, K. S. (1990). Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, *21*, 399–422.
- Bierzychudek, P. (1981). Pollinator limitation of plant reproductive effort. *The American Naturalist*, *117*(5), 838–840.
- Bona, A., Kłosowski, S., Jadwyszczak, K. A., & Petrova, G. (2022). Flowering and quality of seeds and pollen in endangered populations of *Betula humilis*. *Trees*, *36*(1), 313–324.
- Burd, M. (1994). Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review*, *60*(1), 83–139.
- Campbell, D. R., & Halama, K. J. (1993). Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology*, *74*(4), 1043–1051.
- Charalampopoulos, A., Damialis, A., Tsiripidis, I., Mavrommatis, T., Halley, J. M., & Vokou, D. (2013). Pollen production and circulation patterns along an elevation gradient in Mt Olympos (Greece) National Park. *Aerobiologia*, *29*(4), 455–472.
- Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology, Evolution and Systematics*, *18*, 237–268.
- Chen, M., & Zhao, X. Y. (2017). Effect of pollen and resource limitation on reproduction of *Zygophyllum xanthoxylum* in fragmented habitats. *Ecology and Evolution*, *7*(21), 9076–9084.
- Chen, M., & Zuo, X. A. (2018). Pollen limitation and resource limitation affect the reproductive success of *Medicago sativa* L. *BMC Ecology*, *18*(1), 1–10.
- Chen, M., & Zuo, X. A. (2019). Effect of pollen limitation and pollinator visitation on pollination success of *Haloxylon ammodendron* (CA Mey.) bunge in fragmented habitats. *Frontiers in Plant Science*, *10*, 327.

- Colling, G., Reckinger, C., & Matthies, D. (2004). Effects of pollen quantity and quality on reproduction and offspring vigor in the rare plant *Scorzonera humilis* (Asteraceae). *American Journal of Botany*, 91(11), 1774–1782.
- Cosacov, A., Nattero, J., & Cocucci, A. A. (2008). Variation of pollinator assemblages and pollen limitation in a locally specialized system: the oil-producing *Nierembergia linariifolia* (Solanaceae). *Annals of Botany*, 102(5), 723–734.
- Cristóbal-Pérez, E. J., Fuchs, E. J., Olivares-Pinto, U., & Quesada, M. (2020). Janzen-Connell effects shape gene flow patterns and realized fitness in the tropical dioecious tree *Spondias purpurea* (Anacardiaceae). *Scientific Reports*, 10(1), 1–12.
- Csépe, Z., Leelőssy, Á., Mányoki, G., Kajtor-Apatini, D., Udvardy, O., Péter, B., Páldy, A., Gelybó, G., Szigeti, T., Pándics, T., Kofol-Seliger, A., Simčić, A., Leru, P.M., Eftimie, A.M., Šikoparija, B., Radišić, P., Stjepanović, B., Hrga, I., Večenaj, A., Vucić, A., Peroš-Pucar, D., Škorić, T., Ščevková, J., Bastl, M., Berger, U., & Magyar, D. (2020). The application of a neural network-based ragweed pollen forecast by the Ragweed Pollen Alarm System in the Pannonian biogeographical region. *Aerobiologia*, 36(2), 131–140.
- Damialis, A., Fotiou, C., Halley, J. M., & Vokou, D. (2011). Effects of environmental factors on pollen production in anemophilous woody species. *Trees*, 25(2), 253–264.
- Dawson, D. G., & Bull, P.C. (1975). Counting birds in New Zealand forests. *Notornis*, 22(2), 101–109.
- Devaux, C., Porcher, E., & Lande, R. (2019). Mating systems and avoidance of inbreeding depression as evolutionary drivers of pollen limitation in animal-pollinated self-compatible plants. *Annals of Botany*, 123(2), 327–336.
- Devy, M. S., & Davidar, P. (2003). Pollination systems of trees in Kakachi, a mid-elevation wet evergreen forest in Western Ghats, India. *American Journal of Botany*, 90(4), 650–657.
- Dufaÿ, M., Vaudey, V., De Cauwer, I., Touzet, P., Cuguen, J., & Arnaud, J. F. (2008). Variation in pollen production and pollen viability in natural populations of gynodioecious *Beta vulgaris* ssp. *maritima*: evidence for a cost of restoration of male function?. *Journal of Evolutionary Biology*, 21(1), 202–212.
- Duminil, J., Fineschi, S., Hampe, A., Jordano, P., Salvini, D., Vendramin, G. G., & Petit, R. J. (2007). Can population genetic structure be predicted from life-history traits?. *The American Naturalist*, 169(5), 662–672.
