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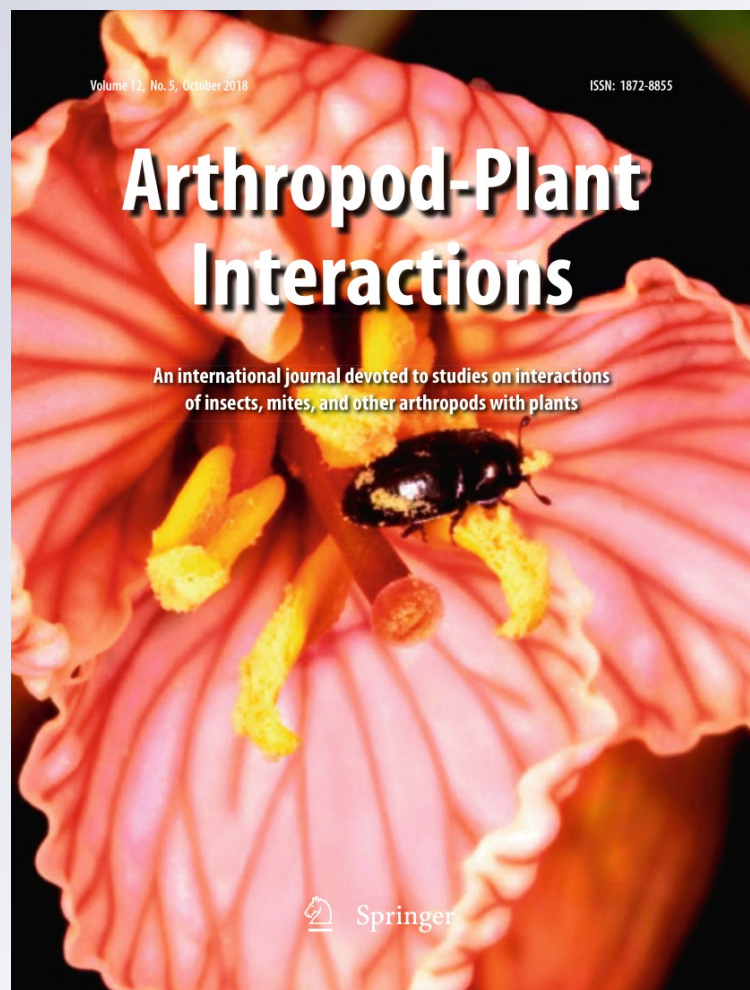
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Ant pollination of *Syzygium occidentale*, an endemic tree species of tropical rain forests of the Western Ghats, India

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Abstract

Although mutualism between ants and flowering plants is wide spread, ant pollination has not evolved as a major pollination syndrome. So far ant pollination has been reported largely in herbaceous species, growing in warm and dry habitats. While studying pollination ecology of *Syzygium* species (Myrtaceae), growing in tropical forests of the Western Ghats, India, we observed one of the ant species, *Technomyrmex albipes*, to be the dominant floral visitor in *S. occidentale* (Bourd.) Chithra among a range of other insect (species of *Xylocopa* and *Trigona*, and *Apis cerana*) and bird visitors. We studied the role of ant species in pollination when compared to other floral visitors. The fruit set in flowers exclusively visited by *T. albipes* was significantly higher than those visited by any other visitor. The day and night exclusive pollination experiments allowing only *T. albipes* indicated diel pollination by *T. albipes*, which was the only active flower visitor during the night. The breeding system of the species was studied through controlled pollinations. The species is partially self-compatible and exhibits considerable autogamy.

Keywords Breeding system · Myrmecophily · Myrtaceae · Pollination efficiency · *Technomyrmex albipes*

Introduction

Mutualism between plants and ants is widespread. Two of these mutualisms involving ants in protecting plants from herbivores, and in seed dispersal (myrmecochory), are well known and intensively studied (Herrera and Pellmyr

2002; Bronstein et al. 2006; Rico-Gray and Oliveira 2007; Schaefer and Ruxton 2011). Ants are closely related to bees and wasps, and have developed very complex social structure. They are the most abundant among the insects, and present in almost all habitats. Ants were present during the explosive radiation of the flowering plants in the late Cretaceous, even before the appearance of bees (Beattie et al. 1984; Beattie 1985). In spite of these positive features for the evolution of a strong ant-pollination syndrome, ant pollination is very rare when compared to their sister groups, bees and wasps (Dutton and Frederickson 2012). So far, ant pollination has been reported in about 46 species of flowering plants (de Vega et al. 2014; de Vega and Gomez 2014). Most of the ant pollination has been reported from dry or cold zones (Hickman 1974; Dutton and Frederickson 2012; de Vega and Gomez 2014; Ibarra-Isassil and Sendoya 2016). Several hypotheses have been put forward to explain the rarity of ant pollination (Beattie 1985; Dutton and Frederickson 2012). Two of the hypotheses that have some evidences are (i) movements of ants are usually restricted to within the plant and thus result largely in geitonogamous self-pollination; and (ii) secretion of antibiotics from metapleural glands (MG), present in most of the ant species, reduce pollen viability (Beattie et al. 1984, 1985; Peakall et al. 1990;

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Rico-Gray and Oliveira 2007; Dutton and Frederickson 2012). However, experimental studies on the effects of ant-secreted antibiotics on pollen germination have shown differential potency among ant species and differential vulnerability among pollen species (see Peakall and Beattie 1989). In vitro germination studies have shown significant reduction in pollen viability in some species, while ant-secreted antibiotics had no effect on pollen of other species (Hull and Beattie 1988). Further, Dutton and Frederickson (2012) suggested that the effect of pollen germination in ant visiting flowers varies in tropical and temperate countries. Although there are not enough evidences available to prove this argument, recently Ibarra-Isassil and Sendoya (2016) found no negative impact on ant-exposed pollen grains of *Blutaparon portulacoides* (A. St.-Hil.) Mears (Amaranthaceae) from Brazil. Ant-pollinated plants are generally herbaceous growing as dense clumps in warm, dry, as well as mountain habitats (see Willmer 2011). So far ant pollination in a tree species has been reported only from *Mangifera indica* L. (Anacardiaceae) from Northern Australia (Anderson et al. 1982). This report was from the human-managed agriculture field. During our studies on pollination ecology of several endemic *Syzygium* species in the evergreen forests of the Western Ghats, we observed that ants are the most abundant floral visitors in *Syzygium occidentale* (Bourd.) D.N. Gandhi (Myrtaceae). Our main objective of this study was to evaluate the role of ants as potential pollinators of *S. occidentale*.

Methods

Study sites and species

Syzygium (Myrtaceae) with over 1200 species (Byng et al. 2015) is one of the most speciose angiosperm tree genus (Parnell et al. 2007). *S. occidentale* is a small evergreen riparian tree species of 4–5 m in height, endemic to the Western Ghats, India (Nayar 1996). The species has so far been reported only along the river Periyar in Kerala. The elevation of its distribution range varies between 70 and 1268 m mean sea level. The present study was carried out on two populations in Edamalayar-Pooyamkutty forests located between 10° 06'27.14"N 76° 53'51.81"E and 10° 13'52.48"N 76° 44'54.86"E along one of the major tributaries of the river Periyar in Central Kerala. The total number of individuals was 38 in population I and 13 in population II. The habitat comprises mostly of moist deciduous trees with patches of evergreen and semi-evergreen trees. The undergrowth is mainly composed of *Ochlandra travancorica* (Bedd.) Gamble (Poaceae), a reed species which is commercially exploited for making paper pulp.

Flowers in *S. occidentale* are produced in terminal cymose inflorescences. The mean number of flowers per

inflorescence was 11.79 (± 4.33 SD, range 3–22, $N=34$). Flowers are large (6.7–8.2 cm long), white, with a pleasant odor and are pendent. The flowers bear four green sepals and four white petals followed by a large number of white stamens (804.95 ± 54.53 SD, range 691–921, $N=62$) (Fig. 1a). The style is long (4.5–5.8 cm) and delicate and terminates in a pointed stigma. The ovary is inferior and bears 56.97 (± 8.87 SD, range 42–81, $N=62$) ovules. However, only one seed is produced in each fruit ($N=68$).

Phenology

The studies were carried out during the three flowering seasons, 2010–2011, 2012–2013, and 2014–2015. The phenological details of flowering were recorded through daily field visits. Floral phenology was studied by tagging flower buds ($N=76$) from 11 trees and recording details of anthesis, anther dehiscence, and longevity of flowers. The volume of nectar was measured using calibrated micro-capillaries and the sugar equivalence of the nectar using portable refractometer. For measuring nectar, the flower buds were bagged the previous evening to prevent insect visits before measurement. Fully opened flowers ($N=227$) from 14 trees were used for one measurement of nectar. Three flowers were used to count the number of pollen grains per anther following the methodology given in Shivanna and Tandon (2014). Flowering initiated in both the populations in mid-December to mid-January, reached the peak by the second week of February, and terminated by the second week of April. All the observational studies were carried out in the peak flowering time. In both the populations, *Entada rheedii* Spreng. (Mimosaceae), *Madhuca nerifolia* (Moon) H.J.Lam. (Sapotaceae), and *Vitex leucoxydon* L.f. (Verbenaceae) were co-flowering during the early flowering of *S. occidentale*; the former two are small trees and the third is a woody perennial.

