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The potential significance of nectar-feeding bats as pollinators of *Sonneratia* species based on pollen load

Abstract

In mangrove communities, bats are believed to be the major pollinators of the mangrove trees from the genus *Sonneratia*, where they visit the flowers to forage for food such as nectar and pollen, in turn pollinating the flowers. However, these bats visit numerous plant species in a feeding night, resulting in mixed pollen loads on their bodies that can potentially affect their role as effective pollinators. In this chapter, I investigated several areas of potential for bats to be effective pollinators. I determined the number of pollen grains that bats carried, and the number of pollen grains they transferred to the stigmata while visiting *Sonneratia* flowers. I also quantified visitation rates of bats to the *Sonneratia* flowers. Despite carrying several pollen types, conspecific *Sonneratia* pollen grains (i.e. grains from the mangrove species that the bats were caught at) were the major pollen grain types carried by *Eonycteris spelaea* (the most abundant bat caught in the study area), and hence the bats are likely to be important pollinators for the three species of mangrove trees in the study area. Even though *Sonneratia caseolaris* flowers received a higher number of visits by bats compared to *S. alba* flowers, multiple visits to the *S. caseolaris* flowers by bats resulted in more heterospecific pollen grains (i.e. foreign grains from the mangrove species that the nets were set at) being found on the stigmata compared with the conspecific pollen grains. Reduced pollinator effectiveness during multiple visits may not reduce reproductive success of the trees however, as conspecific pollen grians usually adhere strongly to the stigmatic surface for germination For *S. alba* flowers, low numbers of conspecific pollen grains were deposited onto the stigmata after the first bat visited the flower as well as after the blooming night. I suggest that lower visitation rates to the *S. alba* flowers by bats are due to the small number of conspecific pollen grains needed for fertilising the small number of ovules. Therefore, my study indicates that bats have good potential to be effective pollinators of these mangroves.

2.1 Introduction

2.1.1 Feeding habits of plant-visiting bats

The plant-visiting bats from the family Phyllostomidae (in Neotropical regions) and Pteropodidae (in Palaeotropical regions) are mainly phytophagous, eating fruit, floral resources such as pollen and nectar, and occasionally leaves (Anthony 1988; Kunz and Diaz 1995; Lim 1970; Parry-Jones and Augee 1991; Phua and Corlett 1989; Ruby *et al.* 2000). Of these, fruit, which is often reported as the most common component of the diet of these bats (Bumrungsri *et al.* 2007; Del Vaglio *et al.* 2011; Giannini and Kalko 2004; Heithaus *et al.* 1975; Hernindez-Conrique *et al.* 1997; Korine *et al.* 1999; Picot *et al.* 2007; Tan *et al.* 1998; Thomas 1984) is generally rich in energy but deficient in protein. Therefore, frugivorous bats consume flower parts and leaves to fulfill their dietary requirements for protein (Lowry 1989; Nelson *et al.* 2005; Rajamani *et al.* 1999; Reiter and Tomaschewski 2003). Frugivorous bats also supply their nutrient needs by feeding on insects (Courts 1997; Giannini and Kalko 2004; Lim 1970; Tschapka 2004; York and Billings 2009).

Even though pollen grains are thought to be consumed accidently while foraging for nectar (Law 1992a), studies by Howell (1974) and Law (1992a; 1992b) showed that bats feed on pollen for its high protein content. The bats that feed almost exclusively on nectar showed specialised morphological adaptations for nectar feeding such as an elongated rostrum, a short digestive tract and a long specialised tongue (Harper *et al.* 2013; Mqokeli and Downs 2013). Moreover, as pollen is usually consumed together with nectar, nectar-feeding bats also have the ability to extract the nutritious content of pollen (Herrera and Del Rio 1998; Mancina *et al.* 2005). Through their feeding habits, plant-visiting bats have been identified as very active and regular visitors to many plant species, transporting abundant pollen loads from their bodies to stigmata while foraging for food from flowers (e.g. for nectar and even flower parts such

the corolla) hence functioning as pollinators (Nathan *et al.* 2009; Singaravelan and Marimuthu 2004).

2.1.2 *The role of flower-visiting bats as plant pollinating agents*

Numerous studies have quantified the role of pollinators in pollination and the consequences for plant mating systems (reviewed in Inouye *et al.* 1994; Ne'eman *et al.* 2010). The term 'pollinator effectiveness' is used to measure pollination success from a single visit by a particular pollinator, mainly from the fruit and seed sets (Mayfield *et al.* 2001; Motten 1986; Spears 1983; Young 1988). King *et al.* (2013) suggested that deposition of pollen grains on stigmata of the flowers from a single-visit is a practical measure of pollinator effectiveness, to avoid misinterpretations arising from simply quantifying visits from pollinators that may not necessarily result in pollen transfer. Fenster (1991) measured conspecific stigma pollen loads and the ratio of pollinator number to the number of flowers as indices of pollinator effectiveness. The definition of 'pollinator efficiency' can be ambiguous as different parameters have been used by different researchers to measure pollination success, including the amount of pollen collected by the pollinator and the number of pollen tubes in a style (Levin and Berube 1972), pollinator visitation frequency (Schemske and Horvitz 1984), and the percentage of stigmata touched in a series of visits by pollinators (Dafni *et al.* 1987). Inouye *et al.* (1994) therefore proposed two general models of pollination systems while Ne'eman *et al.* (2010) provided an integrated conceptual framework and methodology to eliminate the confusion around the concepts and definition, and to clarify the important parameters in pollination. In my study, the effectiveness of bats as pollinating agents was assessed from the amount of pollen deposited onto the stigmata per visit ('quality' component), together with the visitation rate ('quantity' component) following Muchhala *et al.* (2008).

Different pollinators often differ in the quantity and quality of the pollination services they provide (Fishbein and Venable 1996; Schemske and Horvitz 1984). Bats

and hummingbirds for example are similar in visitation rates, although bats are more effective pollinators as they consistently transfer greater amounts of conspecific pollen compared with birds: indeed, bats transferred almost four times as much pollen as birds in flight cage experiments (Muchhala 2007) and in the wild (Muchhala 2006). However, potentially high rates of pollen transfer to the species being visited (conspecific pollen transfer) may be reduced by the remarkably high levels of interspecific pollen transfer by bats (Muchhala 2008) as they are known to visit and forage upon a wide array of plant species (Marshall 1983).

In bat-pollinated plants, the reduction in visitation rates consequently reduced the number of pollen grains deposited on the flowers, and in turn had negative consequences on the reproductive success of the plants (Quesada *et al.* 2003). Frequent visitors however, may increase pollen transfer between flowers on the same plant and therefore result in geitonogamous crosses (Pandit and Choudhury 2001), in which pollination occurs by pollen from different flowers of the same tree and thus leads to the production of less fit offspring through inbreeding depression (de Jong *et al.* 1993). Moreover, the increased time spent by the bats foraging for particular plants may also increase the amount of pollen transferred to the flower of the same plant for geitonogamous crosses. The pollen loads of bats foraging for longer around the same trees consisted of only one or a few pollen donors, therefore reducing their effectiveness as cross-pollinating agents (Arias-Coyotl *et al.* 2006).

2.1.3 Aims of study

In this chapter, I aim to quantify the potential effectiveness of nectar-feeding bats as pollinating agents for *Sonneratia* trees. I assessed the number of pollen grains the bats carried and the number of pollen grains deposited by the bats while visiting the flowers (quality component). Quantification of pollen loads was made by collecting pollen samples from different body parts and by faecal analysis. I documented the frequency and distribution of each pollen type. I also assessed the number of pollen grains

deposited by bats on their first visit to a flower as well as the number of pollen grains deposited onto the stigmata after the blooming night for pollination. I tested the hypothesis that bats carried numerous pollen grains on their bodies and deposited sufficient conspecific pollen grains to the stigmata of the flowers for fertilisation. I recorded the bats' behaviour, frequencies and durations of their visits to the flowers to assess the quantity component of pollinator effectiveness. I tested the hypothesis that bat visitation frequencies to the flowers was associated with the number of pollen grains deposited onto the stigmata.

2.2 Methods

2.2.1 Collection of pollen from Sonneratia and other reference pollen

Pollen grains from flowering trees within 5 km of the study areas were collected to make a reference collection. Plant specimens (usually consisting of flowers, leaves and fruit) of the trees were also collected for species identification. The pollen grains sampled were mainly of mangrove species, as well as non-mangrove species that were abundant in the sampling areas. The reference collection was also supplemented by pollen of the tree species known to be pollinated by bats, collected from various sites in Terengganu. For *Sonneratia* species, pollen grains were collected from nine flowers of three different trees (three flowers per tree) selected randomly from each species for reference. Pollen grains collected were preserved in individual vials containing 75 % ethanol.

Observations of pollen grains were conducted under a light microscope (LM) at x10 and x40 magnification. Pollen grains were extracted from the vials using a micropipette (Eppendorf 1-10 µl). 5 µl of ethanol was extracted, put on a glass slide and covered with a 22 x 24 mm cover slip to spread the pollen grains for observations. A digital microscope eyepiece camera (84 mm length x 23 mm diameter, Dino-eye AM 423X, AnMo Electronics Corporation, Taiwan) with 70x magnification was attached to

the light microscope during observations to obtain a maximum magnification of x2800. The eyepiece camera was connected directly to a computer monitor and controlled by the computer. The images of the pollen grains observed were captured with Dino Capture Software (AnMo Electronics Corporation, Taiwan).

For each *Sonneratia* species, measurements of 90 pollen grains (from nine flowers of three different trees, 10 pollen grains of each flower) from an equatorial view were taken from the images captured using the software. Kelly *et al.* (2002) suggested that strong positive correlations exist between pollen size and viability within species. Observations of pollen viability conducted in Chapter 4 showed that smaller-sized pollen grains (which look 'translucent' when observed under a light microscope) were non-viable pollen (viability is indicated by the growth of pollen tubes after germination). Therefore, the same number of non-viable pollen grains (from the same flowers as above) was also measured for each *Sonneratia* species for species identification. The measurements taken for each pollen grain were the length of the polar axis (P) and the equatorial diameter (E) from the images taken with Dino Capture software to an accuracy of 0.001 µm (Figure 2.1).

Figure 2.1 Measurements of *Sonneratia* pollen grains from equatorial view under a light microscope. DL0 is the equatorial diameter (E) measurement, and DL1 is the polar axis (P) measurement.

For *Sonneratia* pollen, observations were also made with a scanning electron microscope (SEM) for species identification. Anthers from flowers collected were fixed with 2.5 % glutaraldehyde in 0.1M sodium cacodylate buffer, post-fixed with 1 % osmium tetroxide in 0.1M sodium cacodylate buffer and dehydrated using a graded series of ethanol to 100 % (35 %, 50 %, 60 %, 70 %, 80 %, 90 %, 95 % and 100 % for five minutes for each concentration). Samples were then rapidly transferred to a critical-point drying device with liquid carbon dioxide using Baltex 030 apparatus. After the critical point of drying was reached, samples were attached to a 13 mm aluminium stub with ducting paint and coated with gold by using a JEOL JFC-1600 Auto Fine Coater. The aluminium stubs were then observed with a JEOL 6360LA scanning electron microscope (SEM) with magnification between x2000 and x3000.

Identifications of *Sonneratia* pollen grains were also aided by the key to mangrove pollen of southern China (Mao *et al.* 2012).

2.2.2 *Collection of pollen and faeces from bats*

Nettings were conducted on 14 nights between May and November 2010 and 21 nights between May and August 2011 to catch the flower-visiting bats. The bats were captured with nylon mist nets (2.6 x 9 m) set about 3-5 m high in front of flowering *Sonneratia* trees with the aid of aluminium poles. For each trapping night, two to 31 nets were opened between 19.00 h (dusk) and 07.00 h (sunrise) the next day. The nets were tended for netted bats at least hourly throughout the netting period.

When bats were netted, nets were lowered and the bats were screened for pollen loads. The pollen grains adhering to the bat's body were sampled by carefully rubbing the bat's head (chin, crown, face, snout, throat and ear), body (chest, abdomen, back and shoulder) and wings (upper and under surfaces of wing membranes) with separate cotton wool buds individually (Figure S2.1). Voigt *et al.* (2009) recommended a more reliable technique in collecting pollen grains from the bats' bodies by using gelatinous cubes. The glycerine and fuchsin content of the cubes are used for both preservation and colouring of the pollen. The gelatinous cubes however easily melt especially in warm environments. In my study, I used cotton wool buds to collect pollen grains from the bats' bodies, adapting the method used by Singaravelan and Marimuthu (2004). They used separate brushes repeatedly dipped in still water to completely gather pollen grains from pteropodid bats they caught in India. I preserved the pollen grains in small vials (30 ml) containing 75 % ethanol without colouring to observe the pollen features for species identification.

For each part of the body, two to five cotton wool buds were repeatedly dipped in ethanol to gather the pollen grains adhering to the bats as completely as possible. Faeces were also collected if produced by using the cotton wool buds. The pollen grains and faeces collected were preserved in separate vials (for each individual and

different body parts) containing approximately 20 ml of 75 % ethanol for further identification in the lab. Bats were then removed from the net, measured and identified to species following the keys provided by Kingston *et al.* (2006) and Francis (2008). Bats were sexed, weighed using a digital balance (FEJ 600A, 0.1 g accuracy, Colonial Weighing Australia Pty. Ltd, Australia) and measurements of forearm length were taken using plastic vernier callipers (0.1 mm accuracy). Adults and juveniles were distinguished by observing the epiphyseal plates of the phalanges while transilluminating the wings with a head torch. Juveniles were distinguished from adults by the presence of cartilaginous epiphyseal plates in the phalanges (Anthony 1988).

For nettings conducted in 2011, wing biopsies were taken using a 3 mm biopsy punch (Stiefel, UK) for my future research on systematics and population genetics. The biopsy punch and forceps used were wiped with an ethanol swab and dried before conducting the biopsy. The bat's wing was stretched on a white cutting board, and the punch was then pressed down through the wing membrane. The cut tissue was collected and preserved in separate tubes containing 95 % ethanol. The scars from the biopsies were used for individual recognition on recapture (each individual was wing punched at different locations so that individuals could be identified on recapture) (Figure S2.2). The bats were fed with sugar water before being released at the point of capture. To determine whether pollen loss occurred during the collection of pollen from bats (especially to the net and to the fingers of the handler), an additional seven bats were caught and pollen grains from the bats as well as from the net and from the individual handling of the bats were sampled using the same procedure as described above.

2.2.3 Collection of stigmata after the first visit by bats and after the blooming night

Collections of stigmata were conducted only for *Sonneratia caseolaris* and *S. alba* trees between May and November 2012 (stigmata of *S. ovata* were not collected due to an insufficient number of trees; there were only three trees, and the trees were

observed for the phenological study described in Chapter 4). Trees with mature buds were observed during the day prior to night observations. Observations of bats visiting the flowers were conducted starting from 19.00 h with the help of moonlight and dim light from headlamps. Observations were conducted 2-5 m from the trees, and the flowers were observed continuously until 23.00 h for visitation by bats. Watzke (2006) in his study recorded moths (Lepidoptera) as the only visitors (other than bats) that touched and deposited pollen grains to the stigmata during visitations to *S. caseolaris* flowers. However, on only two occasions (26 %) did the moths contact the stigmata from the seven visits recorded by him. On other occasions, moths made contact only with the anthers, and hence did not serve as pollinators. In my study, moths were also observed visiting the *Sonneratia* flowers (from video recordings described in Chapter 3 and from personal observations) usually hovering in front of the flowers and were easily recognized by the red-eye reflection from the dim light headlamp pointed at them. Therefore, flowers seen visited by the moths were excluded from observations, and visitations to the *Sonneratia* flowers by other visitors (such as ants, spiders and bees) in this study were considered negligible and were ignored in subsequent analyses.

Each time a bat visited a flower, the flower was shaken indicating contact and possible pollen transfer to the stigmata by the bat. An aluminium ladder was used to access the flower after the first bat was seen visiting the flowers and the stigmata were immediately removed (usually within 30 s, before other bats visited the flower).

The stigmata of flowers that bloomed on the previous night were examined for pollen deposition (stigmatic surfaces of unvisited flowers were usually sticky) between 07.00 h to 07.30 h to reduce the possibility of pollen deposition on stigmata by the early morning visitors such as bees and wasps. For each *Sonneratia* species, four stigmata were collected from five different trees. All stigmata collected after the first visit by bats and after the blooming nights were preserved in separate 1.5 ml centrifuge tubes containing 75 % ethanol.

2.2.4 Identification of pollen collected from bats and faeces, pollen deposited on stigmata, and identification of food item from faeces

Pollen was identified by comparisons with known pollen types in the reference collection. Pollen load was estimated by counting grains under a light microscope with x10 and x40 magnification. For pollen samples from body swabs, cotton wool buds used to collect the pollen grains were removed from the vials before being thoroughly shaken. 1 µl of ethanol was then extracted from each sample using a micropipette (Eppendorf 1-10 µl) and put on a glass slide for observation. For each slide, the morphologically distinguishable pollen types (morphotypes) were recorded, as well as the total number of pollen grains for each morphotype observed. For each sample, pollen counts were made from 20 replicates of 1 µl samples in ethanol (for each sample, the total pollen count was made from subsamples of 20 µl ethanol from the total 20 ml of ethanol in vials). Pollen counts were conducted using a mechanically operated hand tally counter.

For pollen deposited on stigmata, the centrifuge tubes were vortexed using a vortex mixer (Finevortex mixture 3000 rpm, Fine PCR, Korea) for 2 min prior to pollen observations conducted using the same procedure as with pollen swabs. For each sample, the total number of pollen grains deposited on stigmata was determined based on the ethanol volume. To achieve normality, the number of pollen grains deposited on the stigmata were logarithmically transformed (log base 10). Friedman's ANOVA for repeated measures, Kruskal-Wallis and multiple comparisons (step-down method) were conducted for data that violated the normality assumption. All the analyses were conducted by using the IBM SPSS Statistics ver 19.0 (Chicago, USA).

For identification of pollen from the faeces collected, the samples were mixed with 75 % ethanol in a petri dish to extract the undigestible food fragments under a dissecting microscope with x1 to x4 magnification. The faeces were homogenized and teased apart with a needle and inspected for any indigestible food fragments. The

fragments were then collected from the petri dish with fine forceps and placed in a separate petri dish for further identification. The remaining samples in the petri dish were then kept in a vial containing 75 % ethanol for further examination for the presence of pollen under a light microscope. The identification of pollen from faeces was conducted in the same way as for the analysis of pollen swabs. The food fragments in the petri dish were washed with 75 % ethanol and classified into several categories according to colour, size, texture and shape. For plant material, the fruit fragments were identified from pellet-like soft pulp; the flower parts identified were stamens and petals (based on the colour), while bark was recognized by the presence of dark fragments with a hard texture. The insect fragments encountered consisted of wings, appendages (legs/antennae) and scales. The seeds found were identified based on the morphology of the bean seeds.

The occurrence of each categorized food item was calculated following Thomas (1988). The results were presented as % frequency (number of fragments for a particular food item identified divided by the total fragments, multiplied by 100) and % of occurrence (the number of faecal samples containing a particular food item divided by the total faecal samples, multiplied by 100) for each food item. Pollen grains in faeces were scored using a presence/absence basis to indicate the flower/plant species visited by the bats while foraging.

2.2.5 Observations of floral visiting behaviour

Filming of bats visiting *S. caseolaris* and *S. alba* was conducted between March and December 2012 coinciding with the peak flowering of these species (seven nights between October and December 2012 for *S. caseolaris* and eight nights between March and November 2012 for *S. alba*). During each recording night, two to four nightshot surveillance cameras (1/3" SONY 420 TVL CCTV, Anyon Technology, Malaysia) were used simultaneously. As inflorescences contained only one to two open flowers at any given time, each camera usually recorded a focal one, or occasionally

two flowers. However, for bat visitations to the flowers, three and four flowers were recorded by one camera on single occasions (visits by bats to the flowers were easily detected by their size and their eye shine as shown in the recordings, as compared with other visitors such as insects). The cameras were set up approximately 1 m from flowers with the aid of aluminium poles. The cameras were connected to a digital video recorder (4 channel Crossfire CF1804, Belco, Taiwan) running between 19.00 h and 07.00 h the next morning.

The bats' behaviour while visiting the flower and the time spent at the flower were determined from the video recordings. The recordings were digitized using Windows Live Movie Maker (ver. 2011) for analyses of bat visitations at 0.5 times the original speed. A temporal resolution of 1 s was used as the minimum for measuring the duration of visitation. The duration of a bat's visitation was determined from the time of arrival at the flowers until the time of departure from the time code of the tapes (further results on other floral visitors are in Chapter 3).

2.3 Results

2.3.1 Observations of Sonneratia and other reference pollen

The identification of *Sonneratia* pollen was aided by observations under the SEM. Comparisons of *Sonneratia* pollen in equatorial view observed under both SEM and LM are shown in Figure 2.2. The identification of *S. alba* pollen was based on the presence of distinct meridional ridges extending to the polar areas, a feature which is absent in *S. caseolaris* and *S. ovata* pollen. The *S. caseolaris* and *S. ovata* pollen grains were differentiated by the presence of sharper polar caps in *S. caseolaris* pollen compared with the rounded polar caps in *S. ovata*. Further, the polar region in *S. caeolaris* pollen is distinct and usually thicker, and shows abrupt discontinuities adjacent to the equatorial field, while *S. ovata* pollen grains usually showed thin polar regions. Observations of pollen grains in polar view were uncommon. However, when pollen

grains in polar view were encountered, the cover slip was pressed gently by the fingertip to move the pollen into an equatorial view.

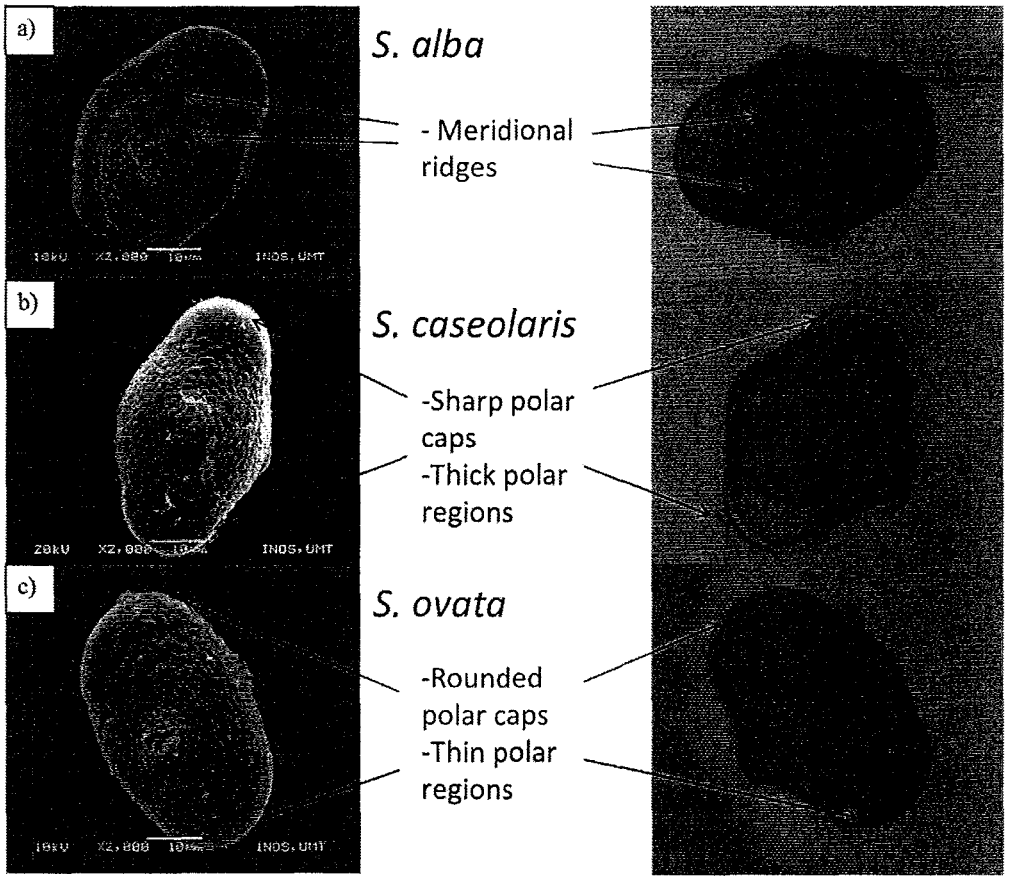


Figure 2.2 *Sonneratia* pollen grains viewed under a scanning electron microscope (SEM) (x2000) and light microscope (LM) (x2800) in equatorial view. a) *Sonneratia alba* b) *S. caseolaris* c) *S. ovata.*

The measurements of pollen grains from an equatorial view conducted for each *Sonneratia* species are as shown in Table 2.1. Results from Kruskal-Wallis tests showed significant size differences in the mean length of the polar axis (P) (H = 121.886, df = 2, $P < 0.001$), equatorial diameter (E) (H = 138.584, df = 2, $P < 0.001$) and P/E ratio (H = 92.334, df = 2, $P < 0.001$) among species. Multiple comparisons showed significant differences in mean length of polar axis and mean of P/E ratio between *S. alba* and the other two *Sonneratia* species, while the differences in mean of

equatorial diameter occurred among all species. Even though *S. alba* pollen was larger than the pollen of the other two species, the identification of pollen grains collected from bats was made using a combination of both pollen morphological characters and size due to considerable overlap in pollen size distributions of the three *Sonneratia* species (Figure 2.3).