- Duminil, J., Hardy, O. J., & Petit, R. J. (2009). Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology*, 9(1), 1–14.
- Etcheverry, A. V., & Alemán, C. E. T. (2005). Reproductive Biology of *Erythrina falcata* (Fabaceae: Papilionoideae) I. *Biotropica*, 37(1), 54–63.
- Etcheverry, A. V., Alemán, M. M., & Fleming, T. F. (2008). Flower morphology, pollination biology and mating system of the complex flower of *Vigna caracalla* (Fabaceae: Papilionoideae). *Annals of Botany*, 102(3), 305–316.
- Faegri, K., Kaland, P. E., & Krzywinski, K. (1989). *Textbook of pollen analysis* (No. Ed. 4). John Wiley & Sons Ltd..
- Ferriol, M., Pichot, C., & Lefèvre, F. (2011). Variation of selfing rate and inbreeding depression among individuals and across generations within an admixed *Cedrus* population. *Heredity*, 106(1), 146–157.
- Filon, F. L., Bosco, A., Barbina, P., Sauli, M. L., & Longo, L. R. (2000). Betulaceae and Corylaceae in Trieste (NE-Italy): Aerobiological and clinical data. *Aerobiologia*, 16(1), 87–91.
- Fleming, T. H., & Muchhala, N. (2008). Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography*, 35(5), 764–780.
- Gioulekas, D., Papakosta, D., Damialis, A., Spieksma, F., Giouleka, P., & Patakas, D. (2004). Allergenic pollen records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki, Greece. *Allergy*, 59(2), 174–184.
- Glémin, S., Bazin, E., & Charlesworth, D. (2006). Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society B: Biological Sciences*, 273(1604), 3011–3019.
- Gómez, J. M., Abdelaziz, M., Lorite, J., Jesús Muñoz-Pajares, A., & Perfectti, F. (2010). Changes in pollinator fauna cause spatial variation in pollen limitation. *Journal of Ecology*, 98(5), 1243–1252.
- González-Varo, J. P., Arroyo, J., & Aparicio, A. (2009). Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*). *Biological Conservation*, 142(5), 1058–1065.
- Hilje, B., Calvo-Alvarado, J., Jiménez-Rodríguez, C., & Sánchez-Azofeifa, A. (2015). Tree species composition, breeding systems, and pollination and dispersal syndromes in three forest successional stages in a tropical dry forest in Mesoamerica. *Tropical Conservation Science*, 8(1), 76–94.
- Hirayama, K., Ishida, K., Tomaru, N. (2005). Effects of pollen shortage and self-pollination on seed production of an endangered tree, *Magnolia stellata*. *Annals of Botany*, 95, 1009–1015.
- Holsinger, K. E. (2000). Reproductive systems and evolution in vascular plants. *Proceedings of the National Academy of Sciences*, 97(13), 7037–7042.
- Hufford, K. M., & Hamrick, J. L. (2003). Viability selection at three early life stages of the tropical tree, *Platypodium elegans* (Fabaceae, Papilionoideae). *Evolution*, 57(3), 518–526.
- Iwaizumi, M. G., & Takahashi, M. (2012). Effects of pollen supply and quality on seed formation and maturation in *Pinus densiflora*. *Journal of Plant Research*, 125(4), 517–525.
- Jadwiszczak, K. A., Kłosowski, S., Zalewska, I., Banaszek, A., & Chrzanowska, A. (2017). Genetic diversity and sexual reproduction in relict populations of *Betula nana*. *Silva Fennica*, 51(2), 5643.
- Kearns, C. A., Inouye, D.W., & Waser, N.M. (1998). Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology, Evolution and Systematics*, 29, 83–112.
- Khanduri, V. P., Sharma, C. M., Kumar, K. S., & Ghildiyal, S. K. (2013). Annual variation in flowering phenology, pollination, mating system, and pollen yield in two natural populations of *Schima wallichii* (DC.) Korth. *The Scientific World Journal*, 2013, 350157.
- Khanduri, V. P. (2014). Annual variation in floral phenology and pollen production in *Lagerstroemia speciosa*: an entomophilous tropical tree. *Songklanakarin Journal of Science and Technology*, 36(4), 389–396.

- Khanduri, V. P., Kumar, K.S., & Sharma, C.M. (2015). Role of pollen production in mating success in some tropical tree species. *Brazilian Journal of Botany*, 38(1), 107–112.
- Khanduri, V. P. (2016). Mating system and seedling growth of five tropical tree species. *Scientia Forestalis*, 44(110), 509–517.