Pollination biology

Floral visitors and their frequency of visits were studied by visually marking a group of 10–15 flowers on each day and recording the number of visits of each visitor to focal flowers, duration of their visit, and their contact with the anthers and the stigma. Diurnal observations were made from dawn (06:00 h) to dusk (18:00 h) continuously for all flying visitors. The visits of ants were recorded in 15-min time slots each hour. All insect visits were observed by sitting 1–3 m away from the marked flowers. Visits of birds were recorded using a pair of binoculars from a distance by sitting around bushes of other plants. Since most of the nocturnal visits have been reported to occur during late evening and early morning, we managed to make night observations from 18:00–20:00 and 04.00–06.00 h. Total time spent to record

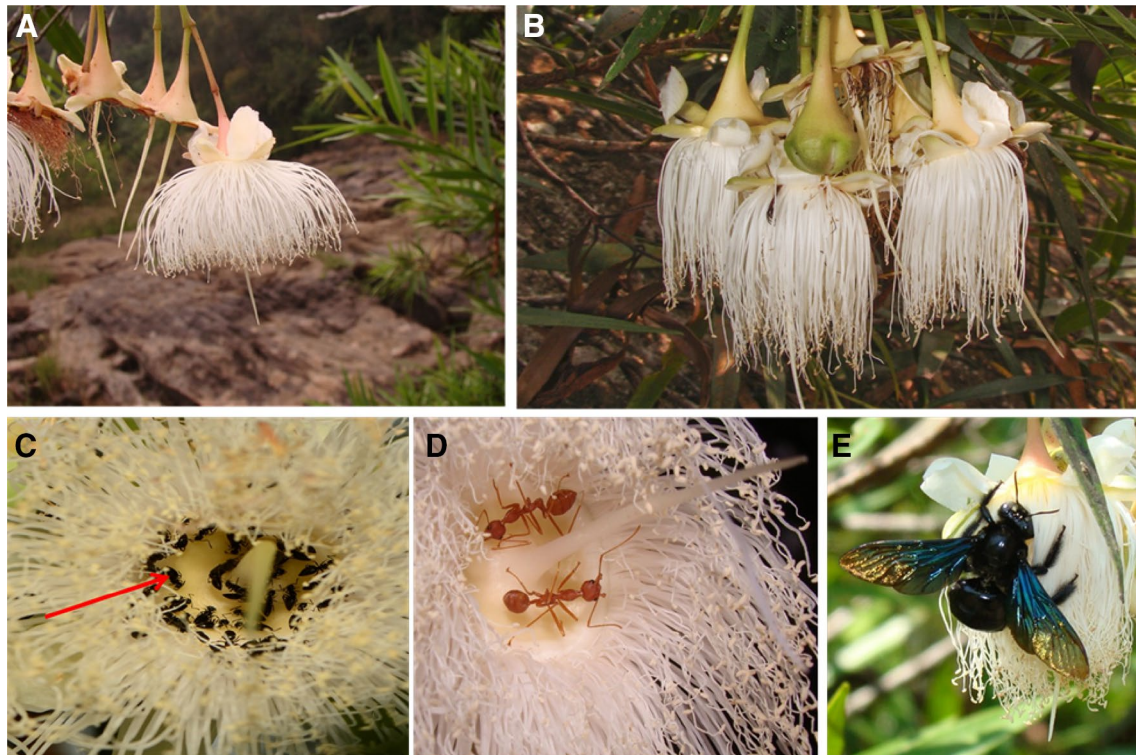


Fig. 1 *Syzygium occidentale* flower morphology and some flower visitors. **a** Fresh flower soon after anthesis; **b** flowers at noon on the day of anthesis; stamens have lost turgidity and sagged; **c** and **d** flow-

ers magnified to show foraging ants, *Technomyrmex albipes* (**e**) and *Oecophylla smaragdina* (**d**). **e** *Xylocopa* sp. foraging the nectar

different floral visitors was 32 h spread over three days for flying insects, 49 h 50 min spread over 18 days and nights for *T. albipes* Smith (Formicidae), other nocturnal insects, and bats, 19 h 25 min spread over seven days for *O. smaragdina* Fabricius (Formicidae), and 44 h spread over 4 days for birds.

Effective pollinators

In order to identify the effective pollinators of *S. occidentale*, we allowed only the visitors of our focal species at a time and excluded all other visitors. To test the efficiency of ants, we covered the terminal flowering branches bearing focal flowers with bags made of pollen-proof cotton cloth. The opening of the bag was tied loosely leaving some space around the stem. This permitted ants to visit bagged flowers freely but effectively prevented all flying insects. After the observation period, the bags were tied firmly to close the gap. The developing fruits mature in 29–32 days and ripen in 50 days. So, we assessed fruit set on 30th day of the experiment. The fruits were dissected to confirm the presence of healthy seeds with embryo. The pollination efficiency of the ant species was examined in two flowering seasons of *S. occidentale*.

To study the role of other diurnal insects in pollination, we standardized a dual method to prevent ant visits to focal flowers. We smeared a strong non-solidifying adhesive (Stikem Special-Hold Fast, Seabright Laboratories, Emeryville, CA, USA) around the stem (2–3 cm long) of branches proximal to the focal flowers. The glue prevented ants from reaching focal flowers. A few that tried to cross got stuck to the glue. Terminal part of the branch bearing focal flowers (distal to the part smeared with the glue) was covered with a bag made up of pollen-proof cotton cloth and its opening was tied firmly around the stem without leaving any space to prevent ants from entering the bagged flowers. During the day, the bags were opened at the tip and folded back to record other insect visitors. After observations, the bags were tied at the tip.

Pollinators' efficiency was studied on the basis of fruit set in flowers visited exclusively by a specific visitor species. We prevented the visits of non-target species by netting them before landing on the focal flowers. However, *Trigona* was very small and it was difficult to prevent its visit to flowers targeted for *Apis cerana* Fabricius (Apidae) and *Xylocopa* sp. (Apidae). Such observations were replicated in sufficient numbers to study the pollination efficiency of combined visits of the two pollinator species (*Apis* + *Trigona* and *Xylocopa* + *Trigona*). All the pollinator-specific

observations were made for one or two flowering seasons of *S. occidentale*.

Our observations showed that the birds followed a sort of punctuality on their bouts to the trees. Most of the birds visited the flowers located at the top of the canopy. We tied the branches likely to receive birds with orange color tags so that they can be seen through the binoculars and ants were prevented to those branches by smearing the non-solidifying adhesive to the stem of those branches. We covered the flowers until the visits of the birds were likely to start using a mosquito net to prevent other flying insects visiting those flowers. Whenever we encountered any other flying insects on the focal flowers before the visits of birds, we abandoned such flowers from recording. The number of flowers monitored to study the pollination efficiency of different floral visitors is mentioned with the respective figures.

The effect of diel period on pollination efficiency was studied on the most abundant visitor of the day and night, *T. albipes*. In both the diel periods, we excluded the visits of *T. albipes* in one set of flowers using glue (see the previous paragraph for the details) and used them as controls. In another set of flowers, we allowed the ants to the flowers, but excluded other visitors as described above. After our observations, we bagged the focal flowers and monitored for fruit set. These observations were made during two flowering seasons of *S. occidentale*.

We hung petroleum jelly-smear slides and net-covered inflorescences to study the role of wind in effecting pollination. We hung the slides on flowering tree canopy 0 m, ($N=45$), 1 m ($N=32$), and 2 m ($N=34$) away from the edge of flowering tree to study the effect of distance on wind-borne pollen dispersal. We covered flower buds after emasculation with mosquito nets, which filter pollinators out, but allowed wind-driven pollen grains and studied resulting fruit set ($N=40$).

Breeding system

To study the breeding system, manual self- and cross-pollinations of emasculated flowers were carried out and bagged. The bags were removed after flower senescence and the flowers were monitored until their abscission/fruit set.

Open pollination efficiency

We tagged inflorescences for 3 years ($N=78$ (mean number of flowers/inflorescence = 9.69; year = 2010), 105 (mean number of flowers/inflorescence = 6.83; year = 2013), and 128 (mean number of flowers/inflorescence = 6.32; year = 2014)) to study annual variation in pollination efficiency in *S. occidentale*. The number of flowers monitored for fruit set for each study year has been given in the respective figure.

