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Original Article

Flower Pollinator Interactions within Two Tropical Tree Species of Mizoram, North East India

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Abstract

Understanding the interactions between plants and pollinators is vital in exploring the structural and functional dynamics of tropical forest ecosystem. Such interactions are also helpful in resolving issues of regeneration, maintenance of biodiversity and conservation of tropical forest resources. The aim of this study was to understand the foraging behaviour of floral visitors of two valuable forest species in North East India, viz. *Schima wallichii* Choisy and *Lagerstroemia speciosa* (L.) Pers. The types and behaviour of pollinators were assessed by counting of the total number of visits by insects during day time (05:00-18:00) per field visit/flower in the peak flowering season, foraging rate (number of flowers visited/minutes) and average time spent per flower. The frequency of pollinator visits was also determined in terms of visits/flower/hour. Ten species belonging to seven families were recorded visiting *S. wallichii* flowers. The flowers of *L. speciosa* were mainly visited by three species in the family Apidae and one species in the family Calliphoridae. Various moth and bee species were involved in pollination of *S. wallichii* flowers. The *Xylcopa* spp. of bee showed a high level of functional synchronisation with the floral morphology of *L. speciosa*.

Keywords: anther dehiscence, anthesis, pollination, pollinators, receptivity, tropical

Introduction

Plant-pollinators interactions are one of the key ecological steps in the reproductive success of plants, particularly in out-crossing species. There has been heightened interest in tropical pollination biology in recent decades due to the phenomenon of global environmental change (Bond, 1994; Murren, 2002). Tropical forest ecosystems are facing accelerated rates of habitat conversion to other land-use systems, leading to loss of biodiversity (Vitousek, 1994). Furthermore, there is a concern regarding the failure of mutualistic interactions (plant-pollinator interaction) due to habitat destruction and fragmentation, which may lead to the cascade extinction of species and loss of valuable biodiversity (Bawa, 1990; Kearns and Inouye, 1997; Renner, 1998). Tropical plant species with disperse and patchy distribution, self-incompatibility, dioecy and high level pollinator specificity are more vulnerable to extinction in case of failed pollination (Bond, 1994; Corlett, 2004). Tropical forest tree species offer a unique opportunity to explore evolutionary tendencies in relation to morphological diversification. Understanding the interactions between plants and pollinators is vital in exploring the structural and functional dynamics of tropical forest ecosystem. Such interactions are also helpful in resolving issues of regeneration, maintenance of biodiversity and conservation of tropical forest resources (Bawa and Headley, 1990; Sharma *et al.*, 2010).

Understanding the diversity and behaviour of floral visitors to different species from a range of ecological characteristics is helpful in evaluating the features of different pollination systems. Key features are: species relationship (Fenster *et al.*, 2004), specific evolutionary tendency between plants and animals (Thompson, 1988; Mitchell *et al.*, 2009) and the structural and functional dynamics of a particular community (Memmott, 1999). Flower anthesis provides a new resource for local floral visitors. Also, local fauna may visit opened flowers for a variety of reasons, such as to feed on nectar or pollen (Bawa, 1990), for shelter (Sakai, 2000) and/or to predate upon other floral visitors. Pollinators are vital in successfully transferring pollen to conspecific individuals at the population level and govern the level of gene flow (House, 1998). Scientific studies are mainly focused on direct observations to record, identify and quantify floral visitors (Momose *et al.*, 1998). For example, even flowers that appear to require specialised pollinators are often visited by a diverse array of faunal visitors (Johnson and Steiner, 2000). Changes in climate are raising concerns about the size of pollinator population and their ecological services (Hegland *et al.*, 2009). Therefore, it is necessary to determine the

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current profile of floral visitors to flowering plants of agricultural and forestry importance (Bronstein 1995; Waser *et al*., 1996).