Table 2.1 Comparison of pollen grain sizes (means ± SE) among three *Sonneratia* species.

Sonneratia species	Polar axis, P (µm)	Equatorial diameter, E (µm)	P/E ratio
S. caseolaris (N = 90)	51.671 ± 0.235[a]	38.223 ± 0.155[a]	1.353 ± 0.008[a]
S. alba (N = 90)	56.811 ± 0.329[b]	45.807 ± 0.374[b]	1.244 ± 0.007[b]
S. ovata (N = 90)	51.910 ± 0.272[a]	38.856 ± 0.282[c]	1.339 ± 0.007[a]

Different letters indicate significant differences from one another

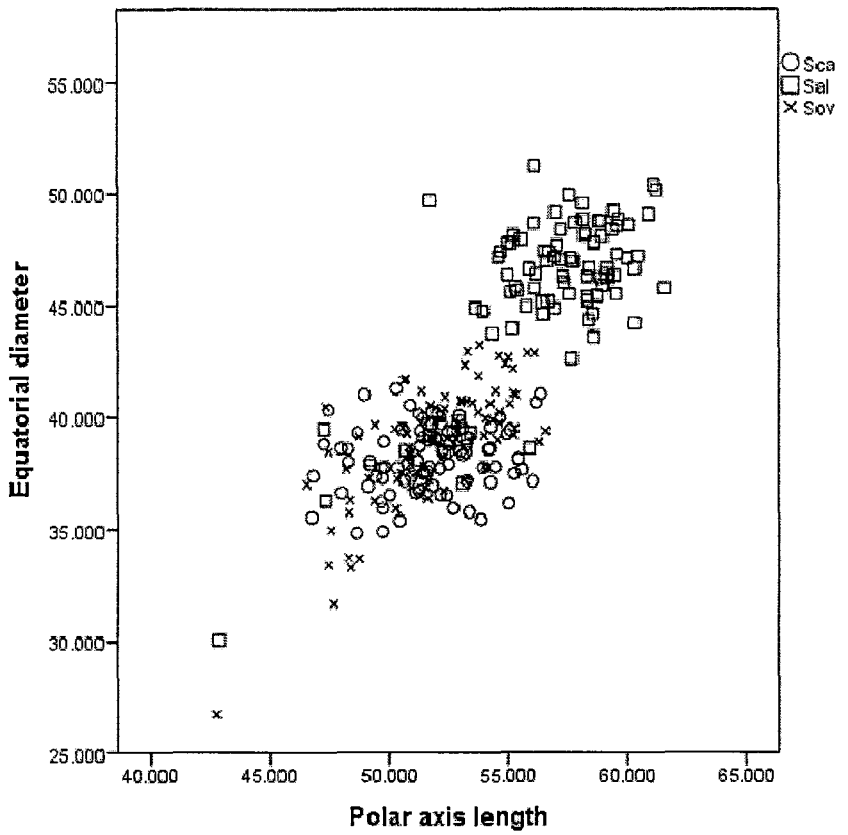


Figure 2.3 Distribution of pollen size between the *Sonneratia* species. All measurements were in µm. Sca = *Sonneratia caseolaris*, Sal =*S. alba*, Sov = *S. ovata.*

Observations of non-viable pollen grains were also conducted to differentiate the species for identification. Even though the observations under SEM showed distinct morphological characters that allowed identification of the three *Sonneratia* species, observations under LM were unable to reveal the same features as the non-viable pollen grains usually appeared 'translucent' under the LM (Figure 2.4). Measurements of the non-viable pollen grains conducted the same way as the viable pollen showed significant size differences in the length of polar axis (P) (H = 77.117, df = 2, P < 0.001), equatorial diameter (E) (H = 77.811, df = 2, P < 0.001) and P/E ratio (H = 39.143, df = 2, P < 0.001) among species (Table 2.2). Multiple comparisons showed significant differences in the mean length of the polar axis between *S. caseolaris* and the other two *Sonneratia* species while the differences in means of equatorial diameter and P/E ratio occurred among all species. The distribution of non-viable pollen size

plotted from the length of polar axis and equatorial diameter measurements also showed considerable overlap between species (Figure 2.5), therefore the non-viable pollen grains were identified and grouped as *Sonneratia* sp. given the uncertainties in identifying non-viable pollen to species.

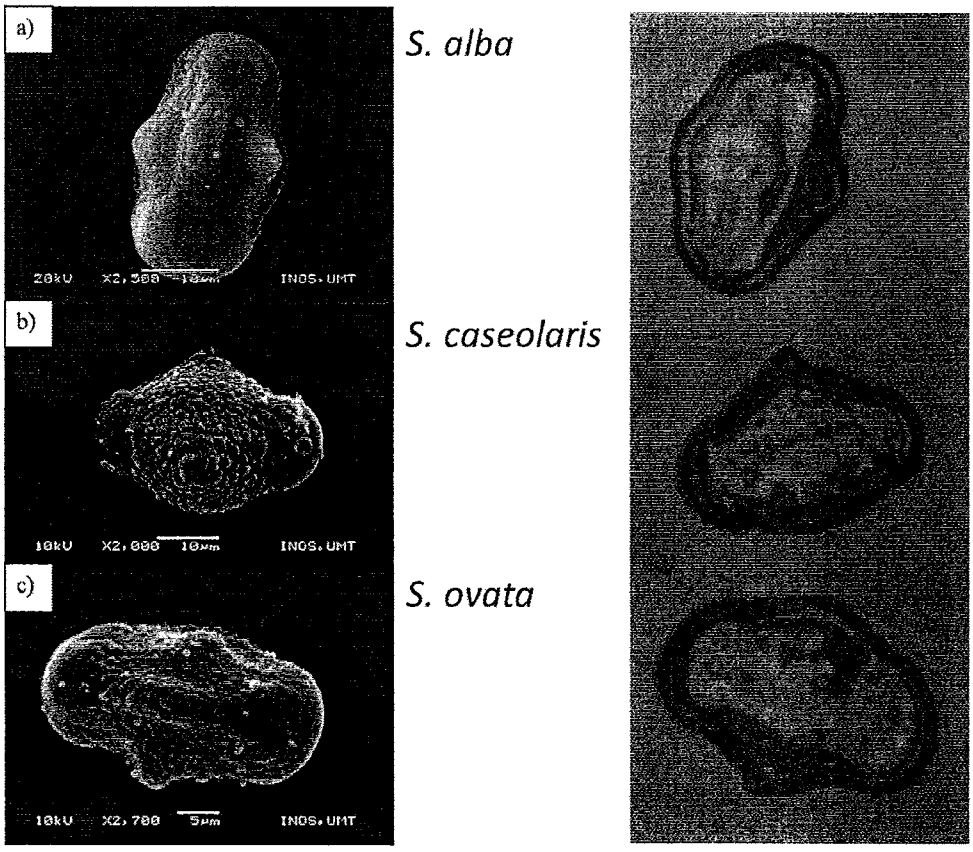


Figure 2.4 Non-viable *Sonneratia* pollen grains viewed under a scanning electron microscope (SEM) (x2000-x2700) and light microscope (LM) (x2800) in equatorial view. a) *Sonneratia alba* b) *S. caseolaris* c) *S. ovata.*

Table 2.2 Comparisons of non-viable pollen grain sizes (means ± SE) among three *Sonneratia* species.

Sonneratia species	Polar axis, P (μm)	Equatorial diameter, E (μm)	P/E ratio
S. caseolaris (N = 90)	39.629 ± 0.275[a]	29.094 ± 0.179[a]	1.365 ± 0.010[a]
S. alba (N = 90)	43.048 ± 0.306[b]	32.841 ± 0.296[b]	1.317 ± 0.011[b]
S. ovata (N = 90)	42.958 ± 0.350[b]	30.842 ± 0.372[c]	1.406 ± 0.016[c]

Different letters indicate significant differences from one another

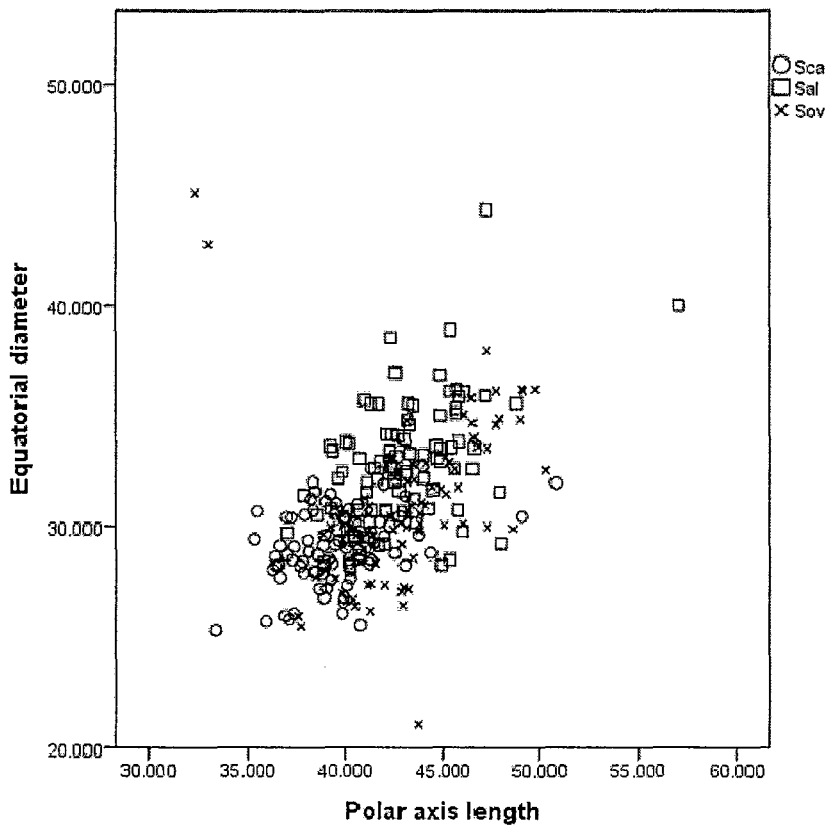
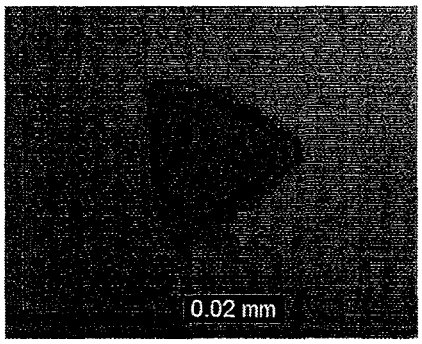
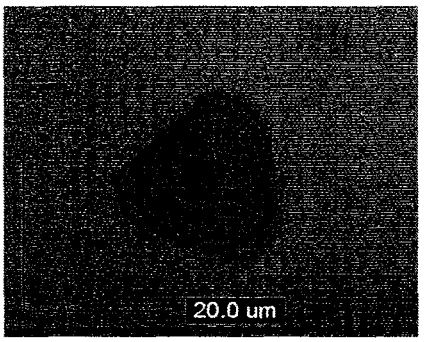


Figure 2.5 Distribution of non-viable pollen size between the *Sonneratia* species. All measurements were in μm. Sca = *Sonneratia caseolaris*, Sal = *S. alba*, Sov = *S. ovata*.

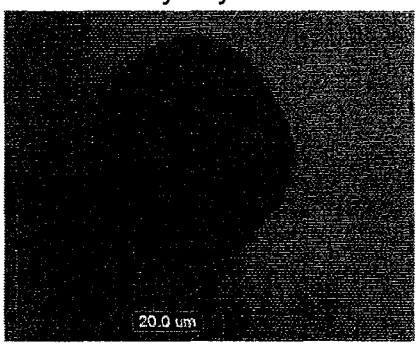
Images taken under a light microscope of pollen (other than *Sonneratia*) collected as reference material to facilitate the pollen identifications are shown in Figure 2.6. The pollen of each species was visually distinguishable under the light microscope either with magnification of x10 and x40. As well as shape, the size of pollen from each plant species was also noted to aid the identification process.



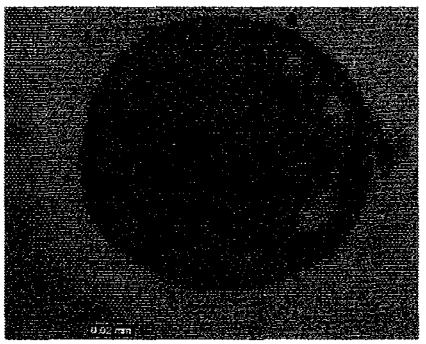
a) *Melaleuca cajuputi* (Cajeput tree)
Family: Myrtaceae



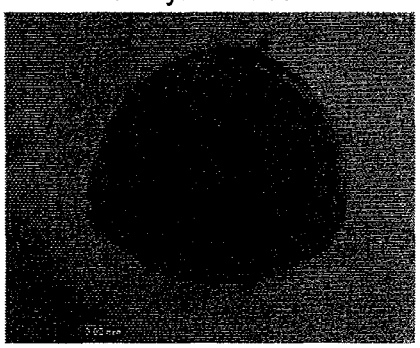
b) *Eugenia aquea* (Water apple)
Family: Myrtaceae



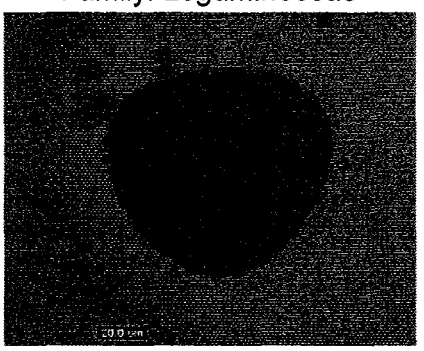
c) *Acacia auriculiformis* (Acacia)
Family: Fabaceae



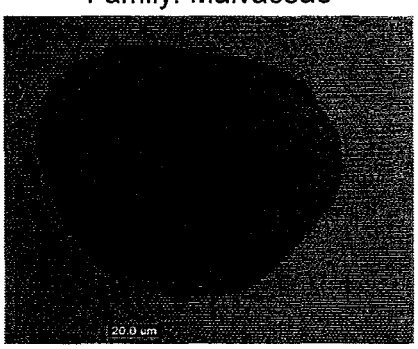
d) *Parkia* sp. (Bitter bean)
Family: Leguminoceae



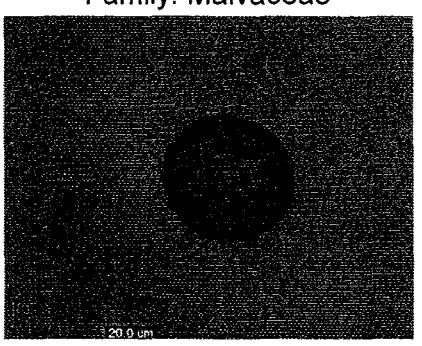
e) *Durio* sp. (Durian)
Family: Malvaceae



f) *Ceiba pentandra* (Kapok)
Family: Malvaceae



g) *Oroxylum indicum* (Indian trumpet)
Family: Bignoniaceae



h) *Musa* sp. (Banana)
Family: Musaceae

Figure 2.6 Pollen from reference collections observed under a light microscope (x2800). a)-c) are the abundant species at the study areas and d)-h) are the bat-pollinated species.

2.3.2 *Bat captures*

From a total of 35 netting nights, 137 individuals from three different species of flower-visiting bats were caught (Table 2.3 and Figure 2.7). The number of individuals caught in 2010 was 63 and 74 in 2011 (each capture in 2010 was considered a single distinct individual as the bats captured during this period were not marked). Over the entire study, eight bats were recaptured once and three individuals were recaptured twice.

Table 2.3 List of bats mist-netted (number of individuals) visiting flowering *Sonneratia* trees.

Bat species/ *Sonneratia* species	*S. caseolaris*	*S. alba*	*S. ovata*	Total
E. spelaea	35[a]	37[a]	45[c]	117
C. brachyotis	14[b]	3	12	19
R. amplexicaudatus	0	0	1	1
Total individuals	49	40	58	137
Total net-nights	84	126	93	303
Total net-hours	951	1505	1116	3572

[a]One individual was recaptured
[b]Two individuals were recaptured
[c]Four individuals were recaptured once and three individuals were recaptured twice

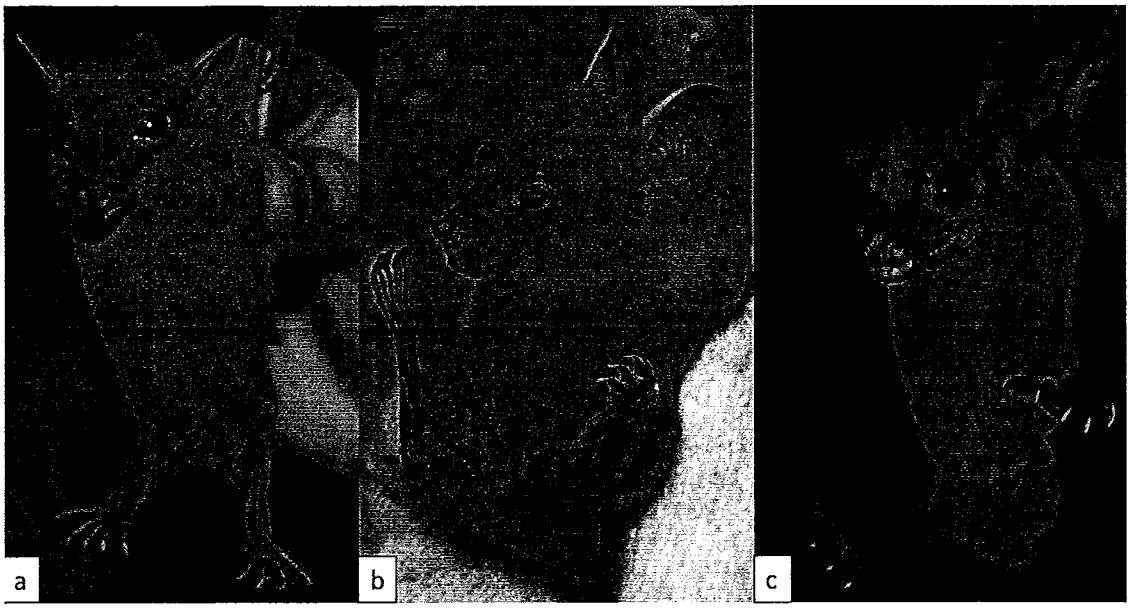


Figure 2.7 Bat species caught at the study areas. a) *Eonycteris spelaea* (cave nectar bat) b) *Cynopterus brachyotis* (short-nosed fruit bat) c) *Rousettus amplexicaudatus* (Geoffroy's rousette).

About 85 % of the total catch was the cave nectar bat (*Eonycteris spelaea*) which was disproportionately the most frequently captured species (χ^2 = 170.686, df = 2, $P < 0.001$), while the short-nosed fruit bat (*Cynopterus brachyotis*) represented 14 % of total captures, and only a single individual of the Geoffroy's bat (*Rousettus amplexicaudatus*) was netted in front of an *S. ovata* tree. The body mass (mean (± SE) recorded was 40.66 ± 1.00 g for *E. spelaea* (N = 106), 33.83 ± 1.60 g for *C. brachyotis* (N = 19), and 91.9 g for a single individual of *R. amplexicaudatus* (data from pregnant females were excluded).

2.3.3 Pollen loads on bats body

From the total captures, pollen swabs were collected on 151 occasions, and only four captures were negative for pollen load on their body at the time of capture (Table 2.4). The four negative captures were two individuals each of *E. spelaea* and *C. brachyotis*, caught visiting *S. alba* trees. The four individuals were excluded from further analysis. The bats were carrying 11 different morphotypes of pollen, of which six were identified

to species level and the remaining five to genera. For *Sonneratia* pollen grains, the non-viable pollen was not identified to species level, and therefore was grouped as *Sonneratia* sp. As the non-viable pollen grains could be from any one of the three *Sonneratia* species, this pollen group was excluded in the analysis of pollen types (2.3.3.1). However, the non-viable pollen group was included in further analysis of total pollen grains (2.3.3.2) on the basis that this pollen does not result in fertilisation (i.e. heterospecific pollen) and contributes to reproductive interference by clogging the stigmata of the flowers.

Table 2.4 Number of bats carrying each pollen type at the time of capture. Percentages in parentheses.

Bat species/ Pollen type	*Sonneratia*			*Parkia* sp.	*Musa* sp.	*Durio* sp.	*Ceiba pentandra*	*Oroxylum indicum*	*Eugenia* sp.	*Acacia* sp.	*Melaleuca cajuputi*	Zero
	caseolaris	*alba*	*ovata*									
E. spelaea (N = 130)	42(32.3)	42(32.3)	70(53.8)	39(30.0)	21(16.2)	27(20.8)	10(7.7)	20(15.4)	7(5.4)	4(3.1)	9(6.9)	2(1.5)
C. brachyotis (N = 20)	15(75.0)	2(10.0)	4(20.0)	7(35.0)	4(20.0)	1(5.0)	1(5.0)	3(15.0)	0(0.0)	0(0.0)	3(15.0)	2(10.0)
R. amplexicaudatus (N = 1)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	57(37.7)	44(29.1)	75(49.7)	47(31.1)	25(16.6)	29(19.2)	11(7.3)	24(15.9)	7(4.6)	6(4.0)	12(7.9)	4(2.6)

2.3.3.1 Pollen types

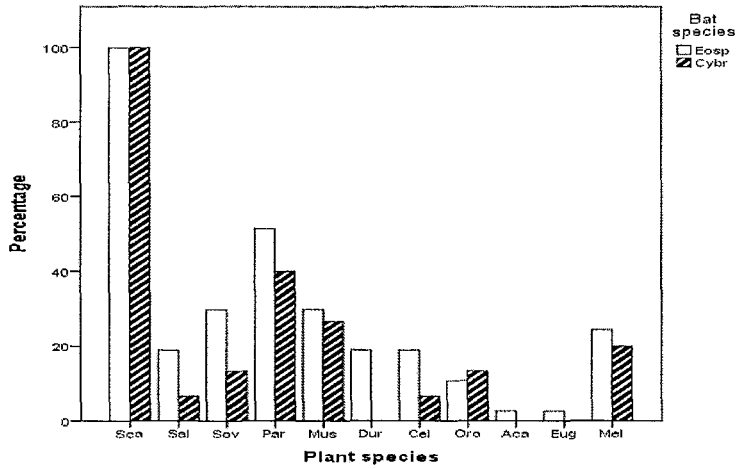
Individual bats were found to carry several types of pollen at the time of capture. The mean number (± SE) of pollen types per individual was 2.40 ± 0.12 (N = 147). *E. spelaea* carried a mean of 2.41 ± 0.13 pollen types per bat (N = 128) with eight being the highest number of pollen types per individual. For *C. brachyotis*, the mean pollen types was 2.28 ± 0.36 per bat (N = 18) and up to six pollen types were found on a single bat. The mean number of pollen types carried by the two species was not significantly different (Mann-Whitney test, U = 1043.000, P = 0.500). The single individual of *R. amplexicaudatus* caught was found with four pollen types.