Statistical analyses

We constructed a generalized linear model (GLM) with fruit set as response variable and the pollinator species or medium as effect type to study the effect of pollinator species or medium on fruit set in *S. occidentale*. We used binomial distribution as error type in the model. *T. albipes* and cockroach were the only nocturnal visitors to the flowers. We studied the effect of diel period on pollination efficiency of *T. albipes* using another GLM. The cockroach spent a long time visiting the flowers with very limited inter-flower movement. Therefore, we excluded the cockroach visits from all the analyses. We specified binomial distribution as the error type in the model. We constructed another model (GLM) to study the effect of pollinators of broad type (ants, ant control, other pollinators, and wind) by studying fruit set in respective categories. We used binomial distribution as an error type in this model. We used Wald's z test available in the R-package "car" to examine the significance of the models.

We used a generalized linear mixed model using number of pollen grains on slides as the response variable, distance as the fixed factor, plant ID and slide number as the random factors, and Poisson distribution as the error type to study the effect of distance on wind dispersal of pollen grains. The open pollination efficiency of 3 years was compared using a generalized linear mixed model (GLMM) using the function 'glmer' available in the R-package "lmerTest." The study year was the fixed factor, the percent fruit set was the response variable, and plant ID and diel period of open pollination were the random factors in the model. The effect of diel period on open pollination efficiency was studied using a generalized linear mixed model, in which percent fruit set was the response variable, diel period was the fixed factor, and study year and plant ID were the random factors. In both the models, we specified binomial error as the distribution. The significance of the model was tested using Wald's z test. All the analyses were conducted in R 3.2.3 (R Core Team).

Results

Flower morphology and rewards

In the bud stage, all the stamens of the flower are compactly arranged and incurved around the basal part of the style; the extended part of the style is bent on outer surface of incurved stamens. Anthers dehisce in the buds 2 days before anthesis. However, dehisced anthers do not come in contact with the stigma in the bud stage because of incurved nature of the stamens and the location of the stigma outside the stamen filaments. Flowers open around 20:00–23:00 h during the night. The stamens and style

unfold during anthesis and become erect. The mean nectar volume per flower at anthesis was 71.01 μl ($\pm 45.2\text{SD}$, $N=227$) and the concentration of sugar equivalence in the nectar was 13.59% ($\pm 6.03\text{SD}$, $N=227$). The stamens spread out and remain fresh, erect, and turgid until about 10:00 h on the day following anthesis (Fig. 1a). Later, the anthers start withering and the stamens become flaccid and sag (Fig. 1b). Mean number of pollen grains per anther was estimated to be 13,596 ($\pm 633.35\text{SD}$, $N=3$). By the second day, all the stamens abscise. The style and the sepals are persistent and remain even in mature fruit.

Floral visitors, visitation frequency, and foraging pattern

The flowers of *S. occidentale* attracted a range of animal species (Fig. 1c–e). Ants were the most frequent visitors to the flowers of *S. occidentale*. Of the 38 trees in population I, 32 had *T. albipes* and the remaining 6 had *O. smaragdina*. In population II, out of 13 trees, 8 harbored *T. albipes* and the remaining 5 harbored *O. smaragdina*. Ants of *T. albipes* foraged only the floral nectar, but *O. smaragdina* foraged both the nectar and the flower visitors. As the stamens are arranged compactly around the nectar cup, ants crawl on the outer surface of the stamens and move all over the dehisced anthers; pollen grains stick to their body during their movement on dehisced anthers. Ants often come in contact with the stigma. Mean frequency of visits of *T. albipes* was very high (136.45 visits/h/flower $N=198$) when compared to the visits of *O. smaragdina* (7.75 visits/h/flower, $N=77$). The visits by *T. albipes* contributed 91% of total visitation by all the floral visitors (Table S1).

Apis cerana and *T. iridipennis* mostly foraged pollen and occasionally the nectar and often came in contact with the stigma, but their frequency of visits in the flowers was very low (Table S1). They consistently avoided the flowers in which ants, particularly *O. smaragdina*, were present. *Xylocopa* sp. (visitation rate = 0.33 visits/flower/h; $N=35$) foraged both the nectar and pollen (Fig. 1e) but did not come in contact with the stigma.

All the three bird species foraged nectar mostly from the side of the flower and caused maximum physical disturbance to the stamens. *Leptocoma minima* Sykes (Nectariniidae) was the most frequent visitor among the birds followed by *Cinnyris asiaticus* Latham (Nectariniidae) (Table S1). *Arachnothera longirostra* Latham (Nectariniidae) was the infrequent visitor to the flowers and they were active only during the late flowering period. As its visits were not at the peak of flowering when the frequency of other visitors were recorded, the frequency of visits of *A. longirostra* was not recorded.

Pollination efficiency of floral visitors and wind

Overall, the pollination efficiency of different pollinator species and wind were different (Chi square = 15.69, d.f. = 7, $p=0.02$) (Fig. 2). The model clearly showed the significant role of ant species *T. albipes* in pollination of *S. occidentale* (Fig. 2 & Table S2). Visits of other species of pollinators and wind were not crucial in pollination when compared to *T. albipes* (Table. S2). When we grouped the pollination types into ants, ant control, all other animal vectors, and wind, we found that the pollination efficiency varied significantly between the groups (Chi square = 14.33, d.f. = 3, $p=0.002$) (Fig. 3). Pollination efficiency of wind (20%) was significantly very lower than that of the ants (Table S3). *Technomyrmex albipes* visited the inflorescences both during the day and the night, but the diel period did not affect its pollination efficiency (Chi square = 0.11, d.f. = 1, $p=0.91$) (Fig. 4). However, the pollination efficiency was high in *T. albipes*-visited flowers both during the day (Chi square = 9.17, d.f. = 1, $p=0.002$) and during the night (Chi square = 30.81, d.f. = 1, $p < 0.0005$) when compared to the ant-excluded flowers of the respective times (Fig. 4). The number of pollen grains on exposed slides drastically reduced with the distance from the flowering tree canopy ($z = -7.825$, $p < 0.0005$) confirming limited efficiency of wind in dispersing pollen grains and bringing out pollination in *S. occidentale*.

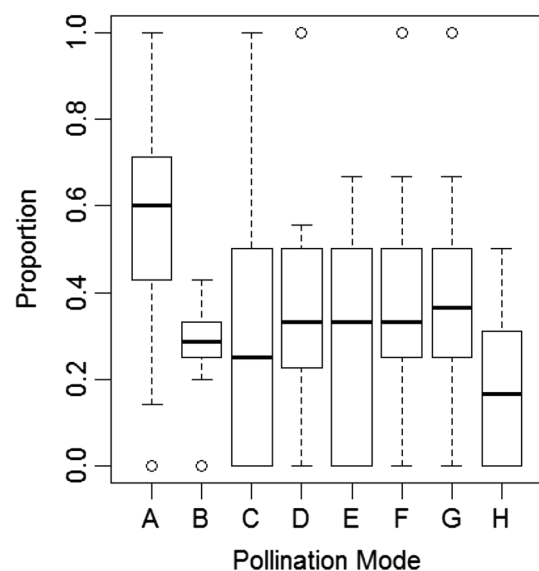


Fig. 2 Box plots show pollination efficiency (fruit set/flower) of different floral visitors and wind in *Syzygium occidentale*. Legend for x-axis (Number of flowers monitored for fruit set are given in parentheses): a *Technomyrmex albipes* (257); b *Oecophylla smaragdina* (134); c *Apis cerana* + *Trigona iridipennis* (64); d *Xylocopa* sp. + *T. iridipennis* (67); e Little Spider-Hunter (42); f Purple Sunbird (51); g Small Sunbird (78); h Wind (68)

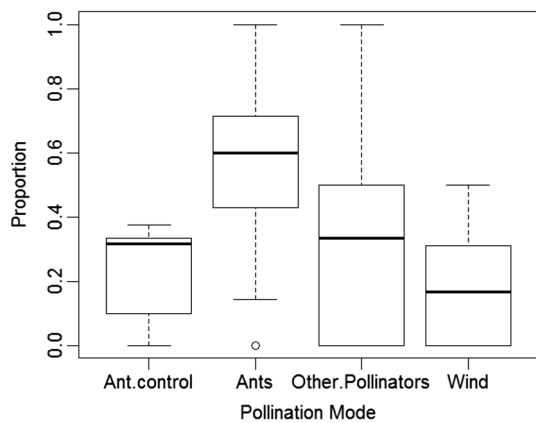


Fig. 3 Box plots show pollination efficiency of ants (fruit set/flower) is significantly higher than that of other combined floral visitors, ant-control flowers, and wind in *Syzygium occidentale*; $N=391$ (ants); 302 (other pollinators); 68 (wind)

