EXPERIMENTAL POLLINATOR EXCLUSION OF *Sonneratia alba* SUGGESTS BATS ARE MORE IMPORTANT POLLINATOR AGENTS THAN MOTHS

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Abstract: *Sonneratia alba* is known to be pollinated by several nocturnal pollinating agents including bats and moths. However, the relative contribution of these pollinators in reproductive success of this mangrove species is unknown. Here, we evaluated the importance of bats and moths as pollinators to *S. alba* flowers by conducting pollinating exclusion experiments in the Setiu mangrove area, Terengganu, Peninsular Malaysia. *Sonneratia alba* was found to be not completely self-incompatible, thus the roles of pollinating agents are still vital. Pollination of *S. alba* by moths produced high quality fruits and seeds. The highest fruit set and high quality fruits and seeds are recorded in open pollination and cross-pollination, respectively. However, our preliminary findings suggest that bats are relatively more important pollinators for *S. alba* than moths. Therefore the conservation of bats is vital to ensure the healthy population of the *Sonneratia* trees, which indirectly will contribute to survival of the mangrove habitats.

Keywords: Bats, fruit quality, mangrove apple, pollinating agents, Setiu Wetlands

Introduction

The mutualistic relationships between pollinators and flowering plants are highly variable (Fenster *et al.,* 2004) and are not altruistic (Willmer, 2011). For animals, pollination of the flowers that they forage at is almost always an irrelevant by-product. For plants however, this interaction may result in a positive effect on their reproductive success, and therefore is important in terms of contributing to their population viability and functioning of their ecosystems.

Interactions in nocturnal pollination have been neglected due to complications of conducting research at night. In some cases where flowers were visited by diurnal and nocturnal pollinators, visitations by nocturnal pollinators accounted for most of the plants' successful pollination (Sazima & Sazima, 1978; Arizaga *et al.,* 2000; Ibarra-Cerdena *et al.,* 2005; Martinell *et al.,* 2010), indicating the significant contribution made by nocturnal visitors for plant fitness. Previous studies show that bats, rodents and moths are among the most important nocturnal pollinators (Baker, 1961; Pellmyr *et al.,* 1996; Fleming & Holland, 1998; Slauson, 2000; Fleming & Kress, 2011).

Sonneratia alba is a mangrove species with flowers of classic bat-adapted traits including large white flowers that are often strongly scented, flowers that last for a single night, are bell-shaped and presented in exposed positions (Faegri & van der Pijil, 1979). However, hawk moths were reported as primary pollinators in the mangroves of Australia (Primack *et al.,* 1981), where the flowers and moths are matched in size. Nor Zalipah *et al.* (2016) recently reported that bats are important pollinators for *S. alba* in Peninsular Malaysia for depositing sufficient number of con-specific pollen grains to fertilise all the ovules of *S. alba* flowers. However, the former researchers do not consider pollen deposition to the stigma of the

flowers from other nocturnal and crepuscular flower visitors such as moth and bees.

Here, we investigate the relative importance of each flower visitors (bats & insects) as pollinating agents of *S. alba* flowers using pollinator exclusion experiments. We hypothesise that bats are relatively more important pollinators of *S. alba* than other nocturnal insects such as moths.

Materials and Methods
Site description

Setiu is located in the northeast of Peninsular Malaysia in the state of Terengganu (5° 40'N 102° 43'E). It is located about 60km from the city of Kuala Terengganu. From the total area of 2400ha of mangrove forest in Terengganu, 470ha is located in the district of Setiu (Mohd Lokman & Sulong, 2001). The mangrove areas of Setiu consisted mainly of nipa palm, *Nypa fruticans* (Arecaceae) and extensive stands of the mangrove, genus *Rhizophora* (Rhizophoraceae) (Nakisah & Fauziah, 2003). Exclusion experiments were conducted at Setiu Lagoon (5° 41'06.79" N 102° 42'29.92" E), where *S. alba* trees grow in the more saline seaward site of the mangrove forests.