E. spelaea carried all of the 11 pollen types recorded while *C. brachyotis* carried nine pollen types. Yates' Chi-square test showed that the number of pollen types carried by the two bat species was not significantly different (χ^2 = 0.250, df = 1, P = 0.655). For both bat species, a single pollen type per individual was the common case observed (35 % in *E. spelaea* and 44 % in *C. brachyotis*) while 86 % of *E. spelaea* individuals and 83 % of *C. brachyotis* caught were found to carry between one to three pollen types. For the bats recorded with a single pollen species, all were carrying *Sonneratia* pollen.

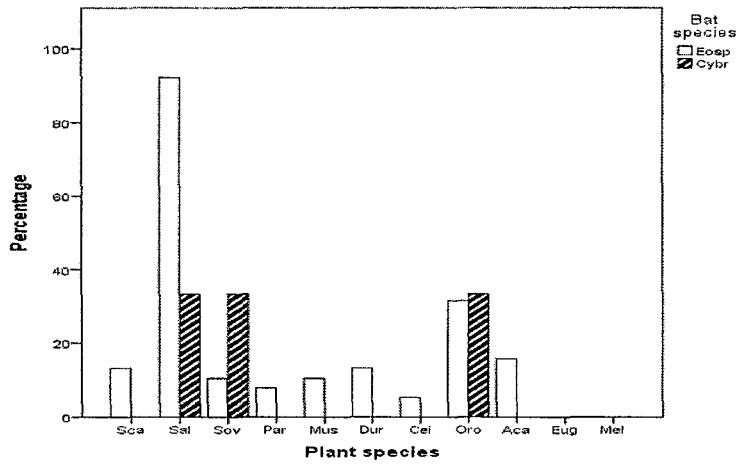
Observations of pollen types on bats according to their netting area showed that the bats were usually bearing pollen of the *Sonneratia* trees they were in close proximity to at the time of capture (i.e. conspecific pollen), with the exception of five cases of bats caught visiting *S. alba* trees (where one case of bat carried *S. caseolaris* together with the non-viable *Sonneratia* pollen grains, and four cases where bats were without pollen loads) (Table S2.1-S2.3). Bats visiting *S. caseolaris* and *S. alba* trees were found to carry heterospecific *Sonneratia* pollen but this was not the case for bats visiting *S. ovata* trees (Figure 2.8). The numbers of individuals carrying conspecific pollen grains of the given *Sonneratia* species (i.e. bats visiting *S. caseolaris* trees

carrying *S. caseolaris* pollen) were associated with the local abundance of the *Sonneratia* trees at all netting sites.

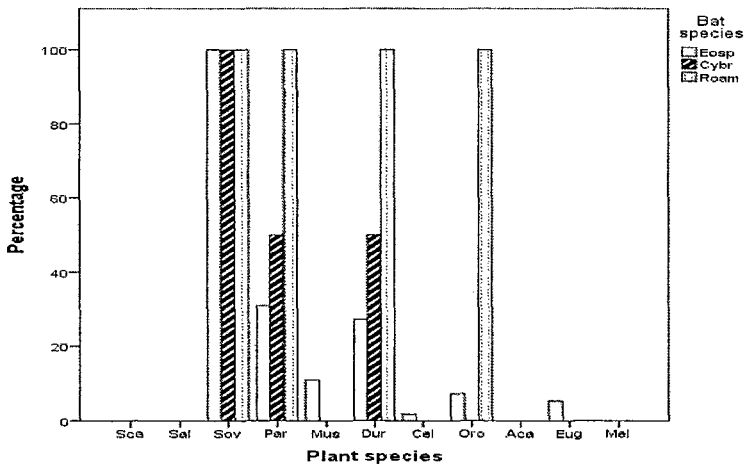
E. spelaea and *C. brachyotis* visiting *S. caseolaris* flowers carried 11 and eight pollen types respectively, and all individuals caught carried the conspecific pollen grains (Table S2.1). *E. spelaea* visiting *S. alba* flowers however carried only nine pollen types (Table S2.2). Ninety-two percent of bats (35 individuals) carried the conspecific pollen, and 26 % (10 individuals) carried only the conspecific pollen. Only one individual from three *C. brachyotis* caught visiting *S. alba* trees carried pollen grains while the other two were negative for pollen loads. As well as the conspecific pollen, the individual was also found to carry *Oroxylum indicum* pollen grains. Three bat species were recorded visiting the *S. ovata* trees and all individuals caught carried conspecific pollen (Table S2.3). The *E. spelaea* captured around *S. ovata* trees recorded the lowest number of pollen types adhering to their bodies (seven) compared with individuals caught visiting the other *Sonneratia* species. Twenty individuals (36 %) of *E. spelaea* caught visiting *S. ovata* trees carried conspecific pollen only. Neither of the two individuals of *C. brachyotis* caught were recorded to carry the conspecific pollen only. The individuals were found with *S. ovata* pollen together with either *Parkia* sp. or *Durio* sp. pollen grains.



a) *S. caseolaris*



b) *S. alba*



c) *S. ovata*

Figure 2.8 Distribution of bat individuals carrying each pollen type according to sampling sites. Sca = *Sonneratia caseolaris* (N = 52), Sal = *S. alba* (N = 41), Sov = *S. ovata* (N = 58), Par = *Parkia* sp., Mus = *Musa* sp., Dur = *Durio* sp., Cei = *Ceiba pentandra*, Oro = *Oroxylum indicum*, Eug = *Eugenia* sp., Aca = *Acacia* sp., Mel = *Melalueca cajuputi,* Eosp = *Eonycteris spelaea,* Cybr = *Cynopterus brachyotis,* Roam = *Rousettus amplexicaudatus.*

Table 2.5 summarises the distribution of each pollen type for samples collected from different body parts of each bat species. The mean number (± SE) of pollen types was highest for swabs taken from the wing for both bat species with sufficient data, 1.78 ± 0.10 (N = 128) for *E. spelaea* (Friedman's ANOVA, $\chi^2 = 8.255$, df = 2, $P = 0.016$) and 1.89 ± 0.33 (N = 18) for *C. brachyotis* (Friedman's ANOVA, $\chi^2 = 12.731$, df = 2, $P = 0.002$) respectively.

Table 2.5 Number of samples containing particular pollen type taken from different body parts of each bat species.

	E. spelaea (N = 128)			*C. brachyotis* (N = 18)			*R. amplexicaudatus* (N = 1)		
Pollen species	Head	Body	Wing	Head	Body	Wing	Head	Body	Wing
S. caseolaris	38	38	41	10	8	14	-	-	-
S. alba	32	37	38	1	1	2	-	-	-
S. ovata	62	64	66	2	2	4	1	1	1
Parkia sp.	16	21	37	2	3	6	1	-	-
Musa sp.	8	7	15	1	2	3	-	-	-
Durio sp.	16	17	31	-	-	1	-	1	1
Ceiba pentandra	6	5	6	1	1	-	-	-	-
Oroxylum indicum	9	15	13	-	2	2	-	1	1
Eugenia sp.	2	2	6	-	-	-	-	-	-
Acacia sp.	2	1	1	-	-	-	-	-	-
Melaleuca cajuputi	4	4	7	3	3	2	-	-	-
Total number of pollen types	11	11	11	7	8	8	2	3	3
Mean number of pollen types per individual	1.52[a]	1.61[ab]	1.78[b]	1.11[a]	1.06[a]	1.89[b]	-	-	-
SE	0.08	0.08	0.10	0.23	0.26	0.33	-	-	-

Different letters indicate significant differences from one another

2.3.3.2 Total pollen grains

Seven individuals of *E. spelaea* were caught to assess the amount of pollen loss to the mist net and to the fingers of the handler. Observations of pollen loss from these seven bat individuals showed that only 3.5 % and 0.7 % of pollen adhering to the bats body were lost to the net and to the fingers of the handler respectively (Table 2.6) during the process of pollen collection using cotton swabs. Therefore, the pollen loss during pollen collection from the bats' bodies in this study was negligible. There is also possible pollen loss to the air and ground while the bats struggle in the net before being tended and also during the pollen collection process. However, no evidence of any substantial pollen loss of this kind was apparent during my study.

Table 2.6 Number of pollen grains collected at different body regions of bats caught as well as pollen loss to net and fingers of the handler during the pollen collection process (N = 7).

Body part	Pollen number (mean ± SE)	%	Range
Head	579.29 ± 195.40[a]	9	57-1618
Body	764.00 ± 0.82[a]	12	98-1724
Wing	4784.00± 1115.75[b]	78	3355-10641
Net	217.86 ± 89.60[a]	3.5*	25-708
Finger	42.71 ± 8.07[a]	0.7*	16-75

*calculated from the total pollen grains collected from bats
One way ANOVA, $F_{4,30} = 14.64$, $P < 0.001$
Different letters indicate means that are significantly different from one another

The total number of pollen grains collected from *E. spelaea* was significantly higher compared with the pollen grains collected from *C. brachyotis* ($t = 6.916$, df = 144, $P < 0.001$). The number (mean ± SE) of pollen grains carried by *E. spelaea* was 2330.80 ± 277.36, compared to only 301.22 ± 95.87 recorded for *C. brachyotis* (Table 2.7). For both bat species, the highest number of pollen grains collected was from the

wings, 83 % of the total pollen grains recorded for *E. spelaea* (Friedman's ANOVA, χ^2 = 182.511, df = 2, $P < 0.001$) and 66 % of the total pollen grains recorded for *C. brachyotis* (Friedman's ANOVA, χ^2 = 15.723, df = 2, $P < 0.001$). The lowest number of pollen grains collected however was from the head region for *E. spelaea* and the body region for *C. brachyotis.*

Table 2.7 Pollen loads (mean ± SE) on bats for each bat species. Percentages in parentheses.

Body part	*E. spelaea* (N=128)	*C. brachyotis* (N=18)	*R. amplexicaudatus* (N = 1)
Total	2330.80 ± 277.36	301.22 ± 95.87	342.00 ± 0.00
Head	138.46 ± 16.99(6)[a]	55.56 ± 19.70(19)[a]	47.00 ± 0.00(14)
Body	247.88 ± 36.97(11)[b]	45.89 ± 11.44(15)[a]	80.00 ± 0.00(23)
Wing	1944.46 ± 242.06(83)[c]	199.78 ± 73.24(66)[b]	215.00 ± 0.00(63)

Different letters indicate significant differences from one another

Pollen grains from the *Sonneratia* group (including *Sonneratia* sp.) were the dominant pollen grains collected from the bats' bodies (Table 2.8). For both *E. spelaea* and *C. brachyotis*, the number of *Sonneratia* pollen grains ranges from 94-98 % of the total pollen grains collected from the three body parts, while for *R. amplexicaudatus,* the range was between 95-100 %. The pollen grains from the non-bat-pollinated flowers (*Eugenia* sp., *Acacia* sp. and *Melaleuca cajuputi*) represented only 0.07 % of the total pollen grains collected from the bats.

Table 2.8 Number of pollen grains (mean ± SE) collected from different body parts of each bat species.

	E. spelaea (N = 128)			*C. brachyotis* (N = 18)			*R. amplexicaudatus* (N = 1)		
Pollen species	Head	Body	Wing	Head	Body	Wing	Head	Body	Wing
S. caseolaris	49.36 ± 12.28	95.99 ± 24.50	945.73 ± 206.78	23.11 ± 7.89	17.39 ± 6.47	82.11 ± 50.40	-	-	-
S. alba	23.84 ± 7.63	20.81 ± 6.62	131.82 ± 29.65	0.06 ± 0.06	2.11 ± 2.11	7.33 ± 6.99	-	-	-
S. ovata	28.25 ± 7.12	69.45 ± 20.44	438.54 ± 97.21	3.39 ± 2.35	2.72 ± 1.89	28.50 ± 25.18	13.00 ± 0.0	30.00 ± 0.0	128.00 ± 0.0
Sonneratia sp.	28.62 ± 3.82	49.04 ± 11.08	350.30 ± 57.88	27.94 ± 18.07	21.22 ± 8.93	75.00 ± 34.18	33.00 ± 0.0	46.00 ± 0.0	84.00 ± 0.0
Parkia sp.	1.11 ± 0.42	2.91 ± 1.09	10.70 ± 4.04	0.44 ± 0.32	0.94 ± 0.74	4.50 ± 2.90	-	-	-
Musa sp.	0.34 ± 0.21	0.19 ± 0.10	0.91 ± 0.66	0.06 ± 0.06	0.22 ± 0.17	0.56 ± 0.34	-	-	-
Durio sp.	1.63 ± 0.65	4.14 ± 2.87	33.69 ± 17.74	-	-	0.33 ± 0.33	-	1.00 ± 0.0	2.00 ± 0.0
Ceiba pentandra	0.35 ± 0.19	0.55 ± 0.32	6.26 ± 5.74	0.06 ± 0.06	0.06 ± 0.06	-	-	-	-
Oroxylum indicum	4.63 ± 4.46	4.55 ± 3.98	33.09 ± 22.85	-	0.50 ± 0.44	0.33 ± 0.23	-	3.00 ± 0.0	1.00 ± 0.0
Eugenia sp.	0.19 ± 0.14	0.15 ± 0.15	0.59 ± 0.30	-	-	-	-	-	-
Acacia sp.	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	-	-	-	-	-	-
Melaleuca cajupu	0.13 ± 0.08	0.08 ± 0.05	0.20 ± 0.10	0.50 ± 0.28	0.72 ± 0.52	1.11 ± 0.83	-	-	-
% of *Sonneratia* pollen grains	93.9	94.9	95.6	98.1	94.7	96.6	100.0	95.0	98.6

E. spelaea carried significantly more conspecific pollen than the heterospecific pollen grains (Wilcoxon signed-rank test, $T = 1490.000$, $P < 0.001$). On average, *E. spelaea* carried 1796.97 ± 238.92 (mean ± SE) grains of conspecific pollen and 533.84 ± 75.16 (mean ± SE) grains of heterospecific pollen at the time of capture. *C. brachyotis* on the other hand carried 165.78 ± 63.61 (mean ± SE) conspecific and 135.44 ± 60.55 (mean ± SE) heterospecific pollen grains (Wilcoxon signed-rank test, $T = 50.000$, $P = 0.122$). Table 2.9 shows the distribution of conspecific and heterospecific pollen grains collected from the three bats' body parts. For *E. spelaea*, the number of conspecific pollen grains collected was between 73-78 % while the heterospecific pollen grains represented about 22-27 % of the total pollen grains collected for the species. For *C. brachyotis,* the number of heterospecific pollen grains was slightly higher at head and body compared with the number of conspecific pollen grains. However, the relative % of conspecific to heterospecific pollen grains was almost equal for all body parts.

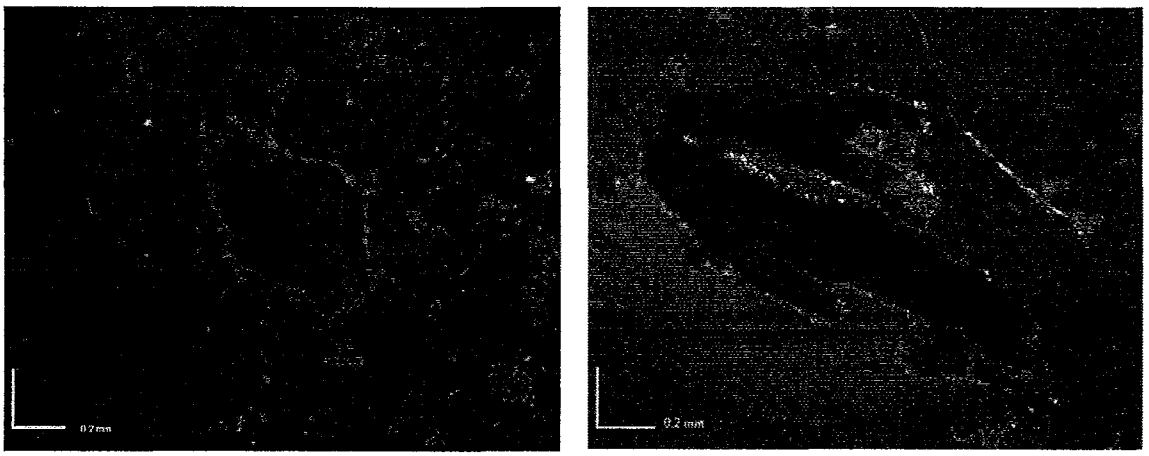
Table 2.9 Number of conspecific and heterospecific pollen grains collected from different body parts of each bat species.

Bat species	Body part	Conspecific		Heterospecific	
		Median (range)	Mean ± SE (%)	Median (range)	Mean ± SE (%)
E. spelaea (N = 128)	Head	33.00 (0-869)	100.96 ± 14.46 (72.9)	20.00 (0-600)	37.50 ± 5.87 (27.1)
	Body	49.50 (0-2167)	185.49 ± 30.08 (74.8)	31.00 (0-1216)	62.40 ± 12.05 (25.2)
	Wing	534.00 (0-11213)	1510.52 ± 209.31 (77.7)	185.50 (0-5356)	433.94 ± 63.55 (22.3)
C. brachyotis (N = 18)	Head	23.50 (0-121)	26.56 ± 7.64 (47.8)	4.00 (0-328)	29.00 ± 18.18 (52.2)
	Body	10.50 (0-85)	22.17 ± 6.28 (48.2)	12.50 (0-145)	23.78 ± 8.95 (51.8)
	Wing	33.00 (0-919)	117.06 ± 53.52 (58.6)	15.00 (0-551)	82.72 ± 34.86 (41.4)
R. amplexicaudatus (N = 1)	Head	-	13.00 ± 0.00	-	33.00 ± 0.00
	Body	-	30.00 ± 0.00	-	50.00 ± 0.00
	Wing	-	128.00 ± 0.00	-	87.00 ± 0.00

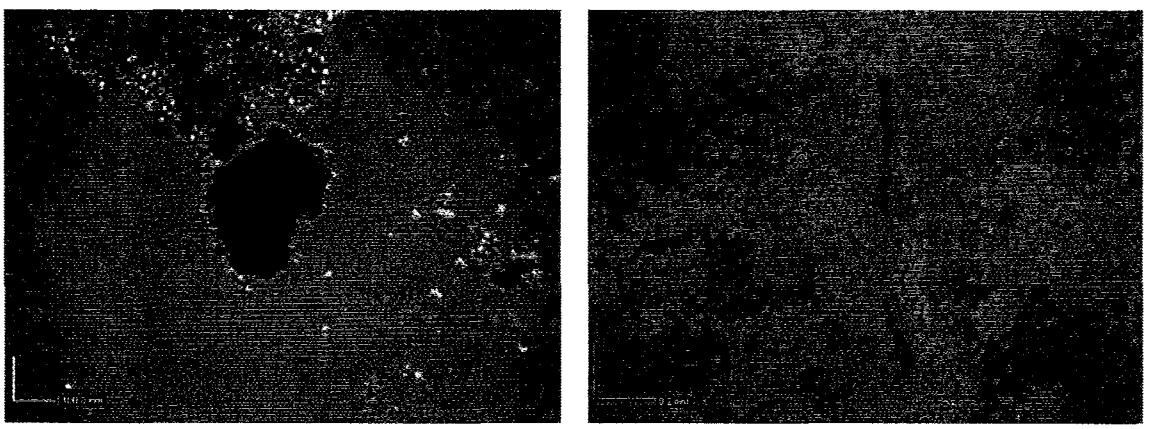
% calculated from the total pollen grains for each body part

2.3.4 *Identification of food items and pollen from faeces*

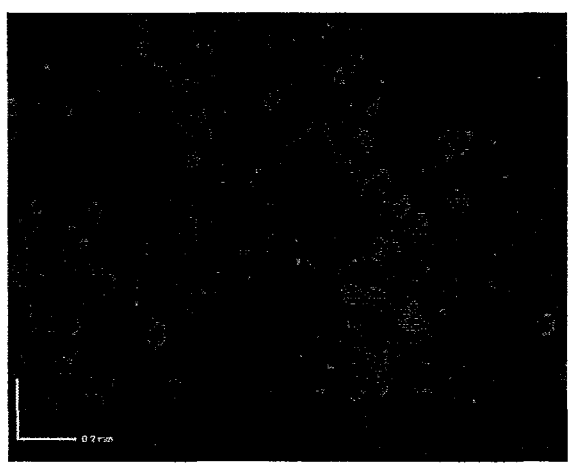
A total of 46 faecal samples were collected from the three bat species caught, with only one sample from *C. brachyotis* and *R. amplexicaudatus* each. All faeces collected were found to contain pollen grains. However, two of the faeces from *E. spelaea* contained only empty pollen shells; therefore, species identification of the pollen grains was not conducted for these samples. Other than pollen grains, the food items identified were fruit fragments, flower parts, barks, insect fragments and unidentified seeds (Figure 2.9).



a) b)



c) d)



e)

Figure 2.9 Food items identified from the bats' faeces collected. a) Unidentified seed b) Fruit pellet c) Bark d) Stamen (flower part) e) Insect fragment. Empty pollen shells are also visible in c)-e).

Table 2.10 presents the results of food items identified only from the faeces containing fruit fragments, flower parts, bark and insect fragments. For *E. spelaea*, fruit fragments were found to dominate the faecal contents examined representing 85.8 % of the total food fragments obtained and being present in 27 faecal samples (64.3 %). Even though the % volume of insects recorded was only 8.4 %, insect fragments were found in the faeces of nine bats (21.4 %), while faeces from only six individuals (14.3 %) contained other plant material such as flower and bark. *C. brachyotis* faeces contains only two seeds of one species and *R. amplexicaudatus* faeces contains only nine fruit fragments.

Table 2.10 The % frequency and % occurrence of each food item in the faeces.

	E. spelaea (N = 42)		*C. brachyotis* (N = 1)		*R. amplexicaudatus* (N = 1)	
Food items	% frequency	% occurrence	% frequency	% occurrence	% frequency	% occurrence
Fruit fragment	85.8	64.3	-	-	100.0	100.0
Flower parts	3.6	14.3	-	-	-	-
Bark	2.2	14.3	-	-	-	-
Insect fragments	8.4	21.4	-	-	-	-
Seeds	-	-	100.0	100.0	-	

A total of nine species of pollen was recorded from 44 faecal samples (Table 2.11) (the two individuals with the empty pollen shells were excluded from this analysis). *E. spelaea* faeces contained all the pollen species recorded. Other than *Sonneratia* pollen, a high percentage of occurrence was also recorded for *Parkia* sp. and *Durio* sp. pollen grains. *C. brachyotis* and *R. amplexicaudatus* faeces contained the *Sonneratia* pollen grains (*S. ovata* and *Sonneratia* sp.), with *O. indicum* pollen

grains present in *C. brachyotis* faeces and *Durio* sp. pollen grains present in *R. amplexicaudatus* faeces.