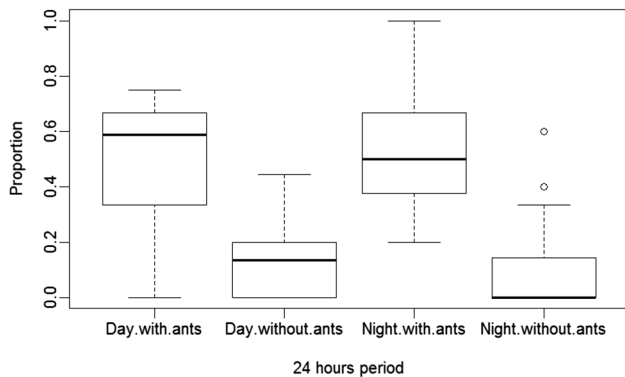


Fig. 4 Box plots show pollination efficiency of *Technomyrmex albipes* (fruit set/flower) is consistent between day and night and that the pollination efficiency of the ant-excluded flowers was significantly lower than that of ant-visited flowers in day and night. $N=136$ (day with ants); 176 (day without ants); 326 (night with ants); 329 (night without ants)

Breeding system

Table 1 gives details of fruit set of different pollination treatments. Apomixis does not seem to occur in *S. occidentale* as most of the emasculated and bagged flowers abscised. A small proportion of fruit set recorded (6.82%) may have been due to pollen contamination as it was very difficult to emasculate such a large number of anthers before they dehisce (2 days before anthesis) without any contamination. The open pollination efficiency was significantly lower than the xenogamous pollination (Chi square = 9.167, d.f. = 1, $p=0.002$). The difference in pollination efficiencies between xenogamy and geitonogamy were marginally significant (Chi square = 3.483, d.f. = 1, $p=0.069$) (Table 1). However, the difference in proportions of fruit set between geitonogamy

Table 1 The proportion of flowers developed into fruits in different pollination experiments

Pollination treatment	<i>N</i>	Fruits developed	Percent fruit set
Open	884	367	41 ^a
Xenogamy	63	39	62 ^b
Geitonogamy	66	29	44 ^{ab}
Apomixis	88	6	7 ^c

Difference in characters in superscript indicates significant differences between percent fruit set

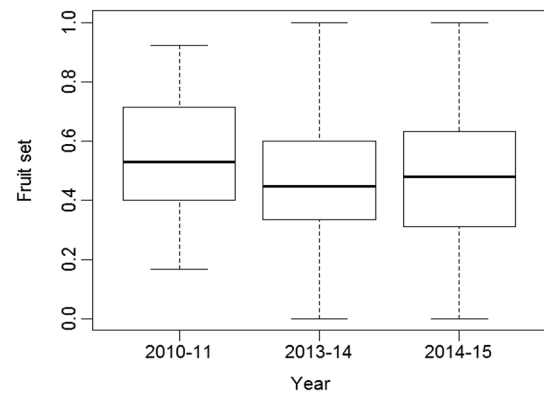


Fig. 5 Box plots show consistent open pollination efficiency (fruit set/flower) in *S. occidentale* across the study years; $N=756$ (2011), 717 (2013), 810 (2014)

and open pollination was not significant (Chi square = 0.066, d.f. = 1, $p=0.7$). The results suggest that *S. occidentale* experiences pollination limitation.

The model showed that open pollination efficiency in *S. occidentale* was consistent across the 3 years of the study (Chi square = 5.26, d.f. = 2, $p=0.07$) (Fig. 5). Open pollination efficiency of day (42.88%) was slightly higher than that of the night (36.07%) (Chi square = 3.66, d.f. = 1, $p=0.05$) (Table S3). This result further strengthens our findings that *T. albipes* is the most efficient pollinator of *S. occidentale* and other diurnal visitors are of less-important pollinators.

Discussion

The genus *Syzygium* is characterized by open flowers with readily available rewards to any visitor. A large number of floral visitors, both diurnal (bees, wasps, ants, butterflies, moths, and birds) and nocturnal (bats and moths), have been reported in several species of *Syzygium* (Crome and Irvine 1986; Hooper 1980; Lack and Kevan 1984; Ludhagha and Proença 1996; Kaiser et al. 2008). However, in most of these reports, the role of individual species in pollination has not

been demonstrated. In *Syzygium tierneyanum*, for example, 45 species of floral visitors have been recorded (Hooper 1980); honey bees and hawkmoths have been inferred to be effective pollinators. Based on the selective bagging studies, bats, large moths, birds, and insects have been reported to be effective pollinators in *S. cormiflorum* growing in Australian rain forest (Crome and Irvine 1986).

The results of the present investigation clearly show that one of the ant species, *T. albipes*, is the major pollinator of *S. occidentale*. Our results are in agreement with the results of Gomez et al. (1996) on pollination of seven Mediterranean species of high mountain and arid habitats in which ants turned out to be the pollinators when they outnumbered other floral visitors. In the present study, the frequency of *T. albipes* visit to the flowers and the extent fruit set in flowers visited exclusively by this ant species was significantly higher than those visited by any other insect or bird species and also the fruit set recorded in open-pollinated flowers. Further, the fruit set in flowers visited by any other visitor was not significantly different from that realized in bagged flowers. It appears that although other insect visitors may bring about pollination to some extent, their efficiency is not better than that in autogamous pollination; at the most, they may increase the number of pollen grains deposited on autogamously pollinated stigma or may bring about limited cross-pollination. In *Cytinus hypocistis*, a Mediterranean holoparasitic plant species, six species of ants were reported to be the pollinators of which five were the diurnal pollinators and one was the nocturnal pollinator (de Vega et al. 2009). The present study has shown conclusively that *T. albipes* is an effective pollinator of *S. occidentale* during the day as well as during the night. So far, ant pollination has been reported largely in herbs and undershrubs. Of the 46 ant-pollinated species listed by de Vega and Gomez (2014), there is only one tree species (*M. indica*) reported from the Northern Australia (Anderson et al. 1982). The flowers of most of the ant-pollinated species listed by de Vega et al. (2014) are white (31.58%) followed by green (26.32%) and pink or red (10.53%); only a few of them are yellow and two species have no perianth. The flowers of *S. occidentale* are also white and are scented (to humans).

Interestingly, ants have been recorded to be floral visitors in two of the *Syzygium* species, *S. tierneyanum* (Hooper 1980) and *S. cormiflorum* (Crome and Irvine 1986); in the latter ants have been reported to carry pollen load on their body, although their involvement in pollination has not been investigated. Therefore, *Syzygium* spp. are likely to be benefited from ant visits, and require in-depth studies in various *Syzygium* spp. across Asia-Pacific region to understand whether these species have evolved ant-pollination syndrome.

We have looked for the presence of ants in flowers of four other species of *Syzygium*, *S. travancoricum*, *S. heyneanum*,

S. laetum, and *S. mundagam*, growing in the tropical forests of the Western Ghats and three co-flowering species (*E. rheedi*, *M. neriifolia*, and *V. leucoxylon*) with *S. occidentale* (unpublished observations). Both the ant species, *T. albipes* and *O. smaragdina*, were present on the flowers of two of the co-flowering species, *E. rheedi* and *M. neriifolia*, but not on *V. leucoxylon*. One of the ant species, *O. smaragdina*, was observed regularly on the flowers of *S. heyneanum* in low frequency. However, the ant was not a pollinator in this species but often preyed upon other insect visitors, particularly *A. cerana* and *A. dorsata* (unpublished data). Ants, particularly *O. smaragdina*, have been reported to be aggressive and to reduce the visitation frequency of other flying insects (Ness 2006; Tsuji et al. 2004; Sinu et al. 2017). In *S. occidentale* also, *A. cerana*, *T. iridipennis*, and other insect visitors avoided visiting flowers in which ants were present. None of the other three species of *Syzygium*, *S. travancoricum*, *S. laetum*, and *S. mundagam* attracted any ant species. It is possible that the flowers of these species either lack any ant attractants or actively repel the visits of ants. There are earlier reports of flower volatiles and nectar components (Stephenson 1982; Willmer and Stone 1997; Junker and Bluthgen 2008; Willmer et al. 2009; Junker et al. 2011) repelling or attracting (de Vega et al. 2014) ant species to the flowers. In *Syzygium* species, floral fragrance components or their role in attraction of floral visitors are not known and thus we do not know whether ants are attracted to the flowers in response to a specific volatile cue emitted by the flowers of *S. occidentale* or opportunistic visits of ants to harvest the nectar have stabilized into a pollination syndrome.

None of the bird species brought about pollination directly in *S. occidentale*. All the bird species forage the nectar from the side of the flowers. We found no considerable improvement in pollination efficiency in bird-visited flowers when compared to bagged flowers. Physical disturbance caused by birds and also *Xylocopa* sp. during their visits may release some pollen grains to the ambient air and some of this pollen is likely to land on the stigma as a result of gravitational movement. Another interesting observation during the study was the visitation by a species of unidentified cockroach during the night. Cockroach pollination is rarely reported in nocturnal flowering plants (Nagamitsu and Inoue 1997). Cockroach was seen visiting the flowers of different individuals and it stays on the nectary of the flower for hours with very limited inter-flower movement. But our experiments showed that it did not bring about pollination in *S. occidentale*.