Studies of pollinators' diversity of forest genetic resources from the North-East India are lacking. The region also covers biodiversity rich habitat of flora and fauna. The rate and quantity of pollinators' visits to plant species is frequently linked with reproductive success through pollen dispersal (Burd, 1994). Different floral visitors disperse pollen across varying distances, thus affecting pollen flow. Exploring the assemblage of pollinators would be helpful in making conservation and management decisions, as reproduction of animal pollinated plants and their demographic growth is significantly influenced by the reduction in pollinator availability (Kearns *et al*., 1998). Perturbation in pollinator communities may not only negatively impact seed and fruit set, but also may reduce the adaptive fitness of progeny through changes in the amount and composition of pollen loads (Burd, 1994). Many studies recently revealed that selfing rates are driven by spatial and temporal variation in the composition and abundance of native pollinating animal visitors (Harrison, 2000; Fenster *et al*., 2001; Christopher *et al*., 2003; Mitchell *et al*., 2009). Therefore, it is important to understand the range of pollinator species and their behaviour with regard to valuable forestry trees.

The tropical forest ecosystem of North East India is extremely rich in floral and faunal diversity, but studies on functional aspects of biodiversity are lacking from this region. In general, there is paucity of basic information on the species of pollinators involved in pollination of valuable forest genetic resources. Therefore, the present study was undertaken to map and understand the foraging behaviour of floral visitors of two valuable forest species, *Schima wallichii* and *Lagerstroemia speciosa*.

Materials and Methods

Study site

The study site was in and around Mizoram University Campus, Tanhril, Aizawl, which is situated about 15 km West of the state capital Aizawl, just below Tanhril village. The study area lies in between 23^0 45'25" and 23^0 43' 37" N latitudes and 92^0 38' 39" and 92^0 40' 23" E longitudes, with the elevation ranging from 300 m to 880 m asl. The area is characterised by a series of undulating slopes with the Western spur fallings under steep slope of the bank of Setlak River. The climate is humid and tropical, characterized by a short winter and long summer with heavy rainfall (2,100 mm). The temperature did not fluctuate much throughout the year, and ranged from 12 °C to 36 °C.

The forest is heterogeneous with 15 species of trees, accounting the majority of individuals over 10 cm dbh. Density was 160 trees per hectare. The forest is dominated by nine species: *Schima wallichii* Choisy, *Callicarpa arborea* Roxb., *Anthocephalus cadamba* (Roxb.) Miq., *Sterculia villosa* Roxb., *Albizia procera* (Roxb.) Benth., *Aibizia chinensis* (Osbeck) Merrill, *Mallotus* spp., *Sepium* spp., *Rhus semialata* Murray and patchy distributed *Gmelina arborea* Roxb. and *Lagerstroemia speciosa* (L.) Pers.

Schima wallichii

Schima is a monotypic, polymorphic genus belonging to the family Theaceae that has a single species, *Schima wallichii* Choisy. It is an evergreen tree native to warm temperate and tropical regions of Southern and South-Eastern Asia. It is locally used for timber, poles, fuel wood, charcoal making and occasionally as fodder (Tamrakar, 1992). Flowering starts at the age of 5-8 years, depending on growth and site. Seasonal flowering occurs in April-early June with most seeds maturing in January to March next year. The development from flower to fruit thus takes about 9 months. The tree attains a height up to 35 m with a dense crown and can attain a stem diameter up to 125 cm. Leaves are elliptic-oblong or lanceolate, 6-15 × 4-6 cm in size; flowers are white, 2.5-4 cm across, scented, axillary solitary or paired. It has a broad distribution occurring from North-Eastern India to Southern China, Thailand, the Malay Peninsula, Sumatra, Java, Borneo and the Philippines, and is adaptive to a wide range of environments (Corlett, 1999; Wu *et al*., 1999). *S. wallichii* is a fire resistant, moderately light demanding and often occurs in diverse primary lowland to mountain forests, scrublands and grasslands, but is particularly common in disturbed and secondary forests (Troup, 1921; Li-Zhen and Li, 1997). Bark contains alkaloids that are used as fish poison and also used for dying (Li-Zhen and Li, 1997). Corollas of the flowers are used to treat uterine disorders and hysteria (Tamrakar, 1992; Siwakoti and Siwakoti, 2000).