- Khanduri, V. P., & Kumar, K.S. (2017a). Reproductive effort and success in *Bombax ceiba* L. in a tropical forest of Mizoram, Indo-Burma region of North-East India. *Brazilian Journal of Botany*, 40(1), 157–166.
- Khanduri, V. P., & Kumar, K. S. (2017b). Pollen production and release in *Mesua ferrea* Linn.(Guttiferae): a spatio-temporal pattern. *Aerobiologia*, 33(1), 13–21.
- Khanduri, V. P., & Sukumaran, A. (2019). Pollen dispersion in *Myrica esculenta* (Myricaceae): a dioecious anemophilous tree species of Himalaya. *Aerobiologia*, 35(4), 583–591.
- Khanduri, V. P., Kumar, K. S., Sharma, C. M., Riyal, M. K., & Kar, K. (2019). Pollen limitation and seed set associated with year-to-year variation in flowering of *Gmelina arborea* in a natural tropical forest. *Grana*, 58(2), 133–143.
- Khanduri, V. P., Kumar, K. S., Sharma, C. M., Riyal, M. K., Kar, K., Singh, B., & Sukumaran, A. (2021). Passerine birds supporting cross pollination in *Erythrina stricta* Roxb. *Dendrobiology*, 85, 117–126.
- Khanduri, V. P., Sharma C. M., Riyal, M. K., & Sukumaran, A. (2022). Racial hybridization and inbreeding depression in *Cedrus deodara* (Roxb.) G. Don. *Kastamonu University Journal of Forestry Faculty*, 22(1), 47–55.
- Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 303–313.
- Knight, T. M., Steets, J. A., & Ashman, T. L. (2006). A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany*, 93(2), 271–277.
- Knight, T. M., Steets, J.A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., & Ashman, T. L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution and Systematics*, 36, 467–497.
- Lande, R., & Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution*, 39(1), 24–40.
- Larson, B. M., & Barrett, S. C. (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, 69(4), 503–520.
- Lázaro, A., & Traveset, A. (2006). Reproductive success of the endangered shrub *Buxus balearica* Lam. (Buxaceae): pollen limitation, and inbreeding and outbreeding depression. *Plant Systematics and Evolution*, 261(1), 117–128.
- Lever, J. J., van Nes, E. H., Scheffer, M., & Bascompte, J. (2014). The sudden collapse of pollinator communities. *Ecology letters*, 17(3), 350–359.
- Liu, M., Compton, S. G., Peng, F. E., Zhang, J., & Chen, X. Y. (2015). Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers?. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150290.
- McKone, M. J. (1990). Characteristics of pollen production in a population of New Zealand snow-tussock grass (*Chionochloa pallens* Zotov). *New Phytologist*, 116(3), 555–562.
- Moles, A. T., & Ollerton, J. (2016). Is the notion that species interactions are stronger and more specialized in the tropics a zombie idea?. *Biotropica*, 48(2), 141–145.
- Moore, P. D., Webb, J. A., & Collinson, M. E. (1991). Pollen Analysis, 2nd edn Blackwell.
- Mu, J., Yang, Y., Luo, Y., Su, R., & Niklas, K. J. (2017). Pollinator preference and pollen viability mediated by flower color synergistically determine seed set in an Alpine annual herb. *Ecology and Evolution*, 7(9), 2947–2955.
- Nakanishi, A., Yoshimaru, H., Tomaru, N., Miura, M., Manabe, T., & Yamamoto, S. I. (2015). Inbreeding depression at the sapling stage and its genetic consequences in a population of the outcrossing dominant tree species, *Castanopsis sieboldii*. *Tree Genetics & Genomes*, 11(3), 1–10.
- Obigba, S. O. (2021). Understanding Past and Present Vegetation Dynamics using the Palynological Approach: An Introductory Discourse. In *Vegetation Index and Dynamics*. Eds. Carmina, E. C., Ortiz, A. C. Canas, R. Q. & Musarella, C. M., PP 1-21, IntechOpen.
- Oldroyd, H. (1958). Collecting, Preserving and Studying Insects. Hutchinson & Co. Publishers Ltd.
- Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution and Systematics*, 48, 353–76.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals?. *Oikos*, 120(3), 321–326.
- Pauw, A., & Stanway, R. (2015). Unrivalled specialization in a pollination network from South Africa reveals that specialization increases with latitude only in the Southern Hemisphere. *Journal of Biogeography*, 42(4), 652–661.