In general, ants are not the exclusive pollinators; they share pollination services with several other visitors. In *Lobularia maritima* (Gomez 2000), for example, over 50 species of animals (made up of largely ants and flies) visit the flowers. The flowers of *Balanophora* attracted a variety of

flying insects, including pyralid moths, craneflies, and cockroaches besides ants (Peakall and Beattie 1989). However, exclusive ant pollination has been reported in four Australian orchid species, *Leporella fimbriata* (Peakall 1989), *Microtis parviflora* (Peakall and Beattie 1989), *Chenorchis singchii* (Zhongjian et al. 2008), and *Chamorchis alpine* (Schiestl and Glaser 2012). In another orchid, *Epipactis thunbergii*, usually pollinated by hoverflies, the contribution of ants is only supplementary (Sugiura et al. 2006). In *S. occidentale* also, similar to several other ant-pollinated species, although ants are the major pollinators, it attracts a range of floral visitors, including flying insects and birds.

Pollination systems are flexible and may change in specific ecological context such as loss or scarcity of pollinators and low density of plants in the population (see Willmer 2011). In *S. occidentale*, flying insects and birds, even when they bring about some pollination, the extent of cross-pollination is very limited. The fruit set is largely the result of autogamy or geitonogamy brought about by ant species. The breeding system with partial self-compatibility in which self-pollination results in considerable seed set seems to have evolved as a means of reproductive assurance in the absence of or limited cross-pollination (see Shivanna 2015).

Large, white flowers of *S. occidentale* with pleasant odor, nocturnal anthesis, and considerable amount of nectar represent bats and/or moths also as effective pollinators. However, we did not observe bats or moths in both the populations we studied during our observation period. Among the two ant species that visited the flowers of *S. occidentale*, *T. albipes* visited flowers both day and night, increasing their chance as efficient pollinators of *S. occidentale*. Additionally, the pollination efficiencies of both nocturnal and diurnal pollinators were similar suggesting limited role of nocturnal visitors in improving pollination efficiency. More studies may be required to infer conclusively the role of bats and moths in pollination of *S. occidentale*.

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Floral traits predict pollination syndrome in *Syzygium* species: a study on four endemic species of the Western Ghats, India

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Abstract. Pollination ecology and the breeding system of four endemic species of *Syzygium* (*S. heyneanum*, *S. travancoricum*, *S. laetum* and *S. mundagam*) of the Western Ghats, India are investigated. The floral traits are used for ordination analysis to understand if the species form any cryptic groups and whether this grouping predicts the pollination syndrome and the breeding system. Pollinators were distinguished from frequent visitors by studying their efficiency to transfer pollen to the stigma or induce fruit set following their exclusive visit to virgin flowers. The species formed two groups in the ordination map: the *S. heyneanum* and *S. travancoricum* group and the *S. laetum* and *S. mundagam* group. The flowers of studied species attracted 3–23 species of animals during the day, but none were encountered during the night. Flowers of *S. heyneanum* and *S. travancoricum* were visited by a large number of insect species of which only a limited number turned out to be the pollinators. *S. laetum* and *S. mundagam* attracted two bird species apart from a few insect species. In *S. laetum*, although birds did not bring about pollination directly, their visits facilitated anemophily by releasing pollen to the air by causing physical disturbance to the flowers. In *S. mundagam*, both birds and wind were involved in pollination. *S. heyneanum* and *S. travancoricum* were fully self-compatible, whereas *S. laetum* and *S. mundagam* were partially self-compatible.

Additional keywords: anemophily, breeding system, endemic species, ornithophily, partial self-incompatibility, plant–pollinator interaction, wet evergreen forest.

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Introduction

Syzygium (Myrtaceae) with over 1200 species is one of the most speciose angiosperm tree genus (Parnell *et al.* 2007). The genus is generally restricted to the tropical evergreen forests and its native range extends from Africa through Madagascar, south and south-east Asia to Australia and New Zealand (Mabberley 2008). Flowers of *Syzygium* are of brush type showing generalised pollination strategy. Studies on pollination biology in several *Syzygium* species especially of Australia and New Zealand have shown that the flowers are visited by several insect as well as vertebrate species (Hopper 1980; Crome and Irvine 1986; Beardsell *et al.* 1993; Law and Lean 1999; Boulter *et al.* 2005). In *Syzygium tierneyanum*, for example, as many as 45 species of animals have been reported to visit the flowers (Hopper 1980). Although the number of floral visitors has been recorded in several species, effective pollinators and their efficiency have not been assessed in most of these studies.

Syzygium is well represented in India with over 75 species. Many of the species of *Syzygium* reported from India are

endemic to the Western Ghats biodiversity hotspot and a few of them are endangered (Nayar *et al.* 2006). Some of the species yield valuable timber and fruits of some species are edible. The leaves of several species are used as green manure by farmers. A few species are also of medicinal importance and some are used to extract natural dyes. Surprisingly, there is very little information on pollination biology of Indian species of *Syzygium*. A few available reports (Raju *et al.* 2014; Geethika and Sabu 2017; Varghese and Sreekala 2017) have largely documented floral visitors without distinguishing pollinators from casual visitors and also there is no information on pollinators' efficiency.

The present study attempts to investigate pollination ecology and the breeding system in four endemic species of *Syzygium*, i.e. *S. heyneanum*, *S. laetum*, *S. mundagam*, and *S. travancoricum* in the Western Ghats. All these four species are rare and distributed in small scattered populations in wet evergreen, riparian or swamp forests. Among these four species, *S. travancoricum* is considered critically endangered (Anonymous 2017). It has

been reported so far only from a few locations in the entire 1600 km² spread of the Western Ghats range (Nair and Mohanan 1981; Chandran et al. 2008).

The studied species show marked variation in the size, colour and orientation of the flowers, their morphological traits, and the nectar dynamics. Our aim was to determine: (1) whether the four species, based on floral traits, form cryptic groups, (2) which are the effective pollinators amongst the floral visitors, (3) whether the trait variations among species predict their pollination syndrome, and (4) the breeding system in these species.

Materials and methods

Some general information regarding the studied species of *Syzygium* and the study sites in the Western Ghats biodiversity hotspot are presented in Table S1, available as Supplementary Material to this paper. All observations and field experiments were carried out on their natural populations between 8 and 11°N (Fig. S1, available as Supplementary Material to this paper). All the species are evergreen and either riparian or swamp inhabitants. Morphological details and floral traits of the four species are given in Table 1.

Flowering phenology and floral morphology

Flowering and fruiting phenology of all the studied species are presented in Fig. S2, available as Supplementary Material to this paper. With the exception of *S. laetum*, in which flowering was initiated in November itself, in all other species flowering was initiated during January–March, before the beginning of the south-west monsoon. In most of the species, flowering was completed during the summer itself before the initiation of the monsoon (June) except in *S. mundagam* in which it continued into the rainy season. In all the species, fruit ripening and dispersal occurred during the peak of the monsoon characterised by heavy rains. The phenology of the flowering and fruiting of the populations was recorded through regular field visits.

The floral phenology was studied by tagging mature flower buds on different trees in different populations and recording details of their anthesis, anther dehiscence, and the longevity of the flowers until they started senescing.

Floral morphology of all the species is typical of Myrtaceae – open flowers with a large number of stamens around a pointed exerted stigma (Fig. 1). Variations in some floral attributes such as orientation of the flower (erect or pendant), the colour of the flower, the number of stamens, the size of different flower parts, the number of pollen grains, the amount of nectar, the number of ovules, and the longevity of the flower were also recorded. The number of pollen grains per anther in each species was counted following Shivanna and Tandon (2016). Floral traits were quantified or measured using newly opened flowers. The volume of nectar was measured by using calibrated micro-capillaries (50 and 100 µL, Drummond microcaps, Sigma-Aldrich, Drummond Scientific, Broomall, PA, USA) and the sugar content of floral nectar was measured by using a hand-held portable refractometer (Model ABT-32, VEE GEE Scientific, Kirkland, WA, USA).