Lagerstroemia speciosa

Lagerstroemia speciosa is a large deciduous tree that grows up to 18 m in height with a diameter of up to 50 cm. Leaves are elliptic-lanceolate. Flowers are mauve coloured in large terminal peduncles up to 25 cm long. Each flower is 5-8 cm in diameter with a calyx of 12-15 mm long, tomentose and ribbed with 6 teeth. There are 6-7 petals per flower, each 3-4 cm long (Sinha *et al*., 2012). Flowers are produced once a year in the height of summer. It is commonly known as Queen's Crape-Myrtle due to its conspicuous beautiful flowers. The species grows naturally in North-Eastern and Southern India, throughout Myanmar, the Philippines and the Chittagong hills of Bangladesh (Troup, 1921). It is a multi-purpose tree species with good quality timber and medicinal values (Suzuki *et al*., 1999). Seeds are narcotic; the bark and leaves are purgative; the roots are astringent, stimulant and febrifuge (fever removing). A decoction of dried leaves is used in diabetes (Mishra *et al*., 1990) and anti-obesity (Suzuki *et al*., 1999).

Floral, pollination and fruit display

This study was conducted during the flowering seasons (mid April-May for *S. wallichii* and mid May-June for *L. speciosa*) of the years 2012 and 2013, which covered about one month period for each species in every year. Floral morphometric traits, pollination and fruit formation were observed during the flowering season on ten randomly selected individuals within each population. On each tree, twenty flower buds of similar stage were marked and subsequently observed for anthesis, anther dehiscence and stigma receptivity at 2-h intervals from dawn (05:00 h) to dusk (18:00 h). Anthesis (the opening of flowers to display its reproductive sexual organs) was observed on the marked flowers on each tree of both study species. Anther dehiscence was assessed at the time of anthesis by using a hand lens (×20) to note the time differences between anthesis and anther dehiscence, and anther dehiscence and stigma receptivity. Receptivity of stigmas was assessed up to 5 days after every 2-h interval during entire day length, by measuring peroxidase activity using the method of Kearns and Inouye (1993). Furthermore, twenty flowers from different trees were harvested to record the flower diameter, sepal and petal length, style, ovary length and ovary circumference, for each species. Similarly, 50 mature fruits from different selected individuals of each species were harvested and the wing length of seeds, seed length (without wing), number of seeds per fruit, number of fruits per inflorescence, length of fruit and circumference of fruit were measured.

Pollinator availability and their frequency

Ten individuals of each study species (*S. wallichii* and *L. speciosa*) were chosen arbitrarily within the populations to record the pollinators. Pollinators and their visitation rates were determined on five randomly chosen branches per tree by counting the individuals of insect species visiting to the selected branches per individual tree of each species. Thus, the sample size represented 50 replications (10 × 5 = 50) in each study species. Pollinator availability was monitored during peak flowering time (mid April-May for *S. wallichii* and mid May-June for *L. speciosa*). Several inflorescences were observed simultaneously to record the number of encounters of pollinator species interacting with open flowers. Each tree was observed for whole day between 05:00 and 18:00 h in 6-h observation blocks, at every full hour interval between 05:00-06:00, 06:001-07:00, 08:01-09:00 h, etc. The observations were recorded for twenty five days during peak flowering time of both species. The sustainable distribution of each observation block was done in such a manner that all the chosen trees in the population would be monitored in each observation block during the course of the study. The pollinators were assessed in respect of total number of visits by insects during field visit/flower (n = 10 trees × 5 branches × 10 flowers per branch = 500 flowers), foraging rate (number of flowers visited/minutes, n = 500) and average time (sec) spent per flower (n = 500). The frequencies of pollinators were also assessed in terms of visits/flower/hour (n = 500).

Results

The visitation rates differed significantly ($p < 0.0001$) within the insect species and also varied significantly ($p < 0.001$) from one year to another. The daily pattern of pollinators' visitation was related to the pattern of anthesis of the flowers of both analyzed species.

In the case of *L. speciosa*, the pattern of anthesis was forenoon (05:00-11:00 hrs) and the peak period of anthesis was recorded between 07:00 and 09:00 hours of the day. The stigma was receptive at the time of anthesis. The anther dehiscence started half an hour after the beginning of receptivity of the stigma. The receptivity continued for 48 hours. The amount of pollen was highest in the morning hours due to high rate of anthesis and anther dehiscence, leading to high visitation rates at that time. Highest quantitative frequency of pollinators was recorded between 07:00 to 09:00 h of the day, which also coincided with the peak timing of anthesis.