Exclusion Experiments

Pollination exclusion experiments of *S. alba* were conducted for five months between June and October 2015. A total of 224 mature flowers from seven trees (1-6 flowers per treatment for each tree) were pollinated using six treatments: (1) open pollination (OP): all potential pollinators were allowed to access the flowers, (2) automatic autogamy (AA): all pollinators were excluded by bagging flowers before anthesis occurred, (3) insect pollination (IP): inflorescences were covered with plastic net (16mm mesh size) allowing access by insects but not bats, (4) hand-cross pollination (CP): anthers were removed before anthesis and stigmas were rubbed directly to anthers and

bagged, (5) facilitated autogamy (FA): stigmas were rubbed on anthers from the same tree and bagged, and (6) emasculation pollination (EP): the anthers were removed by sharp scissor before anthesis and the flowers were left uncovered allowing access by pollinators. For AA, CP and FA treatments, bagging conducted using transparent polythene bags (30cm x 40cm) with small holes to allow only air circulation but not insects and/or bats to visit the flowers. The bags were removed in the evening after blooming nights to prevent early morning flower visitors including bees and birds.

Observations of fruit set were conducted every week and mature fruits were checked after 40 days of pollination. The fruit is considered mature (ripe) when it became soft and easily separated from the calyx and produced sourly smell. Mature fruits were collected, measured and weighed. The seeds were then separated from the fruits and counted to assess the pollination success in each treatment. Fruit set index was calculated by dividing the total number mature fruits to the total flowers used for each treatment. The Index of Self Incompatibility (ISI) was calculated following Bullock (1985) by dividing the fruit set index of AA to CP. Friedman's ANOVA for repeated measures and multiple comparisons (step-down method) were conducted to test for differences in fruit set among treatments (tree as repeated measure). Kruskal-Wallis test and multiple comparisons (step-down method) were used to compare the fruits and seeds produced to locate the differences between the treatments. All the analyses were conducted by using SPSS Statistics ver. 22 (Chicago, USA).

Results and Discussion

From the total flowers used in the experiments, 25.9% developed into mature fruits (58 mature fruits) in all six treatments. The highest fruit set was observed in OP treatment (52.4%), while treatment EP recorded the lowest fruit set with only 4.8% (Figure 1).

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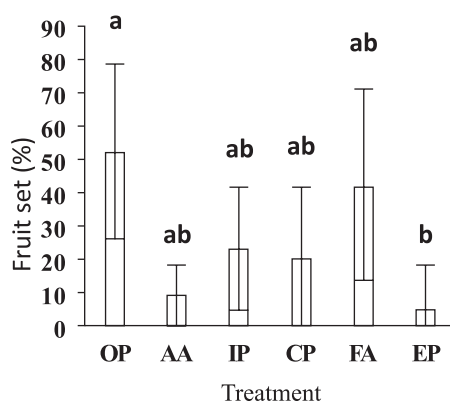*Crustacean Resources for Livelihood Enhancement: 7-13*

Figure 1: Fruit sets of each pollination treatment. OP = Open pollination, AA = Automatic autogamy, IP = Insect pollination, CP = Hand-cross-pollination, FA = Facilitated autogamy, EP = Emasculation pollination. Error bars indicate SD. Different letters indicate significant differences from one another (multiple comparison following significant result from Kruskal-Wallis test).

Pollination treatment	Fruit mass (g)	Fruit volume (cm³)	Seed number
Open pollination (OP), N = 22	7.52 +1.97[a]	12.96 +3.64	32.14 +13.30
Automatic autogamy (AA), N = 4	8.85 + 0.59[ab]	13.22 +2.68	33.00 +17.32
Insect pollination (IP), N = 9	11.94 +4.62[b]	14.10 +4.96	41.22 +19.21
Cross pollination (CP), N = 8	12.13 +4.23[b]	13.36 +2.37	40.38 +16.07
Facilitated autogamy (FA), N = 13	8.78 +3.16[ab]	11.97 +2.48	40.31 +11.11
Emasculation pollination (EP), N = 2	5.40 + 0.28[a]	7.80 + 0.20	15.00 +5.66

Table 1. Fruit and seed produced in each pollination treatment. Mean +SD are used throughout. Different letters indicate significant differences from one another (multiple comparison following significant result from Kruskal-Wallis test).

"Friedman's ANOVA showed a statistically significant difference in fruit set among treatments (χ^2=18.807, df=5, P=0.002). Fruit volume, fruit mass and seed number recorded for each treatment showed that only fruit mass is significantly different between treatments (Kruskal-Wallis test, H=15.206, df=5, P=0.010). Pollination by insects (IP treatment) resulted in the largest fruit in terms of size and the most seed number produced, but CP treatment produced the heaviest fruit (Table 1).