Pollination ecology

We selected 13 trees of *S. heyneanum* in two populations and three trees of *S. travancoricum* in three populations along the river Periyar in Pooyamkutty and Urulathanni, and 13 trees of *S. laetum* and 18 trees of *S. mundagam* in five populations each in Wayanad district to record the floral visitors and their pollination efficiencies. The populations in Pooyamkutty and Urulathanni stood ~15 km from each other. The populations in Wayanad stood ~45 km from each other. Floral visitors and their frequency of visitation were studied by earmarking 10–15 flowers per tree on focal trees in different populations and recording the number of visits of each visitor, duration of their visit and their contact with the anthers and the stigma of the marked flowers. The observations of insects were made by sitting 1–3 m away from the marked flowers. The visitation details of the birds were

Table 1. Flower morphological and functional traits in the four *Syzygium* species investigated in the present study

Variable	<i>S. heyneanum</i>	<i>S. travancoricum</i>	<i>S. laetum</i>	<i>S. mundagam</i>
Length of flower (cm)	0.99 ± 0.005	0.43 ± 0.008	5.64 ± 0.03	3.71 ± 0.02
Width of flower (cm)	1.06 ± 0.13	0.3 ± 0	6.03 ± 0.03	4.38 ± 0.03
Style length (cm)	0.72 ± 0.007	0.2 ± 0	4.49 ± 0.01	2.48 ± 0.03
Ovary length (cm)	0.4 ± <0.00005	0.36 ± 0.009	1.15 ± 0.04	1.23 ± 0.008
Number of stamens	97.46 ± 1.99	50.87 ± 0.82	376.5 ± 4.97	789.09 ± 4.34
Number of pollen grains/anther	816.06 ± 32.42	394.8 ± 16.07	372 ± 19.94	380.34 ± 26.04
Number of flowers/inflorescence	61.78 ± 2.40	73.33 ± 3.28	3.24 ± 1.34	10.29 ± 0.35
Length of stamen (inner whorl) (cm)	0.68 ± 0.06	0.27 ± 0.008	4.36 ± 0.19	3.48 ± 0.27
Length of stamen (outer whorl) (cm)	0.38 ± 0.07	0.1 ± 0	3.57 ± 0.33	2.22 ± 0.05
Length of petal (cm)	Calyptra	Calyptra	1.23 ± 0.02	1.21 ± 0.02
Width of petals (cm)	Calyptra	Calyptra	1.12 ± 0.01	1.39 ± 0.01
Longevity of flower (days) ^A	4	2	5	3
Nectar quantity (µL)	11.06 ± 0.8	C	22.25 ± 0.27	88.42 ± 1.6
Nectar sugar (nm)	3.26 ± 0.61	C	12.03 ± 0.24	10.7 ± 0.24
Flower type	Erect	Erect	Pendant	Pendant ^B
Flower colour	Creamy White	Creamy White	Maroon	White

^AWhile stigma remain fresh.

^BSometimes positioned laterally depending on the orientation of the branch.

^CNot enough nectar in the hypanthium to be measured by capillaries, but trace amount of nectar was present in the soft spongy nectary which the visitors were observed harvesting.



Fig. 1. Flowers of (a) *Syzygium mundagam*, (b) *Syzygium laetum*, (c) *Syzygium travancoricum*, and (d) *Syzygium heyneanum*.

observed using a pair of binoculars from a distance by sitting behind neighbouring bushes or on branches of neighbouring trees. A total of 326 h of visitor observations were made (102 h (9 days) for *S. laetum*, 79 h (8 days) for *S. travancoricum*, 82 h (8 days) for *S. heyneanum* and 63 h (6 days) for *S. mundagam*). Altogether, we spent 139 h in the early morning (between 02:30 and 06:30 hours) and late evening (between 17:50 and 23:30 hours) to observe nocturnal pollinators (34 h (6 days) for *S. laetum*; 51 h (8 days) for *S. mundagam*; 26 h (4 days) for *S. travancoricum*; and 28 h (5 days) for *S. heyneanum*).

Effective pollinators and their efficiency were studied, for the most frequent visitors, on the basis of pollen transfer to the stigma and induction of fruit set following the visit exclusively by an identified visitor to the virgin flower. For this, flower buds were bagged before they opened and the bags were removed in the early morning and were kept under constant observation. After the visit of a specific visitor, one set of flowers was excised and used to confirm the presence of pollen on the stigma under a stereo-zoom microscope and another set of flowers were re-bagged and monitored for fruit set. When non-focal visitors approached the flowers under observation, they were netted before landing on the flowers and later released at a different spot.

Possibility of wind pollination in some species that produced enormous amount of powdery pollen was studied by hanging white petroleum jelly-coated slides, both horizontally and vertically adjacent to the flowers. The slides were exposed for 24 h and observed under a microscope to check the presence of pollen. When present, the number of pollen grains/cm² was counted. Emasculated flowers were also caged with bags made up of mosquito net (that permitted wind borne pollen but not any insects) and monitored for fruit set.

Efficiency of open pollination was studied on the basis of the presence of pollen grains on the stigmas of randomly collected flowers after initiation of senescence and also on the basis of fruit set in tagged flowers. Further, to check nocturnal pollination in the flowers of *S. laetum* and *S. mundagam*, which possess floral traits to attract both diurnal and nocturnal visitors, we bagged flower buds in the previous evening, before anthesis, and opened one set of flowers in the morning and re-bagged before dusk ($n=44$, *S. laetum* and $n=41$, *S. mundagam*); we exposed the second set of the bagged flowers in the evening and re-bagged before dawn the next day ($n=32$, *S. laetum* and $n=28$, *S. mundagam*). These flowers were tagged and monitored for fruit set.

To study the breeding system, manual self- and cross-pollinations of emasculated flowers were carried out and bagged. The bags were removed after the flowers started to senesce and the flowers were monitored until their abscission or fruit set.

Statistical analyses

Pollination efficiencies of individual flowers were used as units of analyses. Thus, for all analyses, we determined percent pollination efficiency across pollination experiments for each plant species. The fruit set was considered as the measure of pollination efficiency throughout the study unless mentioned otherwise in the results. We used binomial test to compare proportions (prop.test) to analyse the results of pollination efficiency of different experiments. We plotted four species of *Syzygium* on flower morphological and reproductive traits in a non-metric multidimensional plot (NMDS) to understand whether the species form cryptic groups. The analysis was performed using the R packages MASS and vegan. All statistical analyses were performed in R ver. 3.2.3 (R Core Team 2014).

Results

Flower morphology

On non-metric multidimensional plot, the four species of *Syzygium* formed two groups based on the floral traits (Fig. 2). *S. heyneanum* and *S. travancoricum* formed one group that produced small erect flowers. *S. laetum* and *S. mundagam* formed the second group with larger pendant flowers. Except for *S. laetum*, the other three species produced white or creamy-white flowers. The two species groups differed significantly on all morphological traits investigated in the present study, but the species within groups did not differ on any of these traits (Table 1).

Pollinators and their efficiency

The number of animal visitors to the flowers of the four species varied from three (*S. laetum*) to 23 species (*S. heyneanum*). The flowers of *S. heyneanum* and *S. travancoricum*, which formed one group, attracted only insect species. The predominant visitors to the species that fell in the second group were the birds. Although we spent considerable amount of time both in late evening and early morning hours, we did not encounter any visitors during the night on flowers of any of the *Syzygium* species investigated in the present study.

Out of the 23 species of insects that visited *S. heyneanum* flowers, only four were frequent (*Apis cerana* (1 visit per flower h^{-1}); one species of halictid bee (0.6 visits per flower h^{-1}); an unidentified *Trigona* species (0.2 visits per flower h^{-1}), and *Apis dorsata* (0.2 visits per flower h^{-1}), and their pollination efficiency did not vary significantly between them (prop. test, $\chi^2 = 5.5$, d.f. = 3, $P = 0.13$) (Table 2). *S. travancoricum* received frequent visits of a syrphid fly (1.22 visits per flower h^{-1}), two species of Muscoid flies (0.47 and 0.44 visits per flower h^{-1}), a halictid bee (0.98 visits per flower h^{-1}), a honey bee (*A. cerana*) (0.42 visits per flower h^{-1}), and a stingless bee (*Trigona iridipennis*) (0.39 per flower h^{-1}) and infrequent visits (<0.005 visits per flower h^{-1}) of two species of wasps and three species

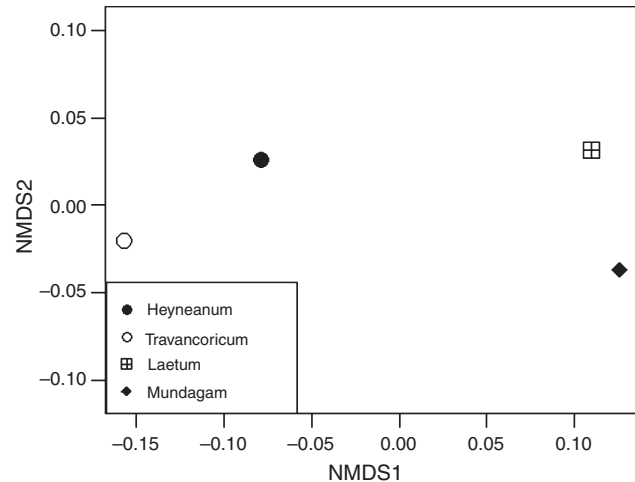


Fig. 2. Nonmetric multidimensional scaling shows distinct species groups in the four species of *Syzygium* investigated in the present study based on the flower morphological and reproductive functional traits.