For *S. wallichii*, anthesis in flowers started from 06:00 h in the morning and reached to its maximum between 07:00 and 09:00 hours of the day. As the day progressed, the level of anthesis decreased and gone down to its minimum level at 11:00 h; the flower openings were again recorded during afternoon hours between 15:00 and 18:00 h, therefore, the pattern of anthesis was bimodal. Anther dehiscence started one hour after anthesis and followed a diurnal pattern. The receptivity of stigma started after 4 h of anther dehiscence and remained receptive up to 60 h. The pattern of pollinators' visitation, particularly moths, in *S. wallichii* was bimodal, which followed the pattern of anthesis.

Anther dehiscence offers mature pollen grains to the insects, to carry them into the receptive surfaces of stigmas, which in turn, utters the pollination success of a species and play an important part for determining the genetic make-up of populations. The detailed morphometrics of the flowers of both study species are presented in Table 1.

Pollinator visitation in Schima wallichii

S. wallichii flowers were visited by 10 insect species belonging to Hymenoptera (2 species of bee), Lepidoptera

Table 1. Floral, pollination and seed display of *L. speciosa* and *S. wallichii*

Floral display	*Lagerstroemia speciosa*	*Schima wallichii*
Sepals	6, fleshy brownish colour	5, persistent grey in colour
Petals	6, Pink to pinkish white in color	5, obovate, creamish white
Average length of petals	5.1 ± 0.24 cm	2.5 ± 0.04 cm
Average width of petals	4.8 ± 0.21 cm	2.03 ± 0.05 cm
Flower diameter	10.5 ± 0.19 cm	3.1 ± 0.05 cm
Stamen	Yellow, many in number	Yellow, many in numbers, to base of corolla
Stigma	Green in colour when receptive	Off white in colour, greenish grey in receptive stage
Style length	2.2 ± 0.03cm	0.82 ± 0.012 cm
Ovary length	0.6 ± 0.09 cm	0.22 ± 0.008 cm
Ovary circumference	2.4 ± 0.02 cm	0.769 ± 0.006 cm
Fruit display		
Circumference of fruit	7.5 ± 0.02 cm	5.32 ± 0.08 cm
Length of fruit	3.2 ± 0.14 cm	1.83 ± 0.03 cm
Number fruits per inflorescence	25 ± 1.68	
Number of seeds per fruit	145 ± 4.03	10-30
Seed length (without wing)	0.69 ± 0.24 cm	Seed length: Winged, 0.72 ± 0.03 cm
Wing length of seeds	0.91 ± 0.06cm	
Pollination display		
Mode of pollination	Entomophilous	Entomophilous
Major pollinators	Honey bee and Carpenter bee	Honey bee and Moths
Type of dichogamy	Slightly protandrous (stigma becomes receptive half an hour prior anther dehiscence	Slightly protandrous (stigma becomes receptive after 4-5 h anther dehiscence
Period of stigma receptivity	48 hours	60 hours

Table 2. Insects visiting the flowers of *Schima wallichii* in tropical forest site of Mizoram

Order	Family	Scientific name	Total number of visits by insects during field visit/flower (n = 500)	Foraging rate (No. of flowers visited/min) (n = 500)	Mean time spent per flower (sec) (n = 500)
Hymenoptera	Apidae	*Apis cerena*	156	1.9 ± 0.23	22.2 ± 0.66
Hymenoptera	Apidae	*Xylocopa* spp.	71	3.6 ± 0.49	6.3 ± 0.32
Lepidoptera	Lycaenidae	*Arhopala eumolphus*	43	1.6 ± 0.22	14.7 ± 0.57
Lepidoptera	Hesperiidae	*Matapa aria*	33	1.5 ± 0.16	18.3 ± 0.55
Lepidoptera	Geometridae	*Cabera pusaria*	50	1.3 ± 0.21	11.0 ± 0.25
Lepidoptera	Geometridae	*Dysphania militaris*	83	2.5 ± 0.34	12.6 ± 0.61
Lepidoptera	Zygaenidae	*Callamesia midama*	44	1.8 ± 0.24	15.2 ± 0.57
Lepidoptera	Zygaenidae	*Eterusia aedea*	38	1.3 ± 0.15	21.8 ± 0.96
Lepidoptera	Noctuidae	*Alypia* spp.	52	2.4 ± 0.16	16.2 ± 0.53
Coleoptera	Scarbaeidae	*Maladera castanea*	17	1.1±0.09	58.8 ± 1.23