The Index of Self Incompatibility showed that *S. alba* is self-incompatible with ISI of 0.34. Not only the highest fruit set was recorded in OP treatment (all pollinators where allowed to access the flowers including bats and insects), IP treatment (only insects were allowed to access the flowers) also resulted in high quality of fruits and seeds, indicating the importance of pollen vectors in pollination of this self-incompatible species. As *S. alba* is known to be visited by nocturnal pollinators such as bats (Nor Zalipah *et al*., 2016) and moths (Primack *et al.,* 1981; Nor Zalipah, 2014), and as crepuscular visitation by hymenopteran insects did not contribute to successful pollination (Nor Zalipah, 2014), we assumed that IP treatment resulted in fertilisation of ovules by moths while OP treatment showed the effect of bat visitation

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to the flowers. When bats were excluded from visiting *S. alba* flowers, fruit set was reduced to more than half, which was only 23.1% in IP compared to 52.4% in OP where bats were allowed to assess the flowers. This result supports our hypothesis that bats are relatively more important pollinators for *S. alba* than moths.

Self-incompatibility in bat-pollinated plants is common (Valiente-Banuet *et al.,* 1997; Ibarra-Cerdena *et al.,* 2005; Arias-Coyotl *et al.,* 2006; Bumrungsri *et al.,* 2008; Srithongchuay *et al.,* 2008; Bumrungsri *et al.,* 2009) and these plants showed a specialised pollination system in which they rely heavily on nectar-feeding bats for pollination. In this specialised pollination system, the floral traits function to facilitate pollination by the primary pollinator and restrict other potential pollinators (Muchhala, 2006). Therefore, despite being visited by a range of taxa, flowers are actually pollinated by only a few functional groups of visitors (Schemske & Horvitz, 1984; Bawa *et al.,* 1985; Johnson & Steiner, 2000), and visits by other taxa often involve theft (nectar & pollen robbers) rather than pollination (Arizaga *et al,.* 2000; Muchhala, 2003). The lowest fruit set recorded in EP treatment where anthers were removed to eliminate self-pollination also might indicate the importance of pollen as a reward in this plant-pollinator interaction (Nor Zalipah, 2014).

High fruit set in FA treatment (about 42%), where stigmas were hand-pollinated with pollen from the flower of the same tree, indicates that *S. alba* is not completely self-incompatible, under scoring the role of bats as important pollen vectors for the plant's fitness. Bats are important pollinating agents, not only because they are known to travel long distances to forage for food (Start & Marshall, 1976), but also transport greater amounts of pollen compared to birds (Law & Lean, 1999; Muchhala, 2006; Muchhala, 2007). The capacity to move high pollen loads over relatively long distances may help to promote out-crossing

in bat-pollinated plant species. Bats usually deposited con-specific pollen grains of several different genotypes (different potential fathers) onto the stigmas of flowers they visited and therefore produced greater genetic variability progeny than pollination by other pollinators (Fuchs *et al,.* 2003; Nassar *et al.,* 2003). High quality of fruits and seeds produced from CP where flowers were hand-cross pollinated with pollen from different tree showed that cross-pollination is important in breeding system of *S. alba* as pollen quality may influence seed set (Crome & Irvine, 1986). Lower fruit sets recorded for CP treatment as compared to OP in this study further confirm the importance of bats in supplying high quality of pollen to *S. alba* flowers for successful regeneration of this mangrove tree species.

Conclusion

Pollination exclusion experiments of *S. alba* show that this species is self-incompatible, thus requiring pollinating agents for pollination. Pollination by bats (from OP treatment) produced the highest fruit set, while pollination by insects (IP treatment) such as moths resulted in high quality of fruits and seeds. As self-pollen also produced fruits and seeds (from AA and FA treatments), *S. alba* however is not highly or completely self-incompatible. Nevertheless, CP treatment produces high quality of fruits and seeds as compared to AA and FA treatments. Therefore, we conclude that bats are relatively more important pollinators for *S. alba* than insects by transporting high quality of pollen grains to the flowers for pollination, which then increase plant fitness. As reproductive success is critical in long-term viability of mangroves, our preliminary study indicates that conserving bat populations will indirectly help in conservation efforts of the degrading mangrove habitats.

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