Table 2.11 The list of pollen species obtained in faeces. % of occurrence in parentheses.

Pollen species	*E. spelaea* (N = 42)	*C. brachyotis* (N = 1)	*R. amplexicaudatus* (N = 1)
S. caseolaris	+ (50.0)	-	-
S. alba	+ (20.4)	-	-
S. ovata	+ (27.3)	+ (100.0)	+ (100.0)
Sonneratia sp.	+ (81.8)	+ (100.0)	+ (100.0)
Parkia sp.	+ (43.2)	-	-
Musa sp.	+ (2.3)	-	-
Durio sp.	+ (25.0)	-	+ (100.0)
Oroxylum indicum	+ (15.9)	+ (100.0)	-
Eugenia sp.	+ (4.5)	-	-
Acacia sp.	+ (2.3)	-	-

+ present, - absent

2.3.5 Pollen deposition on stigmata by bats

A total of 40 stigmata were collected (20 stigmata each for *S. caseolaris* and *S. alba*) after the blooming night and a total of 37 stigmata (20 stigmata of *S. caseolaris* and 17 stigmata of *S. alba*) were collected after the first visit by bats. Overall, pollen from 11 species of plant were found on the stigmata of *Sonneratia* flowers (Table 2.12). The number of species whose pollen was deposited on stigmata after the first visit by bats was five for both species, compared to 10 and eight species for stigmata collected after the blooming night for *S. caseolaris* and *S. alba* respectively. The mean number (± SE)

of pollen grains deposited on stigmata of *S. caseolaris* flowers after the first visit by bats was only 8016.25 ± 1838.06, significantly fewer than the pollen grains on stigmata collected after the blooming night which was 11296.25 ± 1455.38 (t = -2.290, df = 38, P = 0.028). Conversely for *S. alba* flowers, the mean number (± SE) of pollen grains on stigmata after the first visit by bats (11180.88 ± 1797.71) was not significantly different (t = 0.211, df = 35, P = 0.834) from the number of pollen grains on stigmata after the blooming night (10606.25 ± 1992.28).

Table 2.12 Comparisons of pollen deposited on stigmata (mean pollen grains ± SE) of the *Sonneratia* flowers after the first visit by bats and after the blooming night.

Pollen species/ *Sonneratia* species	*S. caseolaris*		*S. alba*	
	After 1st visit by bats (N = 20)	After blooming night (N = 20)	After 1st visit by bats (N = 17)	After blooming night (N = 20)
S. caseolaris	6205.00 ± 1784.19	6408.75 ± 899.49	4.41 ± 2.38	31.25 ± 14.38
S. alba	-	30.00 ± 26.20	2430.88 ± 791.78	1783.75 ± 79.58
S. ovata	20.00 ± 8.23	21.25 ± 10.14	2.94 ± 2.94	-
Sonneratia sp.	280.00 ± 101.77	2203.75 ± 424.14	286.76 ± 91.66	588.75 ± 174.35
Parkia sp.	12.50 ± 12.50	1.25 ± 1.25	-	-
Musa sp.	1497.50 ± 330.52	2612.50 ± 857.14	8454.00 ± 1572.87	8143.75 ± 1724.30
Durio sp.	-	1.25 ± 1.25	1.47 ± 1.47	1.25 ± 1.25
Ceiba pentandra	1.25 ± 1.25	15.00 ± 8.19	-	3.75 ± 2.05
Oroxylum indicum	-	1.25 ± 1.25	-	42.65 ± 12.60
Eugenia sp.	-	1.25 ± 1.25	-	5.00 ± 2.29
Acacia sp.	-	-	-	2.50 ± 1.72
Melaleuca cajuputi	-	31.25 ± 31.25	-	-
Mean	8016.25 ± 1838.06	11296.25 ± 1455.38	11180.88 ± 1797.71	10606.25 ± 1992.28

For *S. caseolaris* flowers, the mean number (± SE) of conspecific pollen grains deposited on the stigmata after the first visit by bats was higher (6205.00 ± 1784.19), representing 77.4 % of the total pollen grains observed as compared with the heterospecific pollen grains (1811.25 ± 302.26) (Table 2.13). However, the number of grains was not significantly different between the two pollen types (t = 1.597, df = 19, P = 0.127). For the stigmata collected after the blooming night, the majority of the pollen grains observed was the conspecific pollen grains (6408.75 ± 899.49) accounting for 56.7 % of the total pollen grains observed as compared with the heterospecific pollen grains (4887.50 ± 985.66) (t = 1.266, df = 19, P = 0.221). The number of conspecific pollen grains was not significantly different on the stigmata collected after the first visit by bats and stigmata collected after the blooming night (t = -1.942, df = 27, P = 0.630); the number of heterospecific pollen grains, however, was significantly higher on the stigmata collected after the blooming night compared to stigmata collected after the first visit by bats (t = -3.689, df = 38, P = 0.001).

For *S. alba* flowers, the mean number (± SE) of conspecific pollen grains deposited on the stigmata was significantly lower than the heterospecific pollen grains for both stigmata collected after the first visit by bats (t = -4.804, df = 16, P < 0.001), as well as for the stigmata collected after the blooming night (t = -6.170, df = 19, P < 0.001). For stigmata collected after the first visit by bats, the number of conspecific pollen grains was 2430.88 ± 791.78 (21.7 % of total pollen grains observed) while for the stigmata collected after the blooming night, the number of conspecific pollen was 1783.75 ± 479.58, accounting for 16.8 % of the total pollen observed. The comparisons of pollen types on stigmata collected after the first visit by bats vs. stigmata collected after the blooming night showed no significant difference in the number of conspecific (t = -0.402, df = 35, P = 0.690) and heterospecific (t = -0.109, df = 35, P = 0.914) pollen grains. Among the heterospecific pollen grains, *Musa* sp. pollen grains were most abundant on the stigmata of both *Sonneratia* species.

Table 2.13 Number of conspecific and heterospecific pollen grains collected from stigmata of the *Sonneratia* flowers.

Sonneratia species	Stigmata	Conspecific		Heterospecific	
		Mean ± SE (%)	Range	Mean ± SE (%)	Range
S. caseolaris	After 1st visit by bats (N = 20)	6250.00 ± 1784.18 (77.4)	200-27150	1811.25 ± 302.26 (22.6)	250-5075
	After blooming night (N = 20)	6408.75 ± 899.49 (56.7)	1175-16275	4887.50 ± 985.66 (43.3)	775-18850
S. alba	After 1st visit by bats (N = 17)	2430.88 ± 791.78 (21.7)	25-9700	8750.00 ± 1584.70 (78.3)	850-20450
	After blooming night (N = 20)	1783.75 ± 479.58 (16.8)	75-8075	8822.50 ± 1768.58 (83.2)	925-32775

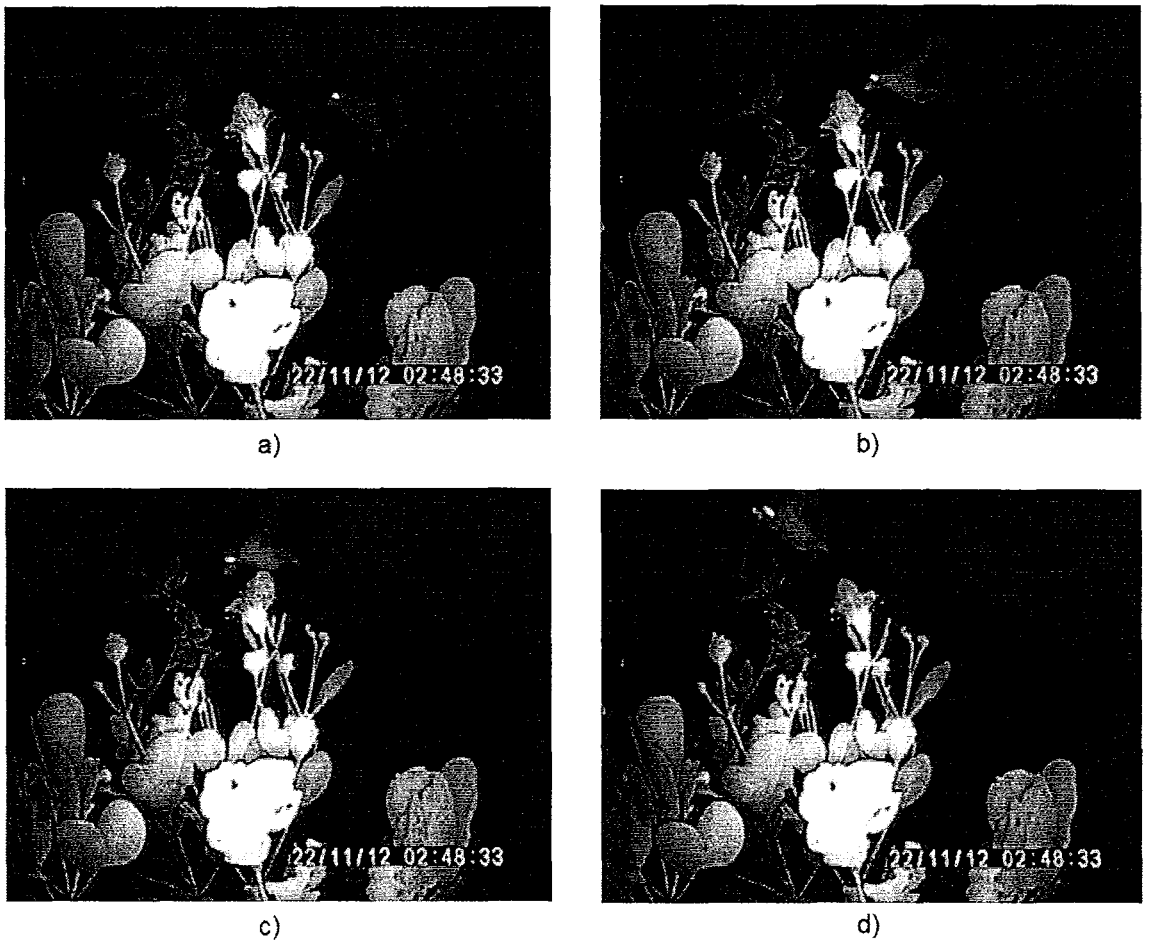
2.3.6 *Bat foraging activities*

Video recordings were conducted on 27 flowers from seven trees of *S. caseolaris* and 24 flowers from 11 trees of *S. alba* for a total of 324 flower-hrs and 288 flower-hrs of filming respectively. A total of 73 observations of bats approaching or visiting the flowers were recorded for *S. caseolaris* and 67 observations for *S. alba* flowers. For most of the recordings, accurate identification of the bat species visiting the flowers was not possible. From the 140 observations in total, only 46 positive identifications of *E. spelaea* were made based on their longer snout compared with the other two bat species (*C. brachyotis* and *R. amplexicaudatus*). Mist netting recorded only these three species at the study areas, and given the high abundance of *E. spelaea* it is likely that most observations relate to this species.

Observations of bat behaviour while approaching the *Sonneratia* flowers showed two distinct activities, which were scouting and feeding on the flowers. For scouting visits (N = 72), bats were found to fly at close proximity to the flower, pausing in flight at the flowers, thrusting their head in front of the flower without touching the flowers (stamens or stigmata), before flying away (Figure 2.10). Some of the bats performed exploratory flights such as approaching the flower before flying away, or passing the flower without pausing or thrusting their head in front of the flowers.

For feeding visits (N = 68), bats were found to either land on the flowers (N = 61) or hover in front of the flowers (N = 7) to feed. For landing, bats were found to thrust their snout between the stamens to explore nectar accumulated at the base of the calyx before landing on the flowers by grasping the flower with their feet, then extending their wings, and pressing the flower to their body. The weight of a bat can bring down a flower, so the bats were seen clinging tightly to the flower while lapping for nectar with their tongues. The durations of bat visits to the *Sonneratia* flowers were between 1-4 s, with the majority of feeding activities lasting 1 s (N = 49) followed by 2 s (N = 8) and 3 s (N = 2). The longest duration of feeding activity observed was 4 s (N = 2). When the

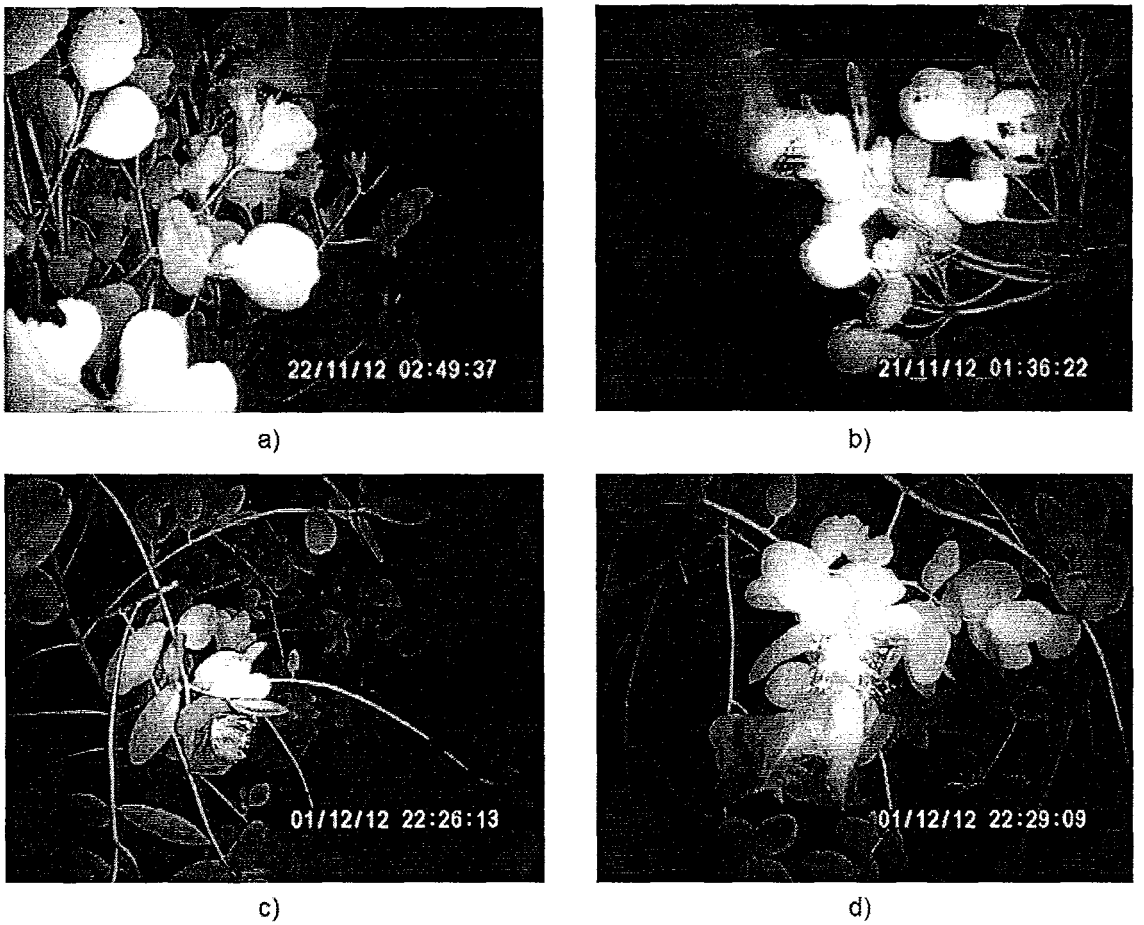
visit was complete, the bats threw themselves off the flower before flying away (Figure 2.11). For hovering activity, bats thrust their head between the stamens up to their ears while wings extended backwards to lap up the nectar, making fleeting contact with stamens and making regular contact with the stigmata (Chapter 3), before finally flying away from the flowers (Figure 2.12). All the hovering visits observed lasted > 1 s.



a) b)

c) d)

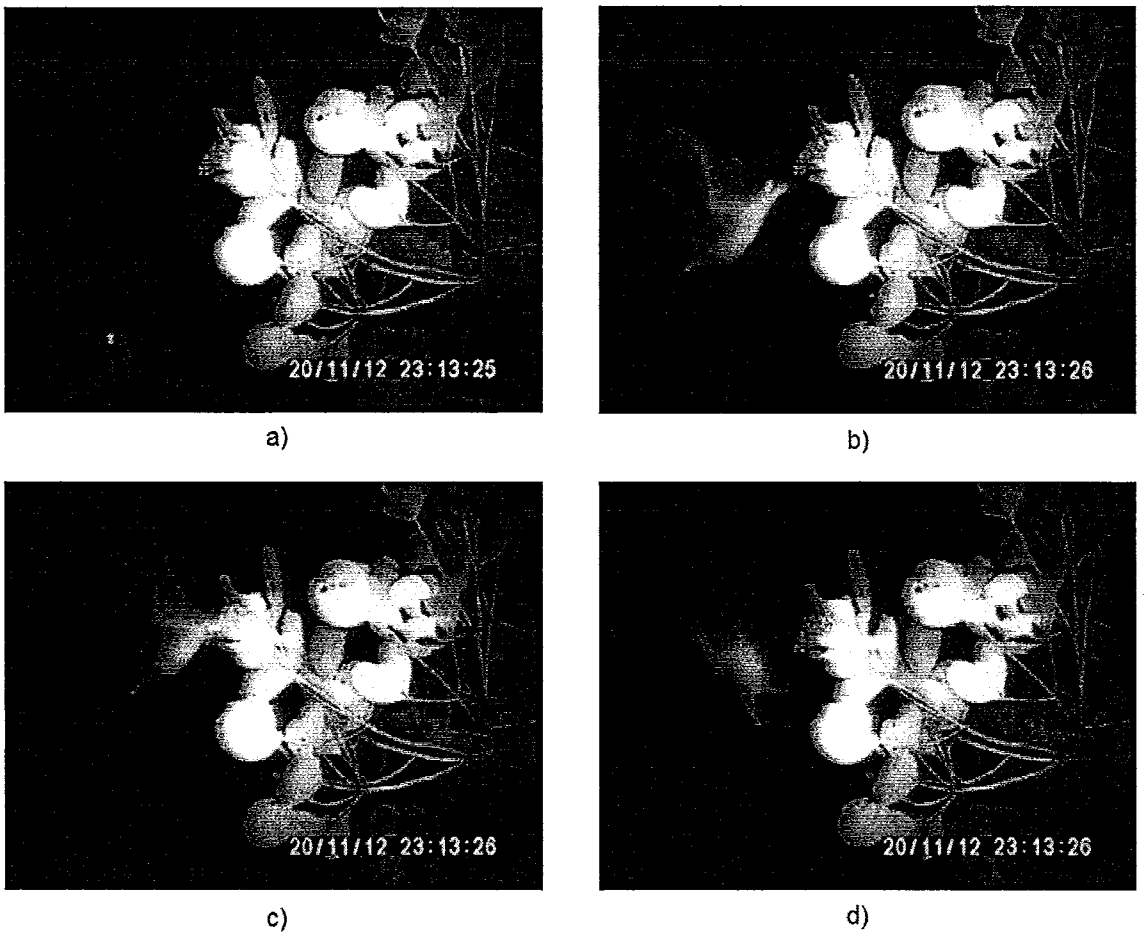
Figure 2.10 Observations of scouting activities by bats visiting *Sonneratia* flowers (in sequence). a) Bat flying at close proximity to the flower b) Pausing in flight at the flower c) Thrusting their head in front of the flower d) Flying away from the flower.



a) b)

c) d)

Figure 2.11 Observations of bats landing for feeding at *Sonneratia* flowers. a) Bat thrusting its snout into a flower to explore the nectar b) Landing with feet c) Clinging to the flower while lapping for nectar d) Launching from the flower before flying away.



a) b)

c) d)

Figure 2.12 Observations of hovering activities by bats visiting *Sonneratia* flowers (in sequence). a) Bat flying at close proximity to the flower b) Pausing in flight at the flower c) Thrusting their snout to lap up the nectar with the wings extended backwards d) Flying away from the flower.

Observations of bats visiting *S. caseolaris* flowers showed 45 feeding events (including three hovering visits) and 28 scouting activities from eight flowers recorded during two recording nights. The highest number of bat activities observed for a single flower was 27, consisting of 18 feeding and nine scouting events. The second highest number of activities recorded was 23, with 13 feeding and 10 scouting events. The number of activities observed for other flowers was very low (between two to nine), and two flowers showed only a single activity (feeding). For other flowers with more than one activity observed, one recorded seven and two feedings while the other two recorded a single feeding. At four of these six flowers activity started with at least one

scouting event before feeding on the flowers. The longest duration of bat feeding was 4 s (N = 2) and a single occasions lasted for 3 s.

Bats visiting *S. alba* showed 23 feeding events (including four hovering visits) and 44 scouting activities from 10 flowers recorded over five nights. The highest number of bat activities for a single flower observed was 15, with only two feeding and 13 scouting events. The highest number of feeding events recorded for a single flower was six, with eight scouting events. One flower recorded only a single activity (scouting), while for the other seven flowers, feeding events occurred between one to three times, with scouting events ranging from 1-9. From the total flowers observed for *S. alba*, only one flower recorded landing without scouting by bats. Compared with *S. caseolaris* flowers, the longest visit was 3 s (N = 1), and four landings recorded 2 s. Whether successive visits were made by the same bat was not determined. Figure 2.13 shows the visitation patterns (from both feeding and scouting of the flowers) of each *Sonneratia* species (mean number of visits $\pm$ SE), with the pattern of visits to the three flowers that received the most visits for each species. The highest activities were recorded between 20.00 h to 24.00 h for *S. caseolaris* and between 21.00 h to 03.00 h for *S. alba* (further analysis on hourly visitation patterns by bats and other floral visitors are in Chapter 4).