Fig.1. Different pollinators of *Schima wallichii*; (A) *Callamesia midama* (Moth); (B) *Alypia* spp. (Moth); (C) *Eterusia aedea* (Moth); (D) *Dysphania militaris* (Moth); (E) *Arhopala eumolphus* (Butterfly); (F) *Matapa aria* (Butterfly); (G) *Maladera castanea* (Bettle); (H) *Apis cerena* (Indian hive bee)

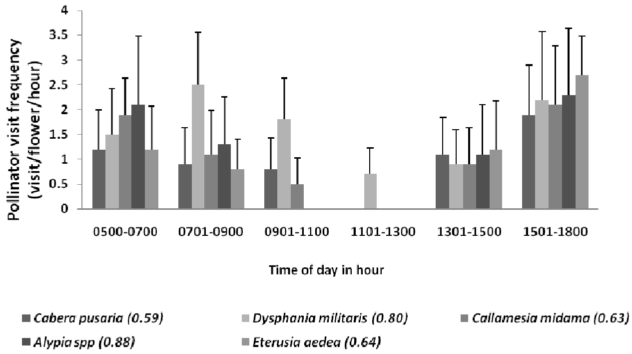

Fig. 3. Frequency of pollinators (moths) visitation in respect to the time of day for *S. wallichii* in the year 2013 (values in parentheses indicate LSD values for 5%)

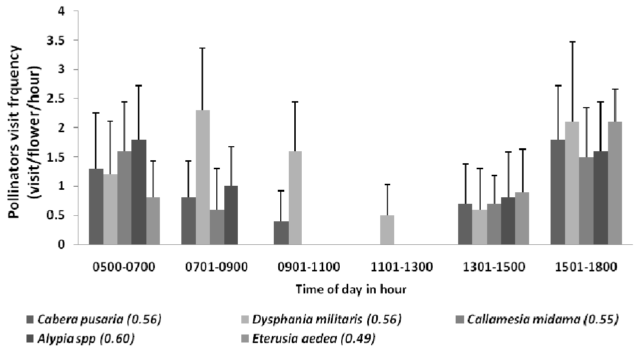

Fig. 2. Frequency of pollinators (moths) visitation in respect to the time of day for *S. wallichii* in the year 2012 (values in parentheses indicate LSD values for 5%)

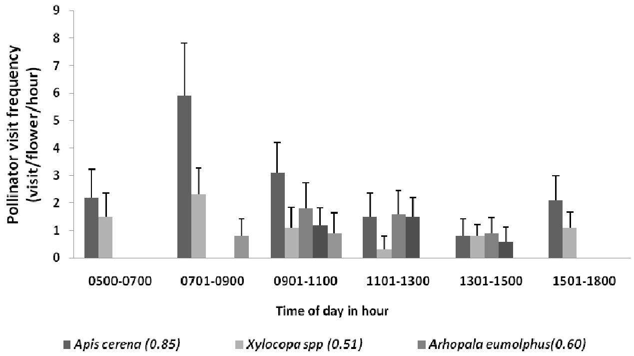

Fig. 4. Frequency of pollinators (bees, butterflies and beetle) visits in respect to the time of day for *S. wallichii* in the year 2012 (values in parentheses indicate LSD values for 5%)

(7 species of moth or butterfly) and Coleoptera (1 species of beetle). Foraging abundance is shown in Table 2. All species visited the reproductive parts of flowers (stamen and stigma) (Fig. 1), suggesting all species were effective in pollinating *S. wallichii*. Moths pollinators (*Cabera pusaria, Dysphania militaris, Callemesia midama, Eterusia aedea* and *Alypia* spp.) followed a bimodal pattern (flower were visited more during morning and evening hours) (Figs. 2 and 3), while butterflies (*Arhopala eumolphus* and *Matapa aria*) generally visited flowers during 09:00 to 15:00 h of the day. Bees (*Apis cerana* and *Xylocopa* spp.) generally visited more often between 07:00 and 11:00 h, but some visited flowers throughout day. The beetle (*Maladera castanea*) only occasionally visited *S. wallichii* flowers (Figs. 4 and 5), but each visit was much longer than for other species (58.8 ± 1.23 sec/flower) compared with the following longest visits such of *Apis cerena* (22.2 ± 0.66 sec/flower), *Eterusia aedea* (21.8 ± 0.96 sec/flower) and