Table 2. Pollination efficiency, on the basis of % fruit set, of the most frequent and efficient pollinator species of four *Syzygium* species

Values in parentheses represent the number of flowers monitored to study fruit set

Pollinator species	<i>S. heyneanum</i>	<i>S. travancoricum</i>	<i>S. laetum</i>	<i>S. mundagam</i>
<i>Apis dorsata</i>	24.3 (37)	–	–	–
<i>Apis cerana</i>	15.2 (46)	28.32 (39)	–	–
Bee-1	7.1 (14)	–	–	–
Halictid Bee	32.4 (37)	60.41 (48)	–	–
Syrphid fly	–	51.8 (27)	23.1 (39)	–
Small Sunbird	–	–	56.25 (32)	52 (77)
Wind	–	–	34.31 (102)	14.5 (394)

of butterflies. Only three of the visitors – syrphid fly, the halictid bee, and *A. cerana* – turned out to be the effective pollinators. The pollination efficiency of *A. cerana* was significantly lower than the other two relatively smaller sized species (prop.test, $\chi^2 = 9.24$, d.f. = 2, $P = 0.009$; Table 2), suggesting that in *S. travancoricum*, foraging behaviour rather than the body size seems to be a predictor of pollination efficiency. Although the other two species of flies and *T. iridipennis* were frequent visitors to the flowers, they foraged nectar and pollen (*T. iridipennis*) by landing on the periphery of the flowers and stayed away from the stigma. The stigma of none of the flowers after the first visitation of these species ($n = 18$ for fly-1, $n = 22$ for fly-2 and $n = 32$ for *T. iridipennis*) had pollen grains.

Among the four *Syzygium* species investigated in the present study, the maroon pendent flowers of *S. laetum* received the lowest number of floral visitors, one species of hover fly (Syrphidae) and two species of birds, small sunbird (*Leptocoma minima*) and oriental white eye (*Zosterops palpebrosus*). *S. laetum*, relative to the other three species, has the lowest number of flowers per inflorescence (Table 1). The small sunbird visited the flowering plants at regular intervals; 4.5 visits per tree day $^{-1}$ (± 2.99 s.d.; $n = 15$ days) were recorded.

The bird also visited almost all the flowers that bloomed on a given tree during each of the visitation bouts. The oriental white eye was very infrequent visitor to the flowers of *S. laetum* (0.1 visits per tree⁻¹; $n = 15$ days). Both the species of birds landed on a branch closer to the flower or the peduncle of the flower and inserted the beak through the side of the pendent slightly tubular wide nectar cup to harvest the nectar. Thus, the bird never came in contact with the anthers or the stigma. However, the flowers get disturbed during nectar harvesting and are likely to release large amount of pollen into the air suggesting the possibility of wind pollination. We have shown the presence of considerable number of pollen grains in the air by exposing the slides (see below). About 56% of the bird-visited flowers ($n = 32$) that were selected randomly all over the trees were pollinated (Table 2), and all fruits developed one seed. The syrphid fly also pollinated 23.1% of the flowers that it visited ($n = 39$). But, its efficiency was significantly lower than the efficiency of the bird (prop.test, $\chi^2 = 6.86$, d.f. = 1, $P = 0.008$).

In *S. mundagam*, another pendent, white, sweet-scented flower bearing species, the flowers received the visits of eight species, two species of birds – small sunbird (*Leptocoma minima*) and small green barbet (*Psilopogon viridis*) – three species of bees, and three species of butterflies. The small sunbird and small green barbet were the frequent visitors (0.34 and 0.21 visits per flower h⁻¹ respectively) than the honey bee (0.06 visits per flower h⁻¹). Although the two bird species visited the flowers, we could examine the pollination efficiency of only the small sunbird in a reasonable number of flowers. The small sunbird proved to be the most effective pollinator (52%, $n = 77$) (Table 2). Small green barbet was visiting the flowers near the top of the canopy that were inaccessible and hence the pollinator efficiency could not be determined. The visits of *A. cerana* did not transfer pollen grains to the stigma as they used to enter the flower away from the style and the stigma. The stigmas examined after the first visit of *A. cerana* showed no pollen grains ($n = 23$). The other five species of insect visitors were not frequent to the flowers, and we did not collect any evidence for their role in transferring pollen to the stigma. Although some bats were observed in the sites where *S. laetum* and *S. mundagam* were growing, none of them visited the flowers during the observation period.

The micro-slides hung adjacent to the flowering branches of *S. laetum* and *S. mundagam* collected pollen grains. An average of 34.9 ± 2.3 (s.e.; $n = 32$) and 36.66 ± 2.65 (s.e.; $n = 65$) pollen grains cm², respectively, were counted on exposed slides. Further evidence for wind pollination came from bagging experiments. Emasculated flowers enclosed by pollen-proof bags yielded 0% fruit set in both the species. However, the net bagged emasculated flowers that permit air borne pollen inside the bag yielded 34.31% ($n = 102$) and 14.5% ($n = 394$) fruit set in *S. laetum* and *S. mundagam* respectively (Table 2). It is unclear whether the pollen grains are released to the air normally or only as a result of disturbance caused by birds to the flowers or due to both.

Breeding system

Results of different pollination experiments and breeding systems are presented in Table 3. Limited fruit set was recorded in emasculated and bagged flowers in three of the species. This may indicate low level of apomixis in these species. Geitonogamous pollinations resulted in fruit set in all the four species. Xenogamous pollinations significantly improved fruit set over that of geitonogamous pollinations in *S. laetum* (prop. test $\chi^2 = 7.32$, d.f. = 1, $P = 0.006$) and *S. mundagam* (prop. test $\chi^2 = 6.31$, d.f. = 1, $P = 0.01$), the two bird pollinated species, indicating their partial self-incompatibility. However, there was no difference between geitonogamous and xenogamous pollinations in *S. heyneanum* (prop. test $\chi^2 = 0.02$, d.f. = 1, $P = 0.8$) and *S. travancoricum* (prop. test $\chi^2 = 0.01$, d.f. = 1, $P = 0.9$) (Table 3).

Open pollination efficiency

The result of open pollination efficiency (fruit set) was comparable to manual pollinations in *S. heyneanum* (prop. test: $\chi^2 = 1.62$, d.f. = 2, $P = 0.4$) indicating absence of pollination limitation. It was significantly lower in *S. travancoricum* (prop. test: $\chi^2 = 6.90$, d.f. = 2, $P = 0.03$), *S. laetum* (prop. test: $\chi^2 = 14.13$, d.f. = 2, $P = 0.0008$), and *S. mundagam* (prop. test: $\chi^2 = 16.64$, d.f. = 2, $P = 0.002$) (Table 3) indicating pollination deficiency in these species under field conditions. The diurnal open pollination efficiency was significantly greater than the nocturnal open pollination efficiency in *S. laetum* (prop. test:

Table 3. Breeding system and pollination efficiency (based on % fruit set) under field conditions in the four *Syzygium* species investigated in the present study

Numbers in parenthesis represent number of flowers used for experiment

Treatment	<i>S. heyneanum</i>	<i>S. travancoricum</i>	<i>S. laetum</i>	<i>S. mundagam</i>
Apomixis ^A	7.6 (79)	4.96 (141)	0 (94)	12.5 (96)
Autogamy ^B	1.04 (385)	5.9 (220)	9.1 (77)	11.0 (190)
Geitonogamy ^C	37.91 (182)	52.12 (94)	42.98 (114)	36.1 (114)
Xenogamy ^D	36.92 (130)	50 (84)	62.03 (108)	51.7 (180)
Open pollination	31.30 (115)	41.26 (4011)	35.26 (346)	56.7 (611)
Open pollination (Day)	–	–	53.6 (41)	47.7 (44)
Open pollination (Night)	–	–	21.4 (28)	18.7 (32)

^AFlowers were bagged after removing the anthers.

^BFlowers were bagged without removing the anthers.

^CPollinated from pollen collected from the same plant.

^DPollinated from pollen collected from other plant.

$\chi^2 = 5.9$, d.f. = 1, $P = 0.01$) and *S. mundagam* (prop.test: $\chi^2 = 5.6$, d.f. = 1, $P = 0.01$) – the two species that have floral traits to attract nocturnal vertebrate visitors. In both the species, the nocturnal pollination efficiency was at par or lesser than the wind pollination efficiency (prop.test: $\chi^2 = 1.14$, d.f. = 1, $P = 0.28$ (*S. laetum*); $\chi^2 = 0.15$, d.f. = 1, $P = 0.6$ (*S. mundagam*), indicating absence of nocturnal visitors to the flowers.

Discussion

In this study, apart from describing floral traits and documenting all the floral visitors, we have distinguished effective pollinators from casual visitors, determined pollination efficiency of the effective pollinators, and the breeding system of the four endemic species of *Syzygium* – *S. laetum*, *S. mundagam*, *S. heyneanum* and *S. travancoricum*.