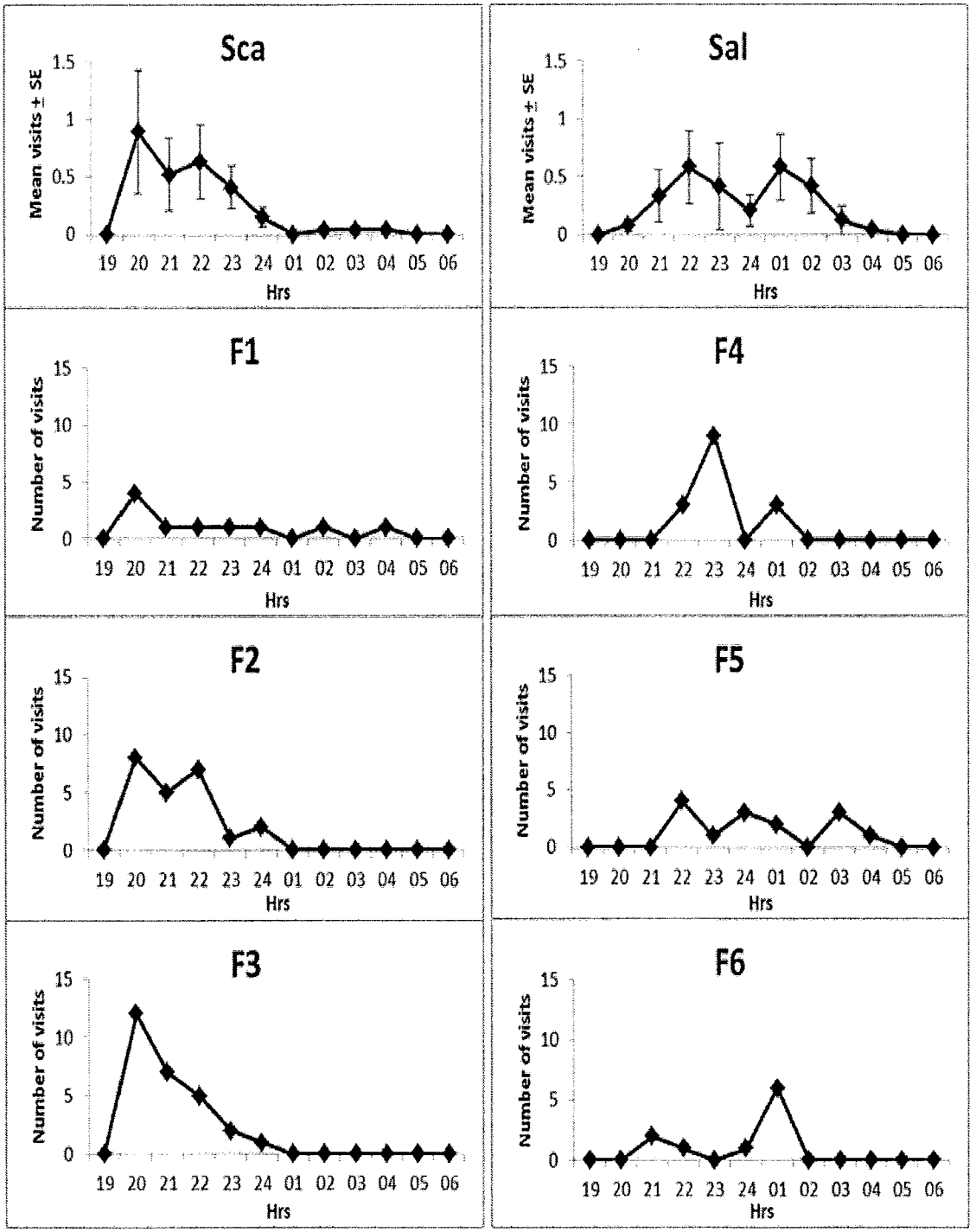


Figure 2.13 Visitation patterns made by the bats to the *Sonneratia* flowers (F1, F2 and F3 are for *Sonneratia caseolaris* flowers while F4, F5 and F6 are for *S. alba* flowers). Sca = *S. caseolaris* (N = 27) Sal = *S. alba* (N = 24). SE calculated from total flowers observed for each species.

2.4 Discussion

2.4.1 Flower-visiting bats as pollinating agents at the Setiu mangrove areas

The number of fruit bat species (Pteropodidae) recorded in this study is lower but broadly comparable with other selected mangrove areas of Peninsular Malaysia such as Larut Matang, Perak (four species, including the island flying fox, *Pteropus vampyrus*), Balik Pulau (five species) and Pantai Acheh (six species) in Pulau Pinang (Shahrul Anuar *et al.*2006; Shahrul Anuar *et al.* 2005). Of the three species (Table 2.3), only *E. spelaea* is considered truly nectarivorous (Start and Marshall 1976), feeding almost exclusively on nectar and in turn probably pollinating the tree species. For the two common species caught visiting the *Sonneratia* flowers at my study sites, not only is *E. spelaea* larger in size (body mass), the wing span (0.4247 $\pm$ 0.0175 m, N = 11) and wing areas (0.02911 $\pm$ 0.00231 m^2, N = 11) are also larger compared with the wing span (0.3734 $\pm$ 0.0167 m, N = 28) and wing area (0.02301 $\pm$ 0.0019 m^2, N = 28) recorded for *C. brachyotis* (Hodgkison *et al.* 2004). *E. spelaea* is a cave dweller while *C. brachyotis* roosts under large leaves of trees, especially palms (Francis 2008; Kingston *et al.* 2006). The feeding roosst and day roosts of *C. brachyotis* are often located about 2-2000 m from fruiting trees (Tan *et al.* 1999).

As well as *E. spelaea*, the *Sonneratia* tree is also visited elsewhere in Malaysia by another specialised nectar-feeding bat, *Macroglossus minimus* (long-tongued nectar bat) (Watzke 2006). The other specialised nectar-feeding bat recorded in Malaysia is *M. sobrinus* (long-tongued fruit bat), which feeds mainly on nectar and the pollen of wild bananas, *Musa* sp. In contrast to *M. sobrinus, M. minimus* is a coastal species, has never been recorded away from mangrove areas and feeds almost exclusively on *Sonneratia* trees. However, neither *Macroglossus* species were recorded in Setiu during my study. Whereas *E. spelaea* travels long distances, up to 38 km in a night to forage for food, *Macroglossus* species roost close to their food resources, only about 2 km, and do not commute long distances to feed (Start and Marshall 1976). Based on

nettings conducted at the study areas since 2009 (for a preliminary study) and their small travelling distance from the roosting site, I suggest that *M. minimus* is probably absent at my study sites.

The other bat species caught in this study, *C. brachyotis* and *R. amplexicaudatus,* feed mainly on fruit (Francis 2008; Tan *et al.* 1998). However, based on the pollen collected from their bodies, both bat species visited flowers for food, including the *Sonneratia* trees (Table 2.4). Both species had pollen grains of *S. ovata* in their faeces, and both species were caught in the vicinity of *S. ovata* trees (Table 2.11 and Figure 2.8). The other pollen grains recorded in their faeces were from *O. indicum* for *C. brachyotis* and *Durio* sp. for *R. amplexicaudatus.* However, only one faecal sample was collected from *C. brachyotis* and *R. amplexicaudatus*; therefore, the importance of floral resources (nectar, pollen and flower parts) in the diets of these bats could not be documented comprehensively.

All faeces collected from *E. spelaea* however showed the presence of pollen grains (including two samples containing empty pollen shells only) indicating the importance of pollen in their diet. Pollen (31 types) was also the major component in *E. spelaea* diets based on the collections of guano from their roosting sites (caves) in Peninsular Malaysia by Start and Marshall (1976). Watzke (2006) in his study at Selangor mangrove areas (west of Peninsular Malaysia), recorded six pollen types collected from *E. spelaea* bodies. In this study at Setiu, *E. spelaea* were found to carry 11 pollen types, of which nine were recorded to be also consumed (i.e. all apart from *Ceiba pentandra* and *M. cajuputi* pollen). All the plant species whose pollen was carried and consumed by *E. spelaea* (Table 2.4 and Table 2.11) were also recorded by Watzke (2006), showing that this bat visits and utilises these plant species as food. Year-long observations on the diet of *E. spelaea* in Thailand concluded that the pollen grains of *Parkia* and *Musa* were the most important food source for this bat in nearly every month (Bumrungsri *et al.* 2013). The presence of empty pollen shells in bat

faeces (Figure 2.9) showed that this bat has the ability to extract the contents of pollen grains and indicates consumption of pollen grains on a regular basis (Herrera and Del Rio 1998; Mancina *et al.* 2005).

All three bat species caught in this study carried pollen grains on their bodies, indicating their important role as pollinating agents at the study areas. From the 151 body swabs conducted, only four individuals (two individuals each of *E. spelaea* and *C. brachyotis*) were negative for pollen load (Table 2.4). As *E. spelaea* travel long distances in a night to feed (Start and Marshall 1976), in addition to visiting the *Sonneratia* trees, the bats also visited plant species away from the mangrove ecosystems, and most of these were of economic importance (Bumrungsri *et al.* 2013; Fujita and Tuttle 1991). Despite the pollen on these plant species making up a small proportion of pollen grains recorded from their bodies (Table 2.8), the bats still have economic importance as pollinators.

Other than the bat-pollinated plants, pollen grains of the non-bat-pollinated plant species (*Acacia* sp., *M. cajuputi* and *Eugenia* sp.) were also found attached to the bats' bodies and two were also ingested (*M. cajuputi* recorded only from body swab) (Table 2.11). Bumrungsri *et al.* (2013) and Start and Marshall (1976) from their studies in Thailand and Malaysia respectively, also reported the presence of *Eugenia* spp. pollen grains (*E. malaccensis* in the case of Start and Marshall (1976)) in *E. spelaea* faeces and concluded that this pollen grain was one of the main dietary components of the bat species. However, this does not mean that these plant species are pollinated by the bats, as the three species are abundant at the study areas, and contamination of pollen from the plants might occur while the bats manoeuvre in areas where these plant species are abundant, particularly for the anemophilous pollen grains (pollen distributed by wind) (Hevly 1979). Of the three species, *Acacia* sp. and *Eugenia* sp. were found both on *E. spelaea* bodies and in faeces. Structural specialisations of the

hairs of bats that feed on plants might aid in the attachment of pollen grains to their bodies (Howell and Hodgkin 1976).

E. spelaea is believed to be a specialist nectar feeder, consuming only nectar and pollen (Gould 1978; Start and Marshall 1976). In my study however, insect fragments were observed in nine of the 42 faecal samples (Table 2.10). The ingestion of insects by the nectar-feeding bats (Herrera *et al.* 2001; Nowak 1994; Tschapka 2004) might not indicate insectivory or perhaps only accidental consumption (Marshall 1983; Start and Marshall, 1976). Accidental consumption is possible as insects such as ants (Family Formicidae) and moths (Family Lepidoptera) were occasionally seen in close vicinity to the *Sonneratia* flowers probably to rob the nectar and pollen (Chapter 3). However, laboratory observations on several species of nectar-feeding bats indicated that insect consumption by these bats does not appear to be opportunistic. Pallas's long-tongued bat *Glossophaga soricina* (Family Phyllostomidae) actively hunts for insect prey (Clare *et al.* 2013), while observations on two *Pteropus* species (*P. livingstonii* and *P. rodricensis*) concluded that these bats also prey on insects (Courts 1997) to obtain sufficient protein (Courts 1998). This behaviour was also observed in captive-bred *P. rodricensis* individuals that had no prior access to insects, further indicating that this behaviour is innate rather than an artefact of captivity.

2.4.2 Pollen types carried and deposited onto the stigmata as an index of pollinator effectiveness ('quality' component)

The bats caught in this study visited several flower species as demonstrated by the mixed pollen loads on their bodies at the time of their capture (Table 2.5). The flower-visiting bats commonly carried several pollen species on their bodies (Heithaus *et al.* 1975; Muchhala and Jarrin-V 2002; Watzke 2006), from their promiscuous foraging on flowers (Sazima *et al.*1999). However, all bat species in my study predominantly carried *Sonneratia* pollen grains (*S. caseolaris, S. alba, S. ovata* and *Sonneratia* sp.) on their bodies (Table 2.8). Small quantities of other pollen types carried by bats

indicated little opportunity for pollen wastage through deposition on other species (Law and Lean 1999). Therefore, from the total number of conspecific pollen grains collected from their bodies while visiting *Sonneratia* flowers, *E. spelaea* is likely to be a more important pollinator of *Sonneratia* species than *C. brachyotis* (Table 2.9).

The distribution of pollen grains on the bats' bodies (highest number of pollen grains at the wing surfaces, Table 2.8) is related to the bats' foraging techniques, as the bats mainly land on flowers (Figure 2.11) instead of hovering in front of them as do many smaller phyllostomids in the New World (Fleming 1982; Fleming *et al.* 2009; von Helversen and Winter 2003). It is also related to the high surface areas of the wings compared to the head and body. The effectiveness of the common blossom bat, *Syconycteris australis* (Family Pteropodidae), as a pollinator of the bumpy satinash (*Syzigium cormiflorum*, Family Myrtaceae) was observed from their foraging behaviour (Law and Lean 1999). Compared to the lesser contact made by birds while hovering in front of the flowers to probe them with their elongated bills, bats landing on flowers were found to carry six times more pollen grains than the birds, making the bats potentially more effective pollinators. As large and mixed parent pollen loads frequently resulted in higher quality progeny (Niesenbaum 1999), extensive pollen carrying by the bats therefore increases the probability of deposition of numerous pollen grains onto the stigmata of flowers. If the number of pollen grains deposited on stigmata was high enough to promote sexual selection, then the service of bats as pollinators could also lead to fitter offspring if the plants select particular qualities in pollen (Nassar *et al.* 1997). Even though the bats collected numerous pollen grains on their wings while visiting *Sonneratia* flowers, the activities during landing however resulted probably only in pollen grains from their face and body being deposited onto the stigmata of the flowers, wasting the large amount of pollen grains on their wings.

Despite carrying numerous pollen grains on their bodies, each visit by bats to the flowers deposits only a small number of pollen grains onto the stigmata of the

flowers. For *S. caseolaris* flowers, pollen deposited on the stigmata per visit by bats (from stigmata collected after the first visit by bats) was significantly lower than the stigmata collected after the blooming night (Table 2.12), suggesting repeated visits by bats to the same flower throughout the blooming night. These results are consistent with the higher number of feeding visits by bats on the *S. caseolaris* flowers which was between 1-18 (N = 8), where two flowers received 13 and 18 feeding visits and the other six received < 10 visits. Therefore, the greater number of pollen grains deposited on stigmata is a result of the greater number of pollinator visits to the same flowers (Quesada *et al.* 2004). On their first visit to the *S. caseolaris* flowers, bats deposited relatively equal numbers of conspecific and heterospecific pollen grains onto the stigmata; however, multiple visits by bats to the flowers throughout the blooming night resulted in relatively more heterospecific pollen being deposited onto the stigmata (Table 2.13). This is consistent with a study by Muchhala *et al.* (2008) that reported multiple visits to the same flowers reduced the quality of bats as pollinating agents as they may deposit relatively more heterospecific pollen onto the stigmata of the flowers.

From personal observations however, stigmata collected after the blooming night were saturated with pollen grains compared to stigmata collected after the first bat visited the flowers, and this trend was more apparent for *S. caseolaris* stigmata than *S. alba* (Figure S2.3). For *S. alba* flowers, the total number of pollen grains (Table 2.12) and the number of conspecific pollen grains (Table 2.13) deposited on the stigmata was almost equal both after the first bat visit and after the blooming night. This result is in accordance with the visitation rate of bats to the flowers, which was usually lower during the anthesis period. *S. alba* flowers received between 1-6 feeding visits (N = 10), with the majority (eight flowers) receiving between 1-3 feeding visits only. These also suggested that bats were responsible for the majority of the pollen deposition onto the stigmata of the *S. alba* flowers despite the flowers being visited by several visitors (Chapter 3). Furthermore, *S. alba* possesses a low number of ovules in the flowers

(Chapter 3) that require only a small number of conspecific pollen grains for fertilisation. Not only that, the numbers of seeds per fruit produced for *S. alba* ranged between 39-83 (mean = 51, N = 13); therefore for this species, few visits would be sufficient to initiate fruit set (more comparisons on the *Sonneratia* breeding systems from pollen-to-ovule ratios and seed-to-ovule ratios are presented in Chapter 3).

Musa sp. pollen grains were the major pollen types deposited on the stigmata of *S. alba* flowers and the second highest in *S. caseolaris* stigmata after conspecific pollen grains (Table 2.12). From their field observations, Start and Marshall (1976) concluded that *Musa sp.* is not a plant genus of major importance to *E. spelaea*. By contrast, Watzke (2006) recorded active visitations made by *E. spelaea* to *Musa* sp. flowers despite recording *S. caseolaris* pollen grains as the major pollen grains collected from their bodies. From the presence of pollen grains in faeces collected in nearly every moth of their study in Thailand, Bumrungsri *et al.* (2013) also concluded *Musa* sp. to be the major food source of *E. spelaea*. Gould (1978) found the trap-line feeding strategies shown by the nectarivorous bats in Malaysia typify schedule visits with visitations early in the evening to certain plants including *Musa* sp. My results suggest that *E. spelaea* may be an important pollinator of *Musa*.

2.4.3 Visitation rates as an index of pollinator effectiveness ('quantity' component)

In this study, two types of feeding behaviour were identified, which were landing on the flowers and hovering in front of the flowers. The landing visits were the more common behaviour observed (90 % from the 68 feeding visits) for these large and heavy pteropodids visiting the *Sonneratia* flowers. The bats were observed landing on and clinging to the flowers while lapping nectar, coating their ventral bodies (particularly the chest and belly) and wings with pollen, and also making contact with the stigmata (Figure 2.11). For the hovering visits which were observed for only 10 % of the total feeding visits recorded, bats thrust their heads (up to their ears) into the flowers to search for nectar at the base of the flowers, briefly making contact with the stamens,

covering their faces with pollen grains before flying away (Figure 2.12). Other than these feeding visits, bats were observed to scout the flowers by making flight passes close to the flowers without making contact with them (Figure 2.10). In Chapter 4, I reported more frequent scouting visits by bats was observed before midnight for both *S. caseolaris* and *S. alba* flowers. According to Horner *et al.* (1998), bats spend the early part of the evening visiting plants without feeding to determine the availability and locations of open flowers and to ascertain good feeding sites; they then do most feeding later, after flowers have accumulated substantial amounts of nectar. This reconnaissance pass might occur repeatedly over the night between visits (Sazima *et al.* 1999) to assess whether a visit to a given flower would be profitable (Sazima *et al.* 1994).

All the stigmata collected after the first bat visited the flowers were positive for pollen grains (Table 2.13), indicating that the bats effectively transferred the pollen grains from their bodies to the stigmata while visiting the flowers to feed, presumably these bats carried the pollen grains of flowers visited beforehand. Even with minimal body contact at the inflorescence while hovering, and the lower number of pollen grains attached to their bodies compared to their perching counterparts, hovering glossophagine bats show high pollination efficiency by frequently switching visits between receptive female phase and male phase inflorescences (Tschapka 2003). In my study, the hovering visits were brief and rarely observed. Nevertheless, pollen deposition by the bats while hovering in front of the flowers to feed might occur given the central position of the stigmata in the flowers surrounded by numerous anthers (more discussion on flower morphology and its pollination is presented in Chapter 4).

Visitation to the *Sonneratia* flowers by bats in this study however was very low compared with the visitation rates reported for bats in Thailand (Bumrungsri *et al.* 2008; Bumrungsri *et al.* 2009; Srithongchuay *et al.* 2008) at the flowers of durian (*D. zibethinus*), two species of bitter bean (*Parkia speciosa* and *P. timoriana*) and Indian

trumpet (*O. indicum*). Lower visitation rates observed for the *Sonneratia* flowers might be due to the abundance of available flowers (recordings were conducted during the peak flowering events, see Chapter 4 for the phenological study); therefore bats may visit more flowers on different plants while visiting each flower less frequently. The same pattern of bat visitations was observed by Arias-Coyolt *et al.* (2006) to the bat-pollinated cactus, *Stenocereus stellatus* (Family Cactaceae). In their study, the total population-wide floral visitation was greater at the sites with more flowers available, but individual flower visitation rates were low because more flowers are available. Therefore on a given night, bats might visit more flowers on different plants while visiting each flower less frequently. If this were the case for *Sonneratia* flowers, even though receiving low visitation rates, might potentially receive pollen from a range of different flowers, increasing genetic diversity of the pollen received. This however contradicts the report by Horner *et al.* (1998) that noted no correlation between visitation rates and the number of open flowers of columnar cacti and organ pipe, or the number of open flowers in a plant's neighbourhood. Nor did the bats spread their flower visits evenly among the flowers they visited. Instead, the visits to the flowers were clumped among plants and flowers, in which many flowers received no or very few visits, whereas a few received many visits. The trees in spatially isolated habitats on the other hand have a tendency to receive high visitations from bats, however they may produce singly-sired fruits because bats tend to forage within the same tree where nectar is concentrated (Fuchs *et al.* 2003).

Even though high visitation rates may indicate the effectiveness of pollinators (Arias-Coyotl *et al.* 2006; Quesada *et al.* 2003), Srithongchuay *et al.* (2008) suggested that a single visit by bats to the flowers of Indian trumpet, *O. indicum* is sufficient to initiate fruit set. Despite the low visitation rates to flowers by two nectarivorous bat species in Australia, Crome and Irvine (1986) suggested that the bats accounted for nearly three times the amount of fruit set compared with four species of honeyeater

birds visiting the bumpy satinash, *S. cormiflorum.* Moreover, visitation rates may reflect the local abundance of pollinating species rather than the attractiveness of the plant to the pollinator. Law and Lean (1999) reported bats carrying six times more pollen than birds, although bats were less frequent visitors to *S. cormiflorum*. Regular visits by birds however were found to compensate for their moderate pollen loads and effectively reduce differences between bats and birds in terms of quantity of pollen reaching a flower.

Under a trap-line feeding strategy, repeated sequential visits to the same feeding sites by the same animals occur. In my study, I cannot confirm that all visitations to the flowers were made by either *E. spelaea* or by the same individuals visiting the same flowers. *E. spelaea* is known to employ a trap-line foraging strategy (Gould 1978; Srithongchuay *et al.* 2008), visiting a series of feeding locations and promoting outcrossing in the plants visited (Heithaus *et al.* 1974; Horner *et al.* 1998). Repeated visits to the flowers by nectarivorous bats always result in successful transfer of conspecific pollen grains, but may also result in stigma blockage by transfer of foreign pollen, subsequently reducing the reproductive success of the plant (Armbruster and Herzig 1984; Bell *et al.* 2005; Caruso and Alfaro 2000; Fishman and Wyatt 1999) by reducing the chances of subsequently deposited conspecific pollen to fertilise the ovules. Multiple visits to the same flower or plant also might result in geitonogamous crosses and set no fruit in self-incompatible plants (Arias-Coyotl *et al.* 2006; Quesada *et al.* 2004).

Even though pteropodid bats usually land while feeding on flowers (Fleming 1982; Fleming *et al.* 2009), a hovering feeding technique was reported in the short-nosed fruit bat (*Cynopterus sphinx*) feeding on *C. pentandra* flowers by Singaravelan and Marimuthu (2004). As with my study, this behaviour was recorded for short durations and only on few visits (8 % in their study and 10 % in my study from the total bat visits recorded) compared with landing behaviour. Flower visiting behaviour may

contribute to the bats' effectiveness as pollinating agents due to the differences in pollen delivery per visit (Frick *et al.* 2013). Bats landing on flowers recorded longer durations of visits hence were more successful in pollen delivery to the stigmata of the flowers compared to the shorter duration of visits by hovering bats. However, no assessment was made in their study to compare the effect of visitation time on the plant fitness to further confirm the effectiveness of bats as pollinators.

In my study, bats feeding on the flowers made brief visits, usually 1 s for landing and < 1 s for hovering visits. The short duration of the feeding visit is a behavioural trait characteristic of the flower-visiting pteropodid bats (Nathan *et al.* 2009; Singaravelan and Marimuthu 2004; Srithongchuay *et al.* 2008). In terms of pollination success, Tschapka (2003) observed no correlation between the fruit initiation rate with total visit duration or number of visits to the flower, and concluded that the intensity of contact between inflorescence and pollinator (number of stigmata touched) and especially the previous flower visits of the visitor are more important than simply the number and duration of visits. From the differences in time spent visiting flowers between the two bat species observed which differ in their effectiveness as pollinating agents, Quesada *et al.* (2004) concluded that the time spent at the flowers per visit is not necessarily an indicator of the frequency of contact with the reproductive parts.