- Portillo Lemus, L. O., Harang, M., Bozec, M., Haury, J., Stoeckel, S., & Barloy, D. (2022). Late-acting self-incompatible system, preferential allogamy and delayed selfing in the heteromorphic invasive populations of *Ludwigia grandiflora* subsp. *hexapetala*. *Peer Community Journal*, 2.
- Ramos, S. L. F., Dequigiovanni, G., Sebbenn, A. M., Lopes, M. T. G., Kageyama, P. Y., de Macêdo, J. L. V., Matias, K., & Veasey, E. A. (2016). Spatial genetic structure, genetic diversity and pollen dispersal in a harvested population of *Astrocaryum aculeatum* in the Brazilian Amazon. *BMC Genetics*, 17(1), 1–10.
- Rao, V. R., & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture*, 68(1), 1–19.
- Rodger, J. G., & Ellis, A. G. (2016). Distinct effects of pollinator dependence and self-incompatibility on pollen limitation in South African biodiversity hotspots. *Biology Letters*, 12(6), 20160253.
- Rodger, J. G., & Johnson, S. D. (2013). Self-pollination and inbreeding depression in *Acacia dealbata*: Can selfing promote invasion in trees?. *South African Journal of Botany*, 88, 252–259.
- Rodríguez-Rodríguez, M. C., Jordano, P., & Valido, A. (2013).

- Quantity and quality components of effectiveness in insular pollinator assemblages. *Oecologia*, 173(1), 179–190.
- Rogers, C. A. (1993). Application of aeropalynological principles in palaeoecology. *Review of Palaeobotany and Palynology*, 79(1–2), 133–140.
- Schoen, D. J., & Lloyd, D. G. (1992). Self-and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences*, 153(3, Part 1), 381–393.
- Sousa-Silva, R., Smargiassi, A., Kneeshaw, D., Dupras, J., Zinszer, K., & Paquette, A. (2021). Strong variations in urban allergenicity riskscape due to poor knowledge of tree pollen allergenic potential. *Scientific Reports*, 11(1), 1–13.
- Sukumaran, A., Khanduri, V. P., & Sharma, C. M. (2020). Pollinator-mediated self-pollination and reproductive assurance in an isolated tree of *Magnolia grandiflora* L. *Ecological Processes*, 9(1), 1–9.
- Tamura, S., & Kudo, G. (2000). Wind pollination and insect pollination of two temperate willow species, *Salix miyabeana* and *Salix sachalinensis*. *Plant Ecology*, 147(2), 185–192.
- Van der Niet, T., Peakall, R., & Johnson, S. D. (2014). Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany*, 113(2), 199–212.
- Van Etten, M. L., Tate, J. A., Anderson, S. H., Kelly, D., Ladley, J. J., Merrett, M. F., Peterson, P.G., & Robertson, A. W. (2015). The compounding effects of high pollen limitation, selfing rates and inbreeding depression leave a New Zealand tree with few viable offspring. *Annals of Botany*, 116(5), 833–843.
- Voukantsis, D., Niska, H., Karatzas, K., Riga, M., Damialis, A., & Vokou, D. (2010). Forecasting daily pollen concentrations using data-driven modeling methods in Thessaloniki, Greece. *Atmospheric Environment*, 44(39), 5101–5111.
- Wesselingh, R. A. (2007). Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist*, 174(1), 26–37.
- Westgate, M. E., Lizaso, J., & Batchelor, W. (2003). Quantitative relationships between pollen shed density and grain yield in maize. *Crop Science*, 43(3), 934–942.
- Wilcock, C., & Neiland, R. (2002). Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, 7(6), 270–277.
- Wolowski, M., Ashman, T. L., & Freitas, L. (2013). Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest. *Annals of Botany*, 112(5), 903–910.
- Wright, S. I., Ness, R. W., Foxe, J. P., & Barrett, S. C. (2008). Genomic consequences of outcrossing and selfing in plants. *International Journal of Plant Sciences*, 169(1), 105–118.
- Yang, C. F., Sun, S. G., & Guo, Y. H. (2005). Resource limitation and pollen source (self and outcross) affecting seed production in two louseworts, *Pedicularis siphonantha* and *P. longiflora* (Orobanchaceae). *Botanical Journal of the Linnean Society*, 147(1), 83–89.
- Zheljazkov, V. D., Semerdjieva, I. B., Stevens, J. F., Wu, W., Cantrell, C. L., Yankova-Tsvetkova, E., & Astatkie, T. (2021). Phytochemical investigation and reproductive capacity of the bulgarian endemic plant species *Marrubium friwaldskyanum* Boiss. (Lamiaceae). *Plants*, 11(1), 114.