Table 3. Insects visiting the flowers of *Lagerstroemia speciosa* in tropical forest site of Mizoram

Order	Family	Scientific name	Total number of visits by insects during field visit/flower (n = 500)	Foraging rate (No. of flowers visited/min) (n = 500)	Mean time spent per flower (sec) (n = 500)
Hymenoptera	Apidae	*Xylocopa* spp.	66	4.9 ± 0.37	5.5 ± 0.42
Hymenoptera	Apidae	*Apis cerena*	147	2.3 ± 0.21	24.2 ± 0.78
Hymenoptera	Apidae	*Apis florea*	115	2.6 ± 0.33	21.3 ± 0.59
Diptera	Calliphoridae	*Chrysomya megacephala*	88	1.3 ± 0.15	59.7 ± 1.07

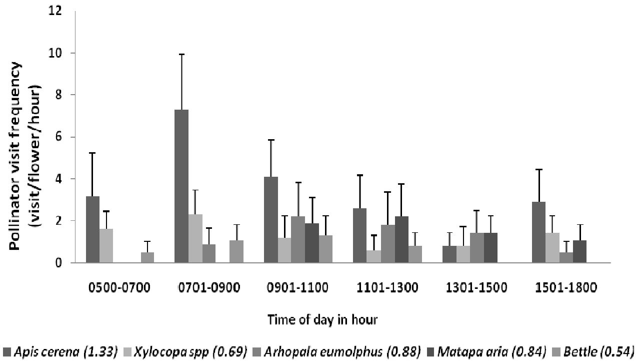

Fig. 5. Frequency of pollinators (bees, butterflies and beetle) visits in respect to the time of day for *S. wallichii* in the year 2013 (values in parentheses indicate LSD values for 5%)

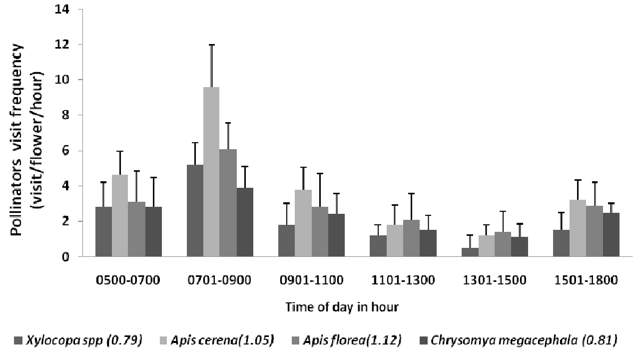

Fig. 6. Frequency of pollinator visits in respect to the time of day for *L. speciosa* in the year 2012 (values in parentheses indicate LSD values for 5%)

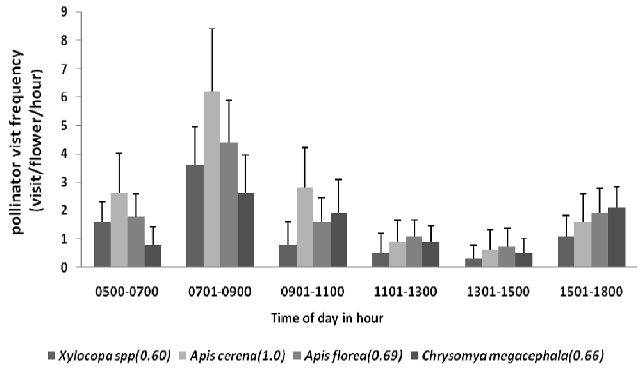

Fig. 7. Frequency of pollinator visits in respect to the time of day for *L. speciosa* in the year 2013 (values in parentheses indicate LSD values for 5%)

Fig. 8. Pollinators of *Lagerstroemia speciosa*; (A) *Apis cerana* (Indian Hive bee); (B) *Apis florea* (Little bee); (C) *Chrysomya megacephala*; (D) *Xylocopa* spp. (Carpenter bee)

Matapa aria (18.3 ± 0.55 sec/flower). However, based on foraging rate, e.g. the mean time spent per flower and visitation frequency in different time blocks of the day, *Apis cerena*, *Xylcopa* spp. and moths species were considered more effective pollinators than others.