2.4.4 Conclusions

All the flower-visiting bats (apart from four individuals) caught in this study showed the presence of pollen grains in their bodies. About 94 % of the total pollen grains the bats carried were from *Sonneratia* species, strongly suggesting that bats, especially *E. spelaea*, are important pollinators of mangroves in Setiu. From the number of pollen types and conspecific pollen grains they carried, and their abundance in the area, *E. spelaea* is likely to be the more important pollinator for the mangrove trees of the genus *Sonneratia*. On each visit, the bats successfully transferred pollen grains from their bodies to the stigmata of the *Sonneratia* flowers by landing on the flowers or hovering

in front of the flowers to feed for nectar. *S. caseolaris* flowers recorded higher visitation rates compared to *S. alba* flowers. However, multiple visits by bats to the *S. caseolaris* flowers may reduce their effectiveness as pollinating agents, yet this may be countered by high pollen-stigmata adhesion of conspecific pollen grains after 12 hrs of anthesis . For *S. alba* flowers, the similarity of pollen loads on the stigmata collected after the first bat visited the flower as well as after the blooming night indicates that bats are principally responsible for pollen depositions to the stigmata of this flower, further confirming their role as effective pollinators for *S. alba.* Based on the number of ovules (as reported in Chapter 3), I suggest that a single visit by bats to this flower should be sufficient to initiate fruit set for this species.

2.5 Supplementary material

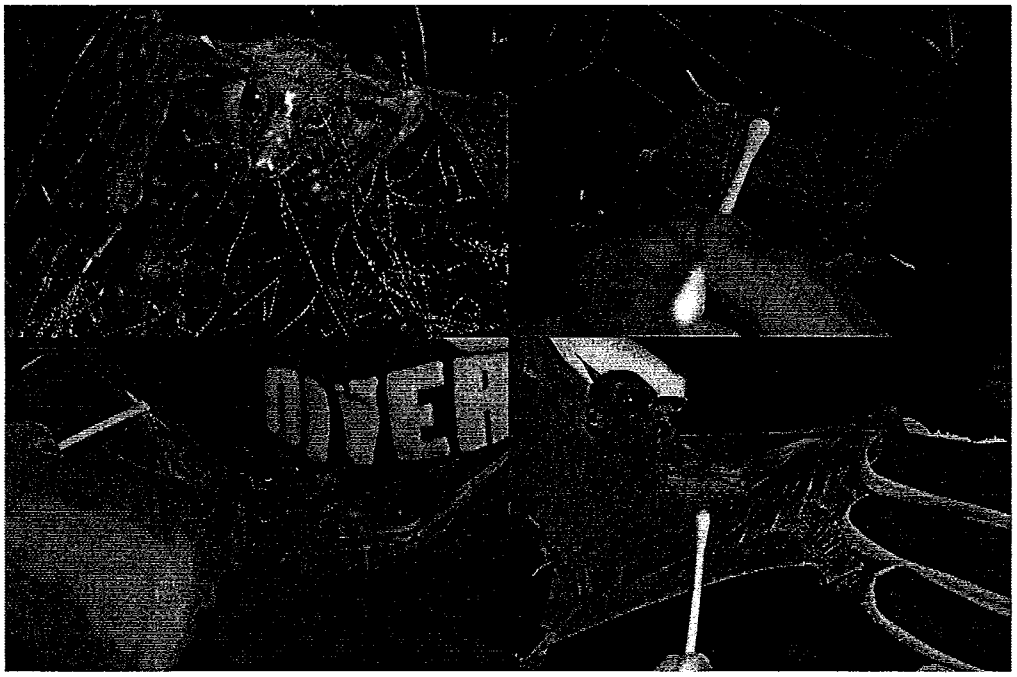


Figure S2.1 Pollen collection conducted by swabbing different body regions with a cotton wool bud.

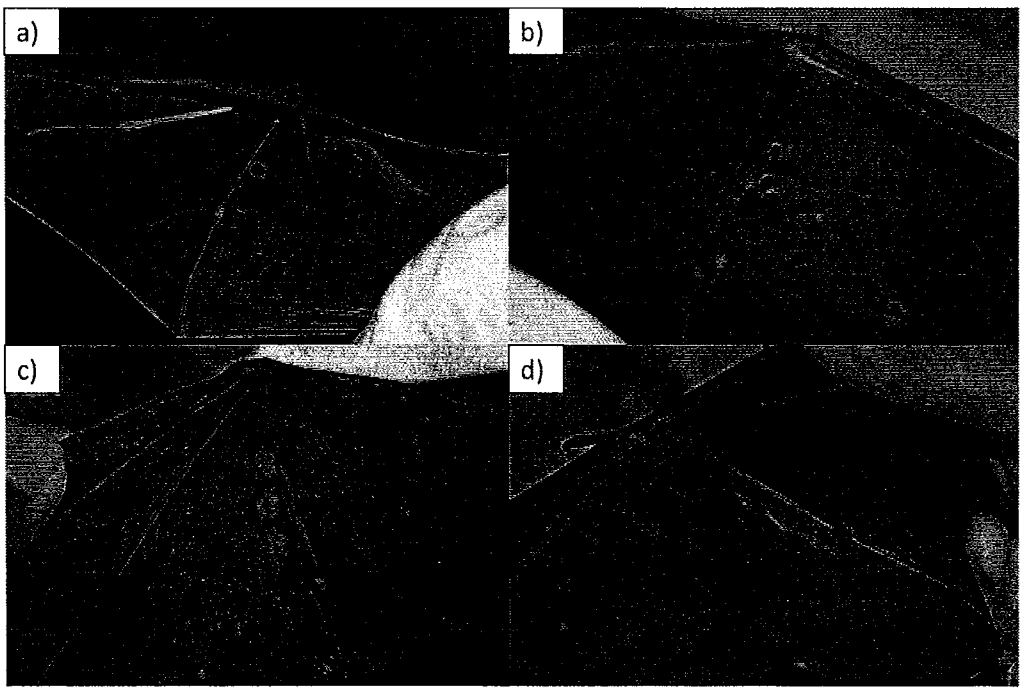


Figure S2.2 Scars from wing biopsies used for individual recognition of bats. a) Punch mark at the wing membrane immediately after wing biopsy b) The wing punches after a week c) Healing scars after three weeks d) Unpigmented scars after four weeks.

Table S2.1 Number of bats carrying each pollen type at the time of capture (for bats captured visiting *Sonneratia caseolaris* flowering trees). Percentages in parentheses.

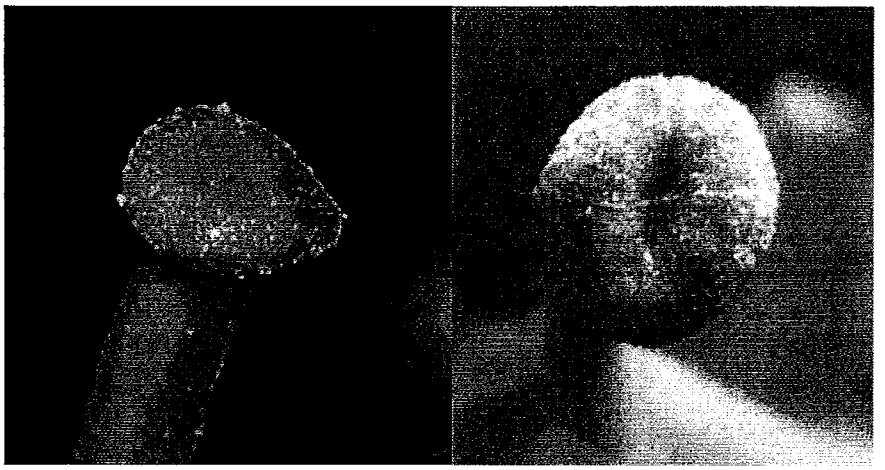
Bat species/ Pollen type	*Sonneratia*			*Parkia* sp.	*Musa* sp.	*Durio* sp.	*Ceiba pentandra*	*Oroxylum indicum*	*Eugenia* sp.	*Acacia* sp.	*Melaleuca cajuputi*	Zero
	caseolaris	*alba*	*ovata*									
E. spelaea (N = 37)	37(100.0)	7(18.9)	11(29.7)	19(51.3)	11(29.7)	7(18.9)	7(18.9)	4(10.8)	1(2.7)	1(2.7)	9(24.3)	0(0.0)
C. brachyotis (N = 15)	15(100.0)	1(6.7)	2(13.3)	6(40.0)	4(26.7)	0(0.0)	1(6.7)	2(13.3)	0(0.0)	0(0.0)	3(20.0)	0(0.0)
Total	52(100.0)	8(15.4)	13(25.0)	25(48.1)	15(28.8)	7(13.5)	8(15.4)	6(11.5)	1(1.9)	1(1.9)	12(23.1)	0(0.0)

Table S2.2 Number of bats carrying each pollen type at the time of capture (for bats captured visiting *Sonneratia alba* flowering trees). Percentages in parentheses.

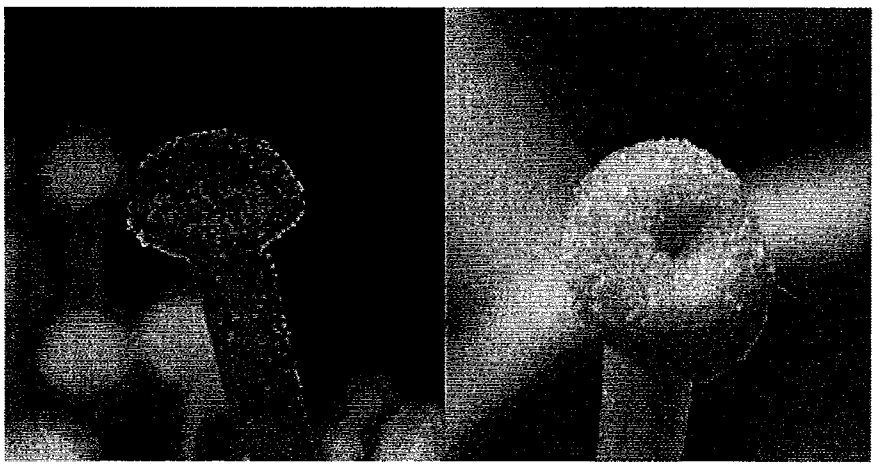
Bat species/ Pollen type	*Sonneratia*			*Parkia* sp.	*Musa* sp.	*Durio* sp.	*Ceiba pentandra*	*Oroxylum indicum*	*Eugenia* sp.	*Acacia* sp.	*Melaleuca cajuputi*	Zero
	caseolaris	*alba*	*ovata*									
E. spelaea (N = 38)	5(13.1)	35(92.1)	4(10.5)	3(7.9)	4(10.5)	5(13.1)	2(5.3)	12(31.6)	6(15.8)	0(0.0)	0(0.0)	2(5.3)
C. brachyotis (N = 3)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	2(66.7)
Total	5(12.2)	36(87.8)	4(9.8)	3(3.7)	4(9.8)	5(12.2)	2(4.9)	13(31.7)	6(14.6)	0(0.0)	0(0.0)	4(9.8)

Table S2.3 Number of bats carrying each pollen type at the time of capture (for bats captured visiting *Sonneratia ovata* flowering trees). Percentages in parentheses.

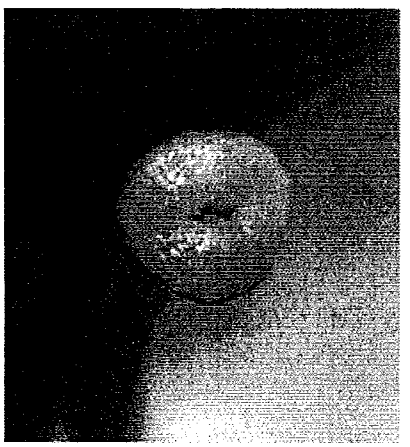
Bat species/ Pollen type	*Sonneratia*			*Parkia* sp.	*Musa* sp.	*Durio* sp.	*Ceiba pentandra*	*Oroxylum indicum*	*Eugenia* sp.	*Acacia* sp.	*Melaleuca cajuputi*	Zero
	caseolaris	*alba*	*ovata*									
E. spelaea (N = 55)	0(0.0)	0(0.0)	55(100.0)	17(30.9)	6(10.9)	15(27.3)	1(1.8)	4(7.3)	0(0.0)	3(5.4)	0(0.0)	0(0.0)
C. brachyotis (N = 2)	0(0.0)	0(0.0)	2(100.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
R. amplexicaudatus (N = 1)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	0(0.0)	0(0.0)	58(100.0)	19(32.8)	6(10.3)	17(29.3)	1(1.7)	5(8.6)	0(0.0)	3(5.2)	0(0.0)	0(0.0)



a) b)



c) d)



e)

Figure S2.3 Stigmata of i) *Sonneratia caseolaris* collected a) after first visit by bats and b) after the blooming night, ii) *S. alba* collected c) after first visit by bats and d) after the blooming night. e) Sticky stigmatic surface of unvisited stigmata.

The breeding system and pollinating agents of three *Sonneratia* species in mangrove habitats in Peninsular Malaysia

Abstract

Measuring pollen-to-ovule ratios (P/Os) is a conservative method for determining the likely breeding system of plant species. However, little evidence exists on the pollination efficiency of the various animal vectors. I examined the P/Os of three *Sonneratia* species to determine their breeding system, and conducted pollination exclusion experiments to evaluate the relative importance of a range of floral visiting taxa by quantifying the fruits and seeds produced in relation to the type of pollinator excluded. As the seed-to-ovule ratio (S/O) expresses the proportion of ovules developing into seeds, I also used S/Os to determine the efficiency of each flower visitor as a pollinator. P/O ratios suggested that all the three *Sonneratia* species should show obligate autogamy (i.e. be restricted to self-fertilisation). Exclusion experiments showed that *Sonneratia caseolaris* is self-compatible yet cross-pollination increased fruit and seed sets. Insect pollination however produced fruit and seed sets only half of the results from to bat pollination. Observations on nocturnal visitors showed large moths (> 20 mm body length), such as sphingids, making regular legitimate visits (by touching anthers and stigmata) to the flowers of *S. alba*. The ability to extract nectar with their long proboscis however limits their effectiveness as pollinators for this species. Bats, on the other hand, are the most important pollinators for both *S. caseolaris* and *S. alba* trees due to their feeding behaviour and body size, effectively contacting both the anthers and stigmata while visiting the flowers to feed.

3.1 Introduction

3.1.1 Plant breeding systems

Quantifying pollen-to-ovule ratios (P/Os) is a conservative way of predicting the breeding system of a plant species (Cruden 1977). The ratios were determined by dividing the estimate of the number of pollen grains per flower by the number of ovules per flower. Charnov (1982) however argued that the P/O ratio might be a result of local mate competition, with resource allocation ratios in hermaphroditic plants being driven by the male/female gain for the plant fitness. Furthermore, Preston (1986) suggested that interpretation of P/O ratio must take into account factors, other than just the breeding system, and any study that includes P/O ratios must discuss the criteria used to evaluate them. P/O ratios however have been widely used as a rough estimate to predict plant breeding systems. For example, Galloni *et al.* (2007), Jurgens *et al.* (2002) and Mione and Anderson (1992) determined the breeding systems of 32 species of legumes (Fabaceae), 79 species of Caryophylloidae and 22 species of Solanaceae respectively, from the P/O ratios.

Indeed, the P/O ratio is a better indicator of a plant's breeding system than floral size and morphology. The P/O reflects the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set. The more efficient the transfer, the lower the P/O should be. Therefore in selfing species, the P/O is lower as the physical distance between the anthers and the stigmata is much shorter and overcoming this distance is less complicated. The P/O in outbreeding plants on the other hand is much higher which ensures efficient pollination by pollen vectors such as animals, wind and water. Although the P/Os are substantially higher in wind-pollinated plants than in animal-pollinated species, little evidence exists on the pollination efficiency of the various animal vectors (Cruden 2000). Besides that, P/O also reflects floral reward type in which species offering only nectar had significantly lower P/O than the species offering only pollen or pollen and nectar (Etcheverry *et al.* 2012). For the flowers that provide pollen as part or all of the reward, the P/O of plants that provide only pollen is

higher than in those that provide nectar as well (Cruden 2000; Dulberger 1981; Mione and Anderson 1992).

Other than pollen ingestion by pollinators, pollen loss due to grooming behaviour may explain a reduction in pollen delivery to the conspecific stigmata hence potentially leading to lowered seed set (Flanagan *et al.* 2009). Therefore, flowers must produce abundant pollen to compensate for its inefficient transfer by pollinators. Also, as cross-pollination requires pollen vectors to visit, xenogamous (cross-pollinated) flowers produce more pollen (compared to ovule number) than autogamous (self-pollinated) flowers (Cruden 1977; Jurgens *et al.* 2002; Lopez *et al.* 1999). The higher P/O ratios in plant species thus may reflect selection to maintain the pollen pool available to the pollen vectors above some critical value (Cruden 2000). However, pollen production cannot be so high that the pollen produced by one plant swamps the stigmata of the next plant visited by a pollinator. Therefore both minimal and excessive pollen production may have detrimental effects on plant fitness.

3.1.2 The importance of pollinating agents

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 they forage at is almost always just an irrelevant by-product. For plants however, this interaction may result in a positive effect on their reproductive success, and therefore is important from a conservation perspective. Reductions in pollinator abundance could have serious implications for a plant's reproductive success (Kearns and Inouye 1997), particularly for plants that are highly specialised for specific pollinators. 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 (Arizaga *et al.* 2000a; Ibarra-Cerdena *et al.* 2005; Martinell *et al.* 2010; Sazima and Sazima 1978), indicating the significant contribution made by nocturnal visitors for plant fitness. Previous studies

have shown that bats, rodents and moths are among the most important nocturnal pollinators (Baker 1961; Fleming and Holland 1998; Fleming and Kress 2011; Pellmyr *et al.* 1996; Slauson 2000).

The identity and behaviour of pollinators are among the main factors that determine the reproductive success and mating system of plants (Herrera 1987; Stebbins 1970). The identity of pollinators shapes floral characteristics that match the morphology and biology of their pollinators (Ibarra-Cerdena *et al.* 2005; Nassar *et al.* 1997; Slauson 2000; Stebbins 1970). When floral visitors forage at flowers for floral resources, deposition of pollen grains on different parts the pollinator's body will determine the subsequent deposition of pollen grains onto conspecific stigmata (Muchhala and Potts 2007). Therefore the fate of many plants may depend on preserving their mutualistic relationships with pollinators. The behaviour of floral visitors while visiting the flowers determines their role as legitimate, effective or efficient pollinators (Fleming and Sosa 1994). Some visitors are only nectar robbers or pollen thieves, taking pollen and nectar without making contact with the reproductive organs and therefore precluding the possibility of pollination occurring (Inouye 1980). Other than by grooming, inconstant pollinator movements can significantly reduce the amount of pollen that reaches conspecific stigmata and hence reduces seed set (e.g. Flanagan *et al.* 2009). Repeated visits to the flowers on the other hand increases the pollen loads on stigmata and results in an increased likelihood of successful pollination (Arias-Coyotl *et al.* 2006; Engel and Irwin 2003; Flanagan *et al.* 2009; Herrera 1987; Quesada *et al.* 2001; Quesada *et al.* 2003; Schemske and Horvitz 1984; Silander and Primack 1978; Stone 1996).

The efficiency of bats as pollinators for four species of Venezuelan columnar cacti was reported by Nassar *et al.* (1997) in which fruit:flower ratios in their pollination experiment varied from 0.46 to 0.76 when bats were allowed to visit the flower. Moreover, the number of seeds produced was also high relative to ovule number (S/O ratios), varying from 0.70 and 0.94. This result indicates that bats are depositing enough pollen on floral stigmas to fertilize most of the ovules. When bats were

excluded from visiting the flowers however, the proportion of flowers producing fruits decreased drastically, and was sometimes even close to zero.

3.1.3 Aims of study

I aim to infer the breeding system of *Sonneratia* species from their reproductive organs by measuring the P/O ratios as well as conducting pollinator exclusion experiments. I tested the hypothesis that the P/O ratios of the three *Sonneratia* species fitted with an outcrossing (xenogamy) breeding system. I also aim to assess the relative importance of bats as *Sonneratia* pollinating agents compared to other nocturnal pollinators from the pollinator exclusion experiments. The flower abortion rate, fruit sets and the quality of fruits and seeds produced from the pollinator exclusion experiments conducted on *Sonneratia caseolaris* flowers were quantified to determine reproductive success. These results, together with the rate of stigmata contact while visiting flowers from video recordings were used to determine the relative importance of bats as pollinating agents for the *Sonneratia* trees species compared with other nocturnal pollinating agents such as moths and bees. As the seed-to-ovule ratio (S/O) expresses the proportion of ovules developing into seeds, the efficiency of each pollinator from the exclusion experiments was estimated from the S/O of each pollination treatment. In addition, the S/O of *S. alba* and *S. ovata* were compared with *S. caseolaris* (from open pollination (OP treatment) or natural pollination) to infer the contribution of bats to their pollination. I tested the hypothesis that bats are relatively more important pollinators to the *Sonneratia* flowers as compared with other floral visitors.

3.2 Methods

3.2.1 Collections of flowers and fruits for breeding system determination

Breeding systems of the *Sonneratia* species were determined from the P/O ratios and S/O ratios. For P/O determinations, a total of 49 flowers (20 of *S. caseolaris*, 20 of *S. alba* and nine of *S. ovata*) were carefully plucked between 20.00 h to 21.00 h when flowers were fully opened. For *S. caseolaris* and *S. alba*, the flowers were collected

from five different trees (four flowers for each tree), while flowers of *S. ovata* were taken from three different trees (one, three and five flowers for each tree respectively). For each flower, 10 anthers were collected and preserved in 1.5 ml micro-centrifuge tubes containing 75 % ethanol to count the number of pollen grains per anther. Flowers were then kept in separate plastic containers and labelled individually. The number of anthers for each flower was determined by carefully removing the stamens from flowers for counting. The ovaries were later kept from each flower to determine the number of ovules present.

In the laboratory, pollen counts for each flower were conducted by using the same procedure used for the identification of pollen grains deposited on stigmata (Chapter 2). For each flower, the numbers of pollen grains were counted from 20 µl of ethanol. The total number of pollen grains per flower was estimated by extrapolating ethanol volume and multiplying by the number of anthers in the flower. To determine the number of ovules for each flower, ovaries were dissected under a dissecting microscope and the number of ovules was counted for each locule at 4x magnification. The P/O ratio for a flower was determined by dividing the estimated number of pollen grains by the number of ovules for the same flower. The mean P/O for each species was generated from individual ratios for separate flowers.

Collections of mature fruits of *S. alba* (13 fruits from seven trees) and *S. ovata* (five fruits from a single tree) were conducted to determine the S/O ratios of both species. For *S. caseolaris*, the estimations were made from the mature fruits collected from open pollination (OP) treatments (natural pollination) in pollination exclusion experiments (37 fruits from eight trees). The seeds were extracted by removing the fruit flesh and then washing with tap water. The seeds were later collected and dried at room temperature for 24 hrs and counted. Duke and Jackes (1987) and Goutham-Bharathi *et al.* (2012) described the shape of *S. caseolaris* seeds as angular irregular, *S. alba* seeds as falcate and *S. ovata* seeds as rounded irregular. An arrowhead shape (concave quadrilateral) however was the shape most commonly found for the three *Sonneratia* species in my study (Figure S3.1), in about 95 % of the total seeds per fruit.

Therefore, 40 arrowhead shape seeds of each fruit were selected for measurement. For small fruits and in cases where fewer than 40 seeds were found, measurements were taken from all the seeds. The measurements were taken using digital calipers to record the length and width (Figure 3.1) to an accuracy of 0.01 mm. The seed size was then presented as an index by multiplying the length and width of the seeds. The seeds were then dried in an oven at 40° C for 24 hrs. The dry mass of seeds was recorded using a digital balance (Sartorius ED 153 CWN, Scientech Inc., USA, 0.0001 g accuracy). The S/O ratios of each species were calculated by dividing the mean number of seeds per fruit by the mean number of ovules from flowers of the species. The breeding system determinations from P/O ratios and S/O ratios of the three *Sonneratia* species were conducted from the transformed data (log base 10) to achieve normality. All the analyses were conducted by using the IBM SPSS Statistics ver 19.0 (Chicago, USA).

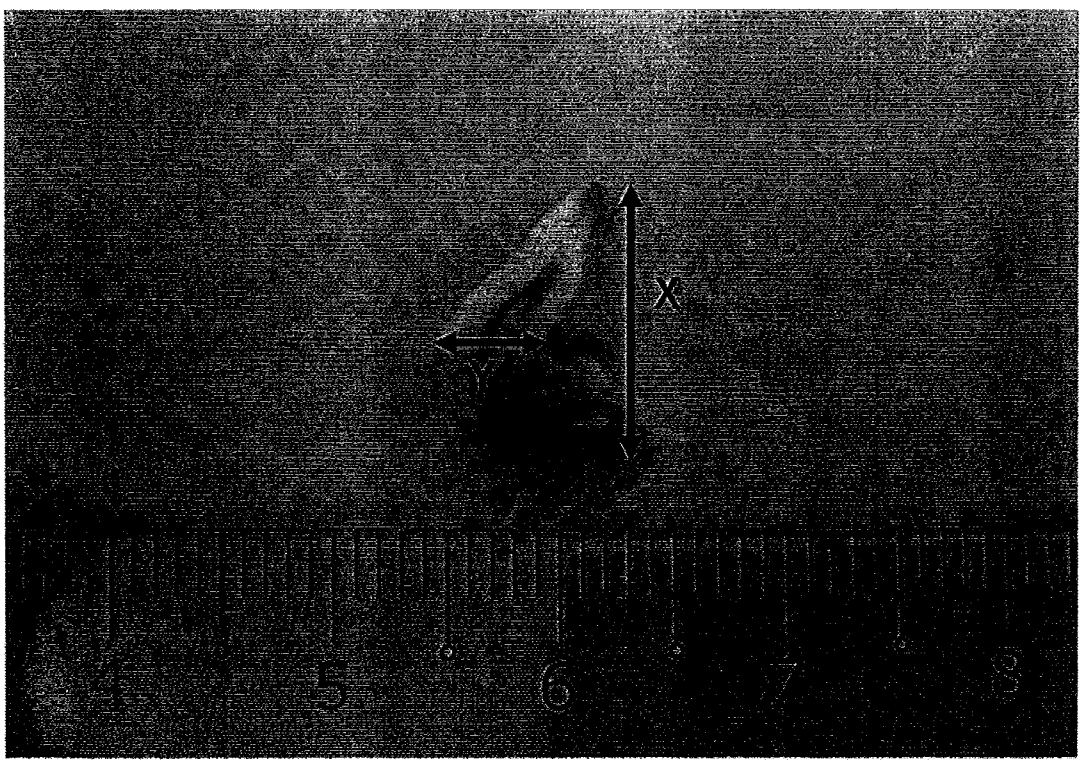


Figure 3.1 Measurements of seeds taken, length (X) and width (Y). Each major mark represents 0.5 cm.

3.2.2 *Pollination exclusion experiments on Sonneratia caseolaris*

The pollination exclusion experiments on *S. caseolaris* were conducted for six months starting from December 2011 until May 2012. These experiments were carried out on 729 flowers selected randomly from 10 trees, where at least six flowers were used for each treatment per tree (at least 36 flowers per tree) (Table 3.1). The six treatments used in this study were (1) open pollination (OP), (2) automatic autogamy (AA), (3) insect pollination (IP), (4) hand-cross-pollination (CP), (5) facilitated autogamy (FA), and (6) emasculation pollination (EP) following Bumrungsri *et al.* (2009). Potential pollinating agents and pollen sources for each treatment are as summarized in Table 3.2. The definition of mature buds and flowers were as described in Chapter 4.

Table 3.1 Total number of flowers used in pollination exclusion experiments for each tree.

Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Total
Open pollination	10	10	14	19	10	10	6	17	10	10	134
Insect pollination	12	11	19	16	11	12	6	26	11	10	116
Automatic autogamy	11	10	14	19	10	16	6	14	11	10	121
Emasculation pollination	12	10	19	21	11	14	6	15	10	10	128
Cross-pollination	10	13	17	10	10	10	6	14	10	11	111
Facilitated autogamy	10	10	13	20	10	10	6	20	10	10	119
Total	65	64	96	105	62	72	36	106	62	61	729

T = tree

Table 3.2 Potential pollinating agent and pollen source for each pollination treatment.

Treatment	Potential pollinating agents	Pollen source
Open pollination	Bats and insects	Cross-pollen and self-pollen
Insect pollination	Insects	Cross-pollen and self-pollen
Automatic autogamy	None	Self-pollen
Emasculation pollination	Bats and insects	Cross-pollen and self-pollen (from different flowers of the same trees)
Cross-pollination	Pollen placed by experimenter	Cross-pollen
Facilitated autogamy	Pollen placed by experimenter	Self-pollen (from different flowers of the same trees)

Mature buds (i.e. those that will open during the night) were selected and treated in the evening between 17.30 h and 19.00 h. For OP, mature buds were marked in the evening and left open to allow all potential pollinators to access the flowers during the blooming night. For AA treatment, mature buds were bagged individually in the evening for self-pollination to occur (automatic self-pollination whereby fertilisation occurs by pollen of the same flower). For IP treatment, mature buds were covered by a plastic cage with a 16 mm mesh in the evening, to allow access by insects but not the bats throughout the blooming night. Bumrungsri *et al.* (2008) showed that the plastic nets (of the same mesh size as in my study) allow most of the nocturnal insects to assess the flowers except the large moths with a wingspan larger than 30 mm. Personal observations however showed low visitation rates by insect visitors (including the large moths of > 20 mm body length from video recordings) to the *S. caseolaris* flowers at the study site.

Mature buds selected for CP, FA and EP treatments were marked and anthers were removed by sharp scissors in the evening. Mature buds for use in the EP treatment were left uncovered allowing access by pollinators throughout the blooming night. In EP, removal of anthers precludes the pollen grains of the same flower to reach

the stigmata for self-pollination (geitonogamy crosses are still possible from pollen grains of other flowers from the same tree). Mature buds of CP and FA treatments were bagged individually after the anthers were removed. The mature buds were visited again at night, between 21.00 h to 23.00 h (where the buds opened to fully bloomed flowers and stigmata were already receptive, Chapter 4) to pollinate the flowers by rubbing the stigmata directly with anthers of flowers from different trees for CP, and flowers from the same tree for the FA treatment. The flowers were then re-bagged to prevent visitors from accessing the flowers.

For the AA, CP and FA treatments, bagging was conducted using transparent polythene bags (30 x 40 cm) with small holes to allow only air circulation but not allowing insects and bats to visit the flowers. The bags and plastic cages were removed in the afternoon (between 12.00 h to 14.00 h) after blooming nights to prevent early morning visitors including bees (except for the IP treatment) and birds from accessing the flowers. The flowers used in the experiments were marked with paper tags for identification (individual numbering and date of treatment). The pollinated flowers were checked every day for abortion and fruit sets (fruit set was recorded on maturing fruits).

3.2.3 Observations of fruit and seed set from exclusion experiments

Observations of fruit set were conducted every week and mature fruits were checked from 40 days after pollination. As mature fruits fall off the trees, the fruits were bagged with mosquito netting approximately 40 days after pollination. The fruit is considered mature (ripe) when it becomes soft and can be easily separated from the calyx, and also produces a sour smell. The fruit set index was calculated as the ratio of mature fruits produced to the total flowers used for each treatment. The index range was from 0 to 1, where 1 shows that all the flowers treated developed into mature fruits and 0 indicates no mature fruits were formed from the treated flowers.

Mature fruits were collected and measured using digital calipers (0.01 mm accuracy). The measurements taken were fruit diameter and height (from the base to

the apex) to determine the fruit volume. The fruit volume was estimated using the ellipsoid volume formula, $4/3\pi a^2 b$, where a = 1/2 of the diameter and b = 1/2 of the height. The fruits were then kept in separate plastic bags and their mass was recorded in the lab using a digital balance (Sartorius ED 153 CWN, Scientech Inc., USA, 0.01g accuracy). The seeds were extracted from the fruits in the same way as for the S/O ratio determinations. The seeds were then counted and measured, and the dry mass was recorded using the same procedure. For pollination experiments, the S/O ratio of each treatment was calculated to estimate the efficiency of pollinators (Nassar *et al.* 1997).

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 tests and multiple comparisons (step-down method) were used to compare the fruits and seeds produced to locate the differences between the treatments. A Spearman rank correlation was conducted to test the relationships between seed number with seed size and mass to determine if trade-offs existed between seed characters. A Spearman rank correlation was also carried out to examine the relationship between seed number and fruit size (mass and volume), in which the size of fruit may increase with the number of seeds produced because fruit flesh is made of enlarged attachment structures of seeds to ovary (funiculi). All the analyses were conducted by using the IBM SPSS Statistics ver 19.0 (Chicago, USA).

3.2.4 Observations of floral visitors

Filming of floral nocturnal visitors was conducted as described in Chapter 2. Floral visitors were divided into functional types given that during the night, species identification of the visitors was not possible for most of the observations. For each moth (Order Lepidoptera) visiting the flowers, photos from digitized recordings (Chapter 2) were used to estimate the total body length relative to the *Sonneratia* flowers

(Chapter 4). The moths were divided into 'small' (< 20 mm) and 'large' moth (> 20mm) categories. Watzke (2006) recorded nocturnal moths from three families; Sphingidae (hawk moths), Arctiidae (tiger moths) and Noctuidae (owlet moths), visiting the *S. caseolaris* flowers at a mangrove habitat elsewhere in Peninsular Malaysia. As a group, the nocturnal moths as pollinators differed in behaviour while feeding on flowers (Faegri and van der Pijl 1979). Typically, noctuid and arctiid moths do not hover and usually land on the flowers, but sphingid moths on the other hand hover in front of the flowers while taking the nectar (Singer *et al.* 2006). In my study, no attempt to identify the nocturnal moths visiting the *Sonneratia* flowers was made. However, from the size (total body length) and their behaviour observed while feeding on the flowers, the large moths are most probably sphingids while the small moths probably comprise mainly arctiids and noctuids Visits were recorded from when the visitor first made contact with the flower, or from the first attempt to make contact by hovering around the flower, until the visitor left the flower. Return visits after a short period of time (at least 5 s) were considered new visits. The time spent at the flowers for each visit was recorded from the time code of the tapes.

Fleming and Sosa (1994) defined legitimate pollinators as the floral visitors that deposit pollen on conspecific stigmata, and efficient pollinators as the floral visitors which deposit more pollen than they lose or consume. I recorded legitimate visits by each floral visitor from the anthers and stigmata contacted during the visits following Ibarra-Cerdena *et al.* (2005). By making contact with anthers, the floral visitors are involved in pollen removal and become a potential pollinator, while making contact with stigmata might result in deposition of conspecific pollen grains. For this purpose, observations of nocturnal visitors other than bats were made from the cameras placed sufficiently close to the flower to allow clear observations of visits to the stigmata and anthers from 19 flowers (from six trees) of *S. caseolaris* and 18 flowers (from seven trees) of *S. alba.* Observations of bats were made from 27 flowers (from seven trees) of *S. caseolaris* and 24 flowers (from 11 trees) of *S. alba* as described in Chapter 2.

3.4 Discussion

3.4.1 Sonneratia breeding system

Following a classification of plant breeding systems based on P/O ratios by Cruden (1977) (Table S3.1), all the three *Sonneratia* species are within the range of species showing obligate autogamy (Table 3.3). All three species showed significant differences in pollen and ovule quantities, but not in the P/O ratios. The similarity in P/O ratios among species reflected more pollen grains produced by the flowers with a larger number of ovules, and therefore suggests that it is not only absolute number, but also the ratio between the pollen and ovule quantity that is biologically meaningful. This result also could be a consequence of a similar pollinator fauna and/or effective pollination rate (Mione and Anderson 1992) between the *Sonneratia* species. Results of pollination experiments on *S. caseolaris* flowers further confirm self-compatibility, at least for *S. caseolaris* (Figure 3.3).

The number of seeds produced showed no consistent association with the number of ovules for each species (Table 3.4). Mean seeds per fruit of *S. alba* and *S. ovata* were about 51 and 43 respectively (Table 3.4), despite *S. ovata* recording a mean number of ovules per flower as high as 1075 as compared to only 301 for *S. alba* (Table 3.3). Although the proportion of seeds produced (S/O ratios) was significantly different between *S. caseolaris* and *S. alba* when compared with *S. ovata*, the P/O ratios were not significantly different among the three species. Nassar *et al.* (1997) reported that S/O ratios corresponded to the P/O ratios of four species of Venezuelan columnar cacti, in which high P/O ratios increased the proportion of ovules successfully fertilised through the increase in pollen produced (high S/O ratios). S/O values < 1 are perhaps due to the fact that not all ovules within a flower are viable and therefore capable of being fertilised (Griffin and Barret 2002). Mione and Anderson (1992) found that species with the lowest ovule and seed quantities have the biggest seed size. My study however found no significant difference in seed size and seed quantities between *S. alba* and *S. ovata* (Table 3.4), even though the ovule quantities were significantly different in the two species (Table 3.3). The smallest S/O ratio calculated was for *S. ovata* and might be affected by the small sample size (five fruits from a single tree).

From the fruit set observed and the ISI index calculated, *S. caseolaris* is found to be self-compatible. Not only was high fruit set (about 45 %) recorded for facilitated autogamy (FA treatment, where stigmata are pollinated with pollen from the flower of the same tree), but automatic autogamy (AA treatment or self-pollination) also yielded fruit for almost 30 % of the total flowers treated (Figure 3.3). For most self-compatible species, hand self-pollination was observed to increase fruit set over spontaneous selfing due to excess pollen loads delivered to the stigmata (Arroyo and Uslar 1993). Watzke (2006) also reported the self-compatibility in the *S. caseolaris* breeding system. From his exclusion experiments, pollination by self-pollen (automatic autogamy and facilitated autogamy) however yielded fewer fruits than open pollination (natural pollination) or cross-pollination (hand-cross facilitated). These results contradict the conclusion of Pandit and Choudhury (2001) who reported that *S. caseolaris* is self-incompatible. As they observed the flowers staying in bloom for 56 hrs compared to the 12 hrs usually reported for *Sonneratia* flowers (Tomlinson 1986), they concluded that *S. caseolaris* in their study area has undergone local ecotypic adaptation in order to make the most efficient use of the pollinator resources available in the area.

A flexible breeding system with autogamy occurring in addition to cross-pollination in other chiropterophilous plants was also reported by Tschapka and von Helversen (2007) for the bromeliad, *Werauhia gladioliflora* (Family Bromeliaceae), and for the terrestrial herb *Irlbachia alata* (Family Gentianaceae) by Machado *et al.* (1998). Bumrungsri *et al.* (2008), Bumrungsri *et al.* (2009) and Srithongchuay *et al.* (2008) however showed other bat-pollinated species such as the bitter bean (*Parkia* sp.), durian (*Durio* sp.) and Indian trumpet (*Oroxylum indicum*) in southern Thailand were highly self-incompatible. The extent of self-incompatibility in the breeding system of another bat-pollinated plant, the silk cotton tree (*Ceiba pentandra*) nevertheless can be flexible (Lobo *et al.* 2005). The species shows self-incompatibility in regions with high pollinator visitation rates, while in regions with low pollinator visitation rates, *C. pentandra* shows a mixed mating system with high levels of self-pollination. Therefore, the patterns of pollinator visitations may modify the mating system of

trees from outcrossing to selfing with a possible reduction in the genetic diversity of the remnant populations for certain species (Quesada *et al.* 2004). The increase in self-compatibility when pollinators become scarce emphasises the importance of global declines in pollinators to the breeding systems of many animal-pollinated plants (Kearns *et al.* 1998). In self-compatible plants, natural seed set may occur as a result of both cross-pollination and autogamy (Tschapka and von Helversen 2007). However, cross-pollination was found to increase the pollination success (Figure 3.3 and Table 3.5). In bat-pollinated plants such as pochote (*Pachira quinata,* Family Malvaceae), Quesada *et al.* (2001) reported that the seeds within the fruits were sired mainly by one or two cross-compatible donors. They also found that only the fruits that resulted from the pollinations performed with large cross-compatible pollen loads developed into maturity. In the columnar cactus, *Pilosocereus moritzianus* (Family Cactaceae), Nassar *et al.* (1997) recorded fruit set from pollination by self-pollen, however the fruits did not reach maturity. For flowers that set fruit, both compatible and incompatible pollen could be found on the stigmata in similar proportions, but the cross-compatible pollen might out-compete the self-pollen, successfully siring seeds in the fruits that developed to maturity.

The fruits (mass and volume) and seeds (size) produced from the exclusion experiments involved two different groups of treatments, i.e. situations where the experimenter placed pollen on the stigmata (CP and FA treatments), and where this was not the case (OP, AA, IP and EM treatments) (Table 3.5). The former group produced bigger fruits and a higher number of seeds compared with the latter. Not only did CP produce most fruits and seeds, the fruits were largest and the total seed mass were the biggest but also the smallest in size. Multiple comparisons showed none of the fruit and seed characteristics of CP and FA treatment differed except for seed number. The higher number of seeds produced from CP compared with the FA treatment might indicate the selection of high quality pollen grains from outcrossing over self-pollen (Crome and Irvine 1986; Hirayama *et al.* 2005), and also the importance of external pollinating agents (Herrera 1987) in the breeding system of *S. caseolaris*. Further, previous studies often show greater survival of

seedlings from crossed than from self-pollinated plants (Colling *et al.* 2004; Niesenbaum 1999). Seedling survival from the seeds collected however was not determined in my study.

A positive relationship between number of seeds per fruit and the seed size in pochote (*P. quinata*) was reported by Quesada *et al.* (2001). The seed mass was also found to increases with seed number. In my study, correlation analyses revealed the seed number was negatively related to the seed size (except for in the IP treatment), though the correlations were significant only for OP and FA treatments (Table 3.6). Strong correlations ($r_s > 0.8$) however were shown between seed number and fruit mass, fruit volume and seed mass for the OP treatment, and between seed number and seed mass for the IP and CP treatments. Therefore in my study, bigger fruits (by volume and mass) produced more seeds (by number and mass), but smaller seed sizes (except for IP). The smaller seed size showed the trade-off with the number of seeds produced by the *S. caseolaris* fruits. Petit (2011) noted the importance of seed mass for seedling survival of a bat-pollinated columnar cactus, *Stenocereus repandus* (Family Cactacea). Smaller size seeds suffered more from decomposition and pathogens than the larger seeds (Crist and Friese 1993). Not only that, seed size also affected the seedling emergence in which larger seeds germinated earlier than the smaller seeds (Hojjat 2011). As larger seeds produced more vigorous seedlings than the smaller seeds, the seedlings from the former also grew more rapidly than those from the latter (Stanton 1984; Winsor *et al.* 1987).

A week after of pollination, flower abortion was observed for all treatments. CP and FA treatments recorded the lowest abortion rate while EP and IP treatments recorded the highest rates (Figure 3.2). Pollen and ovule incompatibility is an important factor in determining fruit abortion after fertilisation in which maternal plants selectively aborted selfed seeds and fruits rather than the outcrossed fruit and seeds to improve fitness (Casper 1988; Herrera 1988b; Levri 1998; Pina *et al.* 2007; Stephenson and Winsor 1986). The timing of fruit abortion on the other hand minimizes the amount of resources wasted by abscission and conserves many resources for the remaining fruits and other growth processes (Stephenson 1981). Lee (1984) hypothesized that plants selectively abort fruits on the basis

of seed number to improve the average quality of their progeny. When the number of pollen grains deposited onto the stigmata exceeds the number of ovules in the ovary, competition for ovules might occur resulting in fertilisation of ovules by pollen grains with the highest fitness (which produces fast-growing pollen tubes). The fruits produced from this intense gametophyte competition (resulting from high pollen loads) then achieve high seed numbers and are more likely to reach maturation (Winsor *et al.* 1987). Quesada *et al.* (2001) demonstrated that the size of pollen loads in stigmata determines the probability of fruit abortion and maturation. Therefore, fruit and seed abortion is not a random process (Stephenson 1981), but rather a mechanism of maternal choice for high quality progeny (Obeso 2004). Selective fruit abortion however could also occur because of resource limitations in the maternal plant as reported by Pellmyr and Huth (1994) and Bookman (1983).

In my study, the number of *S. caseolaris* seeds produced relative to the ovule number (S/O ratio) was low, 30 % in cross-pollination (CP treatment) while only 19 % from the natural pollination (OP treatment) (Table 3.5). For self-compatible plant species, Husband and Schemske (1996) reported a 20 % average reduction in seed set with a maximum of 87 % from the total ovule number in the ovary. The low number of seeds produced (< 40 % of the total ovule number) was not uncommon (Herrera 1987; Quesada *et al.* 2001; Slauson 2000; Winsor *et al.* 1987), even from the flowers saturated with high pollen loads from high numbers of pollen donors. Holland and Chamberlain (2007) observed a maximum S/O ratio of about 60 % in senita cacti (*Pachycereus schottii*, Family Cactaceae) even when pollen loads on the stigmata were 10 times greater than the ovule number. They suggested that even though not all ovules mature into seeds, producing more ovules leads to the probability of producing more seeds.

Exclusion experiments conducted in my study showed that *S. caseolaris* is self-compatible, and therefore geitonogamy crosses by the wind are possible. The use of fabric pollination bags reduced the number of wind-borne pollen grains entering the bags in which pollen grains were blocked by the pore size of fabric or of reduced penetration of the wind

(Neal and Anderson, 2004). In my study, I used transparent polythene bags (30 x 40 cm) with small holes to allow air circulation. Therefore, bagging the flowers (for automatic autogamy) in the exclusion experiment not only resulted in pollen accumulations inside the polythene bags, but also may promote the likelihood of pollen reaching the stigma of the same flower, and therefore resulting in the higher fruit set observed for the AA treatment. However, being in a habitat where wind strongly influences the ecosystem (Tomlinson 1986), wind may principally result in inter-crown pollinations and this might contribute to a high rate of geitonogamy in mangrove trees. The flower morphology study (Chapter 4) showed a long style with short stamens in *S. caseolaris*. In hermaphrodite flowers, spatial separation of style and stamens (herkogamy) is an adaptation to avoid self-pollination (Willmer 2011).

Emasculating flowers eliminates self-pollen deposition by removing the anthers. However, in some plant species, exogenous (external cause of origin) applications of hormones allow apomixis fertilisation in which fruit initiation occurs in the absence of pollen (Coombe 1976). As the emasculated flowers (EP treatment) were left exposed to the visitors in my study, pollination from visitors seeking nectar and also geitonogamy crossed by wind is possible. The lowest fruit set recorded for EP nevertheless might indicate the importance of pollen as a reward in plant-pollinator interactions. Nectar-feeding bats usually consume pollen while they forage for flower nectar and they have the ability to extract the pollen contents efficiently (Mancina *et al.* 2005). Pollen is an important food source as pollen is a rich source of carbohydrates, protein, nitrogen, amino acids, starch, sterols and lipids (Howell 1974; Roulston and Crane 2000). Bat-pollinated flowers therefore presumably produce large amounts of pollen and nectar (Faegri and van der Pijl 1979) for nutritional payoffs to bats for their pollinator services. Moreover, the production of large amounts of pollen by flowers results in large amounts of pollen being deposited on the bats, therefore increasing the probability of deposition of numerous pollen grains on the stigmata by the bats (Nassar *et al.* 1997). Two fruits produced from EP might be the result of pollination by

floral visitors which come to the flowers for the surplus nectar secreted by the emasculated flowers (personal observation).

3.4.2 The relative importance of bats and other visitors as Sonneratia pollinating agents

Sutherland (1986) reported relatively low fruit set values (fruit:flower ratio) from selective abortion in chiropterophilous plants. The fruit set of *S. caseolaris* in my study (26.4 %) from natural pollination (OP treatment) was relatively higher than that recorded by Pandit and Choudhury (2001) (5.8 %), but lower than that documented by Watzke (2006) (58.9 %). Pandit and Choudhury (2001) suggested that their low fruit set was due to high predation on fruit and flowers by rats and monkeys at the study site. Watzke (2006) on the other hand recorded minimal fruit losses to predators (such as monkeys and squirrels) compared with losses from insect damage and parasitism. In my study, fruit losses were avoided by bagging the fruits with mosquito nets. However, insect predation on flowers resulting in ovary damage was observed in my study. The identity of the insects was unknown, but I observed green Coleoptera commonly on the trees, feeding on the leaves and sometimes on the flower calyx (Figure S3.3).

The pollination success of CP however was almost twice that of OP (natural pollination) (Figure 3.3). Since the former received superabundant pollen grains, this result suggests that pollen limitation occurs in breeding systems of *S. caseolaris* at the study area. Pollen limitation is a relatively common phenomenon and is often assumed to result from a low frequency of pollinator visitation (Bierzychudek 1981). Observations of visitors showed some of the *Sonneratia* flowers were not visited, while others were visited by either single or numerous pollinator types on single occasions or repeatedly during the recording nights. Visitors other than bats to the *Sonneratia* flowers however made more contact with anthers than with stigmata (Table 3.7). The high number of illegitimate visits recorded by bats was from their scouting activities (Chapter 2), in which they gathered information on flower opening and nectar accumulations particularly during the early part of the night (Chapter 4).

I observed between 1-18 feeding visits to flowers by bats, with two flowers receiving only a single visit for *S. caseolaris*, and 1-6 for *S. alba* with three flowers recorded in a single visit (Chapter 2). Repeated pollinator visits to the same flowers nevertheless provide ample pollen to initiate pollination and compensated for the negative impact of interspecific pollen loads under pollen limitation (Petit 2011). The increases in seed set were also related to the number of compatible microgametophytes deposited on the stigmata (Quesada *et al.* 2001). As pollen quality may influence seed set (Crome and Irvine 1986), low numbers of fertilised ovules in the natural pollination (OP treatment) might indicate genetic selection in the breeding system of *S. caseolaris*, resulting from unsuccessful fertilisation of the ovules by many of the pollen grains deposited or many of the fertilised ovules not maturing into seeds (Silander and Primack 1978). Quesada *et al.* (2001) recorded low numbers of ovules maturing into seeds (only about 14 %) in pochote, *P. quinata*, due to limited resources provided by the maternal plant. Resource limitation was also reported by Slauson (2000) in the pollination of both *Agave chrysantha* and *A. palmeri* (Family Agavaceae). Therefore I suggest that the low success of natural pollination of *S. caseolaris* in my study is not only due to pollen limitations resulting from the low number of visits by bats at the study area, but also might be due to other factors such as pollen quality (genetic factors) and resource limitation.

The minimum number of pollen grains required to fertilise ovules for fruit set, expressed as the ratio of pollen load size to the number of ovules, varied between plant species from 1:1 (Silander and Primack 1978) to 1:12 (Snow 1982). Therefore, the minimum number of pollen grains required for pollination is equal or less than the number of ovules in the ovary. In my study, the ratio was not calculated for either *S. caseolaris* or *S. alba*. However, each contact with the stigmata made by bats was estimated to deposit about 6205 and 2431 conspecific pollen grains to *S. caseolaris* and *S. alba* flowers respectively (Chapter 2). Compared to the number of ovules estimated for each species (3229 for *S caseolaris* and 301 for *S. alba*) (Table 3.3), a single visit by bats was sufficient to fertilise all the ovules if each pollen grain deposited onto the stigmata results in a fertilised ovule. Cruden (1977)

however reported that several pollen grains per ovule must reach the stigmata to ensure maximum fruit set, therefore large quantities of pollen grains produced by the bat-pollinated flowers may be necessary to ensure deposition of sufficient pollen grains to fertilise as many of the ovules of the flowers (Heithaus *et al.* 1974). For bat-pollinated plants, the ratio of pollen load size to the number of ovules calculated however was higher, 3:1 in the Indian trumpet (*O. indicum*) (Srithongchuay *et al.* 2008) and 2.6:1 in pochote (*P. quinata*) (Quesada *et al.* 2001). I suggest that the number of pollen grains deposited on the stigmata in my study might be underestimated due to strong pollen adherence to the stigmatic surface (Chapter 2). Therefore, to further confirm the effectiveness of a pollinator agent, not only is the number of pollen grains deposited onto the stigmata by the pollinator important, but also determination of the minimum pollen load required by a particular plant species to initiate fertilization.

From video recordings, *Sonneratia* is not exclusively pollinated by bats (Figure 3.4). Bats however were often legitimate pollinators, making contact with the stigmata, either while landing on the flower or hovering in front of the flowers for pollen and nectar (Table 3.7). During the feeding visits to the flowers, bats successfully deposited sufficient conspecific pollen grains for pollination (Chapter 2). I observed only one illegitimate visit to the flowers by a bat that made contact with only the anthers while hovering for feeding (Figure S3.4). Due to their large body size and behaviours (high visit frequency, large movement areas), bats are capable of transporting pollen over long distances and thus promote cross-pollination, making them important pollinating agents for *S. caseolaris* (Watzke 2006).

From the total stigmata contact (TSC) made by the floral visitors, bats are the single most important pollinators of *S. caseolaris* but not for *S. alba* (bats contribute 88.7 % of the TSC made by floral visitors to the *S. caseolaris* flowers, but only 31.4 % in *S. alba*). Bats were the most important pollinator of *S. alba,* successfully making contact with stigmata during its feeding visits to the flowers (SC = 0.96). In some cases, the capability of transferring compatible pollen grains to the stigmata is more important than the pollinators'

visitation rates (Crome and Irvine 1986; Herrera 1987; Schemske and Horvitz 1984). In Chapter 2, I reported that bats are principally responsible for pollen deposition onto the stigmata of *S. alba* flowers, and a single visit by bats deposited adequate conspecific pollen grains (relative to the number of ovules) to initiate pollination of the *S. alba* flowers. Not only that, the high capacity of pollen loads on their bodies due to their bigger size compared to the other important *S. alba* floral visitors (large moths and hymenopterans contribute to 34.3 % and 24.8 % of the TSC made by floral visitors to the *S. alba* flowers, respectively), make bats successful and also important pollinating agents of *S. alba*.

Fruit and seed production are directly related to the pollen load on the stigmata (Snow 1982; Winsor *et al.* 1987). When bats were excluded from visiting the flowers (IP treatment), the fruit set reduced to almost half compared to fruit set recorded when bats were allowed to assess the flowers (OP treatment) (Figure 3.3). Moreover, the seeds produced were also significantly higher (by number and mass) in OP compared to the IP treatment (Table 3.5). However in my study, video recordings showed low visitation rates by insect visitors (including the large moths of > 20 mm body length) to the *S. caseolaris* flowers at the study site (Table 3.7). Therefore the higher fruit and seed set in OP treatment showed the greater importance of bats as pollinating agents for *S. caseolaris* compared to pollination by insects only (IP treatment), and low successful pollination in IP was not from the consequences of the insect being deterred by the netting. Quesada *et al.* (2003) concluded that the pollen received by the stigmata was jointly determined by pollinator visitation rate and by pollinator efficiency. The greater the visitation rate, the greater the number of pollen grains deposited onto the flowers, and therefore the greater the fruit set (Arias-Coyotl *et al.* 2006; Herrera 1987; Quesada *et al.* 2001; Quesada *et al.* 2003; Schemske and Horvitz 1984; Silander and Primack 1978; Stone 1996) and seed set (Engel and Irwin 2003; Flanagan *et al.* 2009).

For self-compatible species, the role of bats as pollen vectors is important for the plant's fitness. The importance of bats as pollinating agents is not only because they are known to travel long distances (Start and Marshall 1976) to forage for food, but they also

transport greater amounts of pollen compared to birds (Law and Lean 1999; Muchhala 2006; Muchhala 2007). The capacity to move high pollen loads over relatively long distances may help to promote outcrossing in bat-pollinated plant species. Bats usually deposited conspecific pollen grains of several different genotypes (different potential fathers) onto the stigmata of flowers they visited and therefore produced greater genetic variability progeny than pollination by other pollinators (Fuchs *et al.* 2003; Nassar *et al.* 2003). The visitation rate correlated with the breeding system and the level of relatedness of the progeny produced was observed in several Neotropical bat-pollinated trees by Collevatti *et al.* (2001) and Lobo *et al.* (2005). They concluded that the number of sires from outcrossed progeny was significantly greater in seeds that received bat visits than in seeds that did not receive bat visits.

As well as bats and rats, video recording showed the *Sonneratia* trees at the study sites were visited by various groups of arthropods including moths, hymenopterans, bush cricket, praying mantises and spiders (Figure 3.2). Most of the arthropods however acted as pollen and nectar robbers and were poor pollinators. Other than the arthropods observed from the video recording, I also observed several species of ants visiting the *Sonneratia* flowers (personal observations). Ants from the genera *Camponotus*, *Crematogaster* and *Tetraponera* are among the common species found nesting in the *Sonneratia* trees (Nielsen 2000; Tokeshi *et al.* 2007). I observed the ants foraging around the flowers to feed on the remaining nectar in the morning after the anthers wilted and dropped, and some species were seen to visit the flowers during the blooming night (Figure S3.5). The ants were observed to feed at the flowers without ever making contact with the reproductive parts of the flowers, thereby precluding the possibility of them being pollinating agents.

Pandit and Choudhury (2001) recorded 35 species of floral visitors to *S. caseolaris*. From the total visitors recorded in their study, three species of Lepidoptera and a rodent (Family Muridae) were the only nocturnal floral visitors, and bats were not recorded visiting the flowers in their study. From their pollinator exclusion experiments, they concluded that both nocturnal and diurnal floral visitors were important in the pollination success of *S.*

caseolaris. Watzke (2006) in his study listed various groups of insects (families Lepidoptera, Diptera, Hymenoptera, Planipennia, Coleoptera, Hemiptera), spiders (Order Araneae) and nectar-feeding bats as floral visitors to *S. caseolaris* during night time. Only the nectarivorous bats however were found to be effective pollinators due to their capability of transferring pollen over long distances to promote cross-pollination.

The large and small moths observed differ in their foraging behaviour therefore affecting their roles as pollinators. Visitation to the *S. alba* flowers by the large moths resulted in regular contact with stigmata and anthers (Table 3.7) from their matched size with the flowers (Chapter 4). A long proboscis however allows the large moths to feed from distance (Figure S3.6), therefore limiting their potential for carrying pollen grains on their bodies, carrying only a small amount of pollen grain on their feet while awkwardly perching on the flowers. Thus, visitations to the flowers by large moths might not always result in pollen deposition to the *S. alba* flowers, despite making regular contact with the stigmata of the flowers. Not only that, on four occasions large moths were observed not touching the stigmata by perching on the outer anthers of *S. caseolaris* flowers and successfully avoiding the stigmata.

Small moths were the most frequent visitors to *S. alba* flowers and the second most frequent visitors to *S. caseolaris* flowers (Table 3.7). Compared to large moths, small moths made contact with the anthers more frequently, occasionally landing on the flower calyx between foraging bouts to the anthers or stigmata (Figure S3.7). As pollen is usually more exposed than nectar in *Sonneratia* flowers (Chapter 4), small moths collected pollen directly from the anthers but without making contact with the stigmata due to their small size. In fact, the legitimate visits by small moths were mainly from making contact with anthers and stigmata only on three and five occasions to *S. caseolaris* and *S. alba* flowers respectively. The mismatch with flower morphology resulted in visitors failing to make contact with the reproductive organ of the flowers while taking nectar thus reducing their potential as pollinators (Muchhala 2003; Pandit and Choudhury 2001). Due to their small size compared to the *Sonneratia* flowers, small moths therefore were considered as pollen thieves (Inouye

1980) rather than effective pollinators (further analysis on *Sonneratia* flower size and its pollinators can be found in Chapter 4).

Hymenopterans were also relatively important visitors contributing about 25 % of the total stigmata contact of *S. alba* flowers. The hymenopterans however may not be effective pollinators because they usually forage for nectar early in the morning, when rapid loss of pollen viability occurs (Chapter 4) as temperature rises (pollen viability factor) and stigmata receptivity decreases during the day (Dafni *et al.* 2005). Despite landing on the flowers of *S. alba*, hymenopterans generally are unable to penetrate the tangle of filaments and do not make contact with stigmata (Primack *et al.* 1981). Ibarra-Cerdena *et al.* (2005) found that hymenopterans such as the carpenter bee *Xylocopa* sp. (Family Apidae) was potentially a legitimate pollinator for a bat-pollinated cactus *Stenocereus queretaroensis* (Family Cactaceae) because this bee spent little time in each flower and flew long distances. The carpenter bees caught at two of my three study sites, *X. varipuncta* carried 33 pollen types including *S. caseolaris* and *S. ovata* pollen (Wahizatul Afzan *et al.* 2012). The *Sonneratia* pollen grains however contributed only 0.1 % of the total pollen grains carried by the bee.

A bush cricket, praying mantis and spiders visited the flowers very infrequently, only to capture other insects visiting the flowers for pollen and nectar. A single bush cricket and praying mantis showed extremely long residence times (Table 3.7), usually at the calyx and occasionally crawled up to the top of the flowers when their prey visited. During their attempt to catch the insects visiting the *Sonneratia* flowers, they made contact with the anthers but not the stigmata (Figure S3.8). Quesada *et al.* (2003) noted that the time spent at the flowers per visit by floral visitors is not necessarily an indicator of the frequency of making contact with the reproductive parts. In my study, bats spent the shortest time at the flowers for feeding compared to other legitimate visitors of *Sonneratia* flowers (moths and hymenopterans), but recorded the highest SC for both *S. caseolaris* and *S. alba*.

Several times I observed visitations by rats to the *Sonneratia* flowers. However, from the video recording, I did not observe the rats making legitimate visits to the *Sonneratia* flowers. Rats damaged the flowers by chewing the calyx, probably to take the nectar at the

base of the flowers, and possibly making contact with the anthers and therefore potentially becoming pollinators. In one event, I observed a rat chewing the calyx and anthers from a *S. alba* flower and the flower was aborted after the visit (Figure S3.9). Most of the time however, the gynoecium remained intact, and the flowers with damaged calyxes developed into mature fruits. As the visits by rats occurred only occasionally, it is unlikely that rats are effective pollinators of *Sonneratia* flowers. Pandit and Choudhury (2001) however reported the rat (*Rattus rattus*, Family Muridae) visiting *S. caseolaris* as a flower predator, visiting the flowers for rewards (pollen and nectar), damaging floral tissues and not performing pollination.

3.4.3 Conclusions

The P/O ratios calculated for the three *Sonneratia* species does not support the hypothesis that the three *Sonneratia* species are xenogamous; indeed, all three *Sonneratia* species are predicted to show obligate autogamy. Pollinator exclusion experiments and observations however suggested facultative autogamy for *S. caseolaris*, in which bats are the major cross-pollinating agents. Even though *S. caseolaris* was found to be self-compatible, reproductive output (number of fruits and seeds produced) showed cross-pollination increased pollination success. The fruit set recorded for CP was almost twice that of the OP treatment, and the fruit and seed characteristics recorded showed CP produced bigger fruits (by volume and mass) and more seeds (by number, mass and S/O ratio). Therefore the determination of plant breeding system from P/O ratios should preferably be supplemented by exclusion experiments. Bats are the predominant visitors to *S. caseolaris*, and from the relative % of total stigmata contact (TSC) made by all visitors to the flowers, were the most important pollinators of *S. caseolaris* trees. When bats were excluded from visiting the flowers (IP treatment in exclusion experiments), not only was the fruit set reduced to almost half compared to when bats were allowed to access the flowers (OP treatment), but the seeds produced from OP were also significantly higher (by number and mass). Small moths were the most common visitors to *S. alba* trees, and made regular contact with the anthers

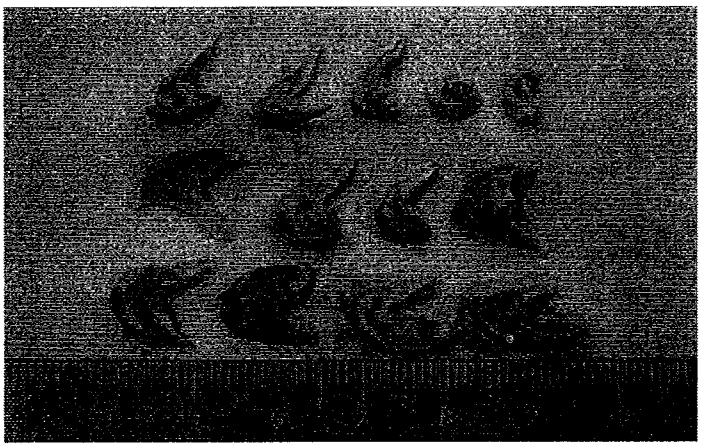
but very rarely touched the stigmata. Large moths such as the sphingids on the other hand, recorded the highest TSC to the *S. alba* flowers. The feeding behaviour of the large moths however reduced their ability to collect and deposit pollen grains despite making regular contact with both the anthers and stigmata of *S. alba* flowers. Visits by large moths to the *S. alba* flowers therefore did not always result in pollen deposition to the stigmata (Chapter 2 reported bats responsible for most of the pollen deposition on stigmata of *S. alba* flowers), and they are considered more as pollen and nectar thieves than as pollinators.

3.5 Supplementary material

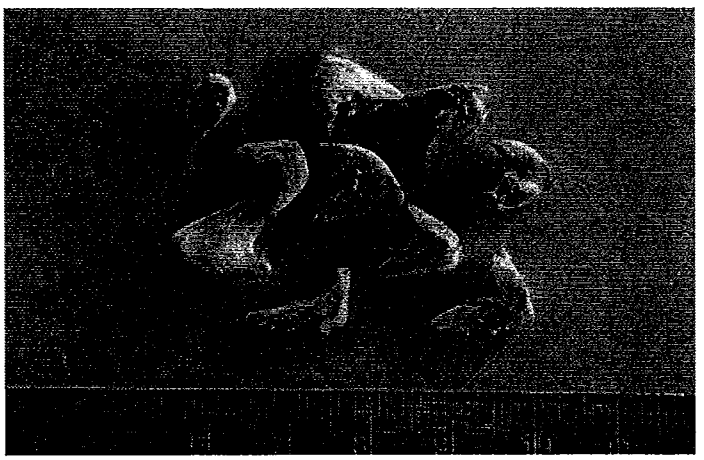
Table S3.1 Classification of plant breeding system from pollen-to-ovule ratio (P/O ratio) as reported by Cruden (1977).

Breeding system	Number of plant species examined	Mean P/O (± SE)
Cleistogamy	6	4.7 ± 0.7
Obligate autogamy	7	27.7 ± 3.1
Facultative autogamy	20	168.5 ± 22.1
Facultative xenogamy	38	796.6 ± 87.7
Xenogamy	25	5859.2 ± 936.5

a)



b)



c)

Figure S3.1 The irregular shapes of *Sonneratia* seeds. a) *Sonneratia caseolaris* b) *S. alba* c) *S. ovata.*

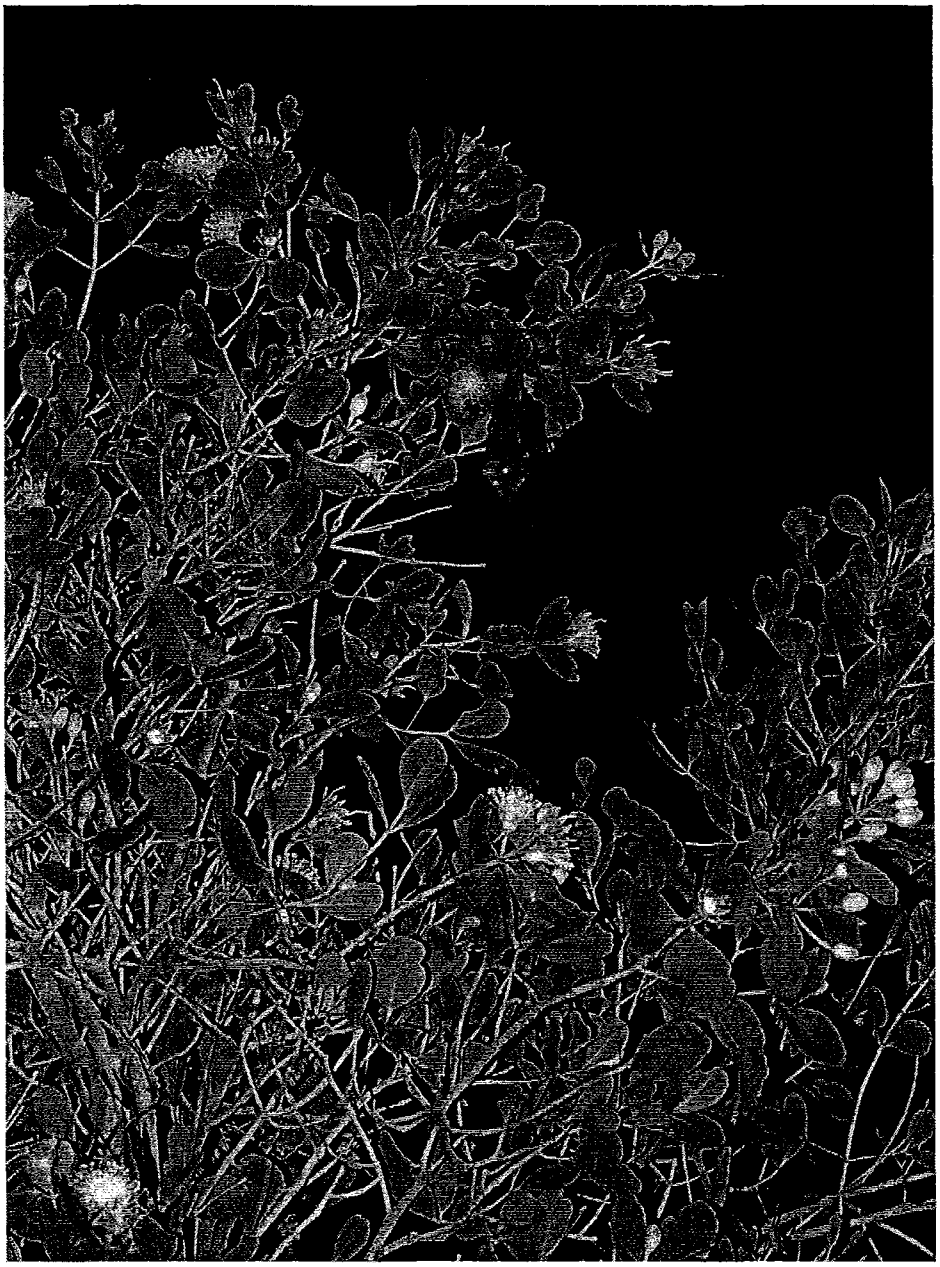