Floral traits and pollination ecology

Floral traits of the flowers are important predictors of plant-pollinator interactions (Faegri and van der Pijl 1979; Neal *et al.* 1998; Sakai *et al.* 1999; Garibaldi *et al.* 2015; Crea *et al.* 2016). In the present study, the floral traits placed the four species of *Syzygium* in two groups, one group (*S. laetum* and *S. mundagam*) adapted to attract and exploit vertebrates, largely birds; and the other group (*S. heyneanum* and *S. travancoricum*) adapted to attract and exploit insect pollinators. Copious amount of nectar with a low concentration of sugar is a predictor of visits of birds and bats to the flowers of *S. laetum* and *S. mundagam*. We found that in *S. laetum*, birds harvest nectar through the sides of the pendant flowers using a long beak without coming in direct contact with the anthers. Since wind pollination in *S. laetum* was high, we speculate that the bird visits to the flowers might enhance the number of pollen grains in the air. Although the flowers of *S. laetum* were pendant, not all were drooping at 90° angle (Fig. 1b). So we assume that the pointed stigma of the flowers oriented in different directions were able to capture the wind-borne pollen grains; however, the exact mechanism of pollen deposition on drooping stigma needs to be studied. There are several examples in which both animals and wind bring about pollination; the syndrome has been termed ambophily (Sacchi and Price 1988; Vroege and Stelleman 1990; Dafni *et al.* 2012). In *S. laetum* although we could demonstrate wind pollination, we have not been able to identify any legitimate animal pollinator including the minute thrips. However, the pollination in *S. mundagam* is ambophilous; the birds as well as the wind are involved in pollination, although the efficacy of wind in terms of fruit set was only 14.5%. Both these species of *Syzygium* did not attract bats. One possible reason for lack of bat visits might be the presence of densely arranged, cluttered stamens that do not allow the wide mouthed bats to reach the nectar (Boulter *et al.* 2005).

S. heyneanum and *S. travancoricum* produce cream coloured flowers and the amount of nectar produced by the flowers was comparatively limited in quantity with high sugar concentration when compared with the other two species, characteristic of insect pollination syndrome. The stamens in these species were also arranged on the margins of the hypanthium cup exposing the nectary and allowing the visitors to easily access the nectar (see Fig. 2c, d). Insects are the predominant visitors of cream

coloured flowers (Boulter *et al.* 2005). As predicted, a total of 11 and 23 species of insects (bees, flies, beetles and butterflies) were attracted to the flowers of *S. heyneanum* and *S. travancoricum*. In contrast, *S. mundagam* flowers, although cream coloured, attracted only six species of insect visitors. The crowded staminal filaments might have limited many insect species from accessing the nectar. The insect visits were highly infrequent when compared with the birds. *S. laetum* flowers attracted no insect pollinators. Among the four *Syzygium* species studied, only *S. laetum* had maroon flowers, a typical feature of bird-pollinated flowers.

Many floral characters that include late evening or nocturnal anthesis, white or creamy flowers and floral longevity lasting for 2–5 days, suggest that all the species studied except *S. laetum* are likely to be adapted for diurnal as well as nocturnal visitors. Although we spent a considerable amount of time in late evenings and early mornings to monitor nocturnal visitors, no visitors were recorded. Further, the pollination efficiency of flowers exposed to nocturnal pollination was not significantly different from that of the diurnal wind pollination confirming absence of nocturnal visitors to the flowers.

Pollinators and pollination efficiency

A major outcome of the present investigation is the demonstration that the number of effective pollinator species is much less when compared with the floral visitor guild. Of the 11 species of insect visitors in *S. travancoricum* and 23 species in *S. heyneanum* only three species in the former and four species in the latter turned out to be the effective pollinators. However, the flowers of the other two species, *S. laetum* and *S. mundagam*, attracted largely the birds. In *S. laetum*, although the bird did not bring about pollination directly, their visits appeared to facilitate wind pollination by releasing pollen from the anthers to the air by physical disturbances. In *S. mundagam*, the bird brings about pollination directly, and it may also facilitate wind pollination. It may be more appropriate to label pollination in *S. mundagam* as ambophily, although the extent of fruit set through wind pollination is much smaller than that of bird pollination (Friedman 2011). Very few investigations have studied about the pollination efficiency of birds; for example, in *S. mamillatum*, a rare endemic species of Mauritius (Kaiser *et al.* 2008), only generalist birds have been reported to visit and pollinate the flowers.

Earlier reports on pollination biology of *Syzygium* spp. in South-east Asia and Australia on the visits of bats and moths are highly variable; some studies reported no nocturnal visitors (Lack and Kevan 1984), whereas others have reported the visits of both bats and nocturnal moths (Crome and Irvine 1986; Boulter *et al.* 2005). However, pollination efficiencies of nocturnal visitors, especially bats have not been categorically documented (Boulter *et al.* 2005) or documented as an unimportant (Hopper 1980) or even important pollinators (Crome and Irvine 1986). It is not unusual that the flowers having traits to attract both day and night pollinators are mostly pollinated by diurnal pollinators as we have observed in our present study. For instance, the flowers of *Luebea seemanii* have floral traits adapted for nocturnal pollination, but pollination is performed largely by diurnal pollinators

(Haber and Frankie 1982). Therefore, predicting pollinators from the floral traits in plants that have both day and night visitors in flowers might have limitations (Crome and Irvine 1986).

Only very few investigators have been so far made to study the reproductive biology of Indian *Syzygium* species growing in the Eastern Ghats and in Botanical Gardens and parks (Raju *et al.* 2014; Geethika and Sabu 2017; Varghese and Sreekala 2017). Although bees, ants, moths, butterflies and birds have been reported to be the floral visitors, none of the earlier studies investigated pollination efficiency of the visitors. These studies also suggest that bats are less frequently attracted to *Syzygium* flowers, although they are the predominant frugivores of *Syzygium* fruits (Ganesh 1996). The bats we encountered amidst the populations of *S. mundagam* and *S. laetum*, did not visit the flowers, but they could be the insectivores. Since these *Syzygium* species produce non-pulpy fruits, bats are unlikely to be the frugivores of the developing fruits. Further studies using camera surveillance methods might be able to throw some light on the nocturnal pollinators of *Syzygium* species of India.

Ants are often reported as nectar foragers of *Syzygium* spp. in their native ranges in India and Australia (Boulter *et al.* 2005; Williams and Adam 2010; Kuriakose *et al.* 2018). We have recorded both *Oecophyla smaragdina* and *Technomyrmex albipes* on the flowers of *S. occidentale* (Kuriakose *et al.* 2018). Although the former ant species preyed on the legitimate pollinators (see also Rodríguez-Gironés *et al.* 2013), the latter foraged nectar and was an effective pollinator (Kuriakose *et al.* 2018). Although the trees of *S. heyneanum* are growing in the same location as of *S. occidentale*, only *O. smaragdina* infrequently visited the flowers of *S. heyneanum*, but did not bring about pollination and *T. albipes* never visited the flowers of *S. heyneanum*. The role of ant attractants and deterrents in the flowers of these two species might throw some light on the causes for the absence of ants in the flowers of *S. heyneanum* (Raine *et al.* 2002).

Breeding system

We recorded some fruit set in emasculated and bagged flowers in three of the species investigated. Many species of *Syzygium* show apomixis (Lughada and Proenca 1996). Further studies are needed to confirm and ascertain the type and extent of apomixis in these species. Two of the species investigated, *S. travancoricum* and *S. heyneanum*, are fully self-compatible; there was no significant difference in fruit set between self- and cross-pollinated flowers. The other two species, *S. laetum* and *S. mundagam*, are partially self-compatible. In these species, the extent of fruit set after self-pollination, though considerable, was significantly less than after cross-pollination. Earlier studies on the breeding system in many other *Syzygium* species have also reported the species to be fully or partially self-compatible (Hopper 1980; Lack and Kevan 1984; Crome and Irvine 1986; Payne 1991; Lughada and Proenca 1996; Boulter *et al.* 2005).

Self-incompatibility (SI) is an important outbreeding device and is genetically controlled. Most of the SI species have been reported to be leaky and produce seeds to different degrees upon self-pollination (de Nettancourt 2001; Goodwillie *et al.* 2005;

Shivanna 2014). Full or partial self-compatibility seems to be the most successful breeding strategy, particularly in tropical tree species such as *Syzygium*, in which the plants are sparsely distributed and the chances of interplant movements of pollinators, particularly insects, are limited (Bawa *et al.* 1985; Shivanna 2014). In *S. tierneyanum*, for example, over 99% of inter-flower movements of birds and hawkmoths have been reported to be within the same plant (Hopper 1980). Unlike strictly self-incompatible systems, self-compatible and partial self-compatible breeding systems enable species to set seed even in the absence of outcross pollen. The prevalence of partial self-compatibility has so far been underestimated and future investigations on the breeding systems should be given greater importance to the operation and significance of partial self-compatibility.

In conclusion, the present study covered the details of pollination ecology of four endemic species of *Syzygium* in the Western Ghats biodiversity hotspot. Based on the floral traits, the four species fall into two groups, as reflected by their mode of pollination: *S. heyneanum* and *S. travancoricum* group and *S. mundagam* and *S. laetum* group. The former two species were pollinated by insects and the latter two were pollinated largely by birds. The number of effective pollinators, particularly of insects, turned out to be much less when compared with the number of floral visitors. There was no evidence for the visitation by specialist pollinators such as moths or bats to the flowers of any of the studied species.

Conflicts of interest

The authors declare no conflicts of interests.

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