Pollinator visitation in Lagerstroemia speciosa

The flowers of *L. speciosa* were observed to be visited by 4 insect species belonging to Hymenoptera (*Apis cerena, Apis florea* and *Xylocopa* spp.) and Diptera (*Chrysomya megacephala*). Insects' visits to *L. speciosa* occurred throughout the day, but the time of maximum visitation frequency was between 07:00 and 09:00 h of day (Figs. 6 and 7). Interestingly, the rate of visitation varied among individuals within the population. There was a definite interaction of floral visitors with the reproductive floral parts of *L. speciosa* while foraging, as shown in the Fig. 8. On the basis of visitation frequency, foraging rate and average time spent per flower, *Apis cerena* were the most effective pollinators, followed by *Xylocopa* spp., *Apis florea* and *Chrysomya megacephala*.

Discussion

Both tree study species were visited by members of bee family Apidae, with *Xylocopa* spp. found to be an important pollinator on the basis of its visitation frequency and rate. Flower size (large for *L. speciosa* and moderate for *S. wallichii*) along with large number of anthers per flower, copious pollen production (Khanduri *et al*., 2015) and morphological fitness of *Xylocopa* bee to the flowers (Khanduri, 2014) were the characteristic features of both species to be adapted to pollination by *Xylocopa* bee. Although *Xylocopa* bees spent less time per flower while foraging than other species, they made frequent inter-flower and inter-tree movements. This characteristic, along with its large size, ability to force into flowers, fast flight, foraging activity during strong winds (Somanathan and Borger, 2001) and long flight range of around 20 km (Kapil and Dhaliwal, 1969) are the important features for its effectiveness as a pollinator.

Apis cerena visited flowers of both study species with high frequency and thus was another important pollinator. It is one of the most widespread species of canopy pollinator along with *Apis dorsata* in the rainforests of Western Ghats and Srilanka (Dayanandan *et al*., 1990; Devy and Davidar, 2003). *Apis florea* was also identified as an important floral visitor of *L. speciosa* in the present study, as this species is known to be a key pollinator of field and orchard crops in South-Asia (Batra, 1967; Rehman *et al*., 1990; Sihag, 2000). However, its role in pollination of wild plants has not been documented previously (Corlett, 2004). Moreover, pollinators, especially bees and moths visitations, were significantly coincided with the pattern of anthesis in both *S. wallichii* and *L. speciosa*. *Chrysomya megacephala*, the Diptera belonging to the family Calliphoridae, was a significant floral visitor in terms of dispersed visitation frequency throughout the day and maximum mean time spent per flower in *L. speciosa*. Calliphoridae are regular visitors and important pollinators of *Avecenia officinalis* (a mangrove species) in South India (Aluri, 1990) and *Mangifera indica* in sub-tropical India (Bhatia *et al*., 1995). Calliphoridae are strong fliers and transmit fairly large quantities of pollen (House, 1989; Aluri, 1990), but their function as pollinators in tropical tree species are largely unexplored and underestimated.

Many members of family Lepidoptera have been reported as casual floral visitors and are considered to be less important as pollinators in lowland rainforest compared with the neotropics (Bosch, 1997; Sakai, 2001). In *S. wallichii,* five species of moths visited flowers irregularly, mainly during early morning and late evening hours. In the Western Ghats region of India, nine tree species have been shown to be pollinated by moths of the family Geometridae (Devy and Davidar, 2003).

Conclusions

Various moth and bee species were involved in pollination of *Schima wallichii* flowers. The flowers of *S. wallichii* showed physical fitness towards functional group of moth and bee in respect to mating success. The *Xylcopa* spp. of bee showed a high level of functional synchronisation with the floral morphology of *L. speciosa*. The smaller number of species attracted to *L. speciosa* means that the loss of any one or more would have a negative effect on pollination of this species. Tropical forest ecosystems sustain high degree of ecological specialisation of plant-pollinator interactions and have a highly coupled relationship between reproductive phenology and pollinators' availability. Anthropogenic pressure may decouple species interactions during pollination, which may lead to accelerated species extinction. Therefore, studies that combine observation of functional groups of pollinators with examination of pollen-mediated gene flow would greatly improve the understanding of the mechanisms involved in tree pollination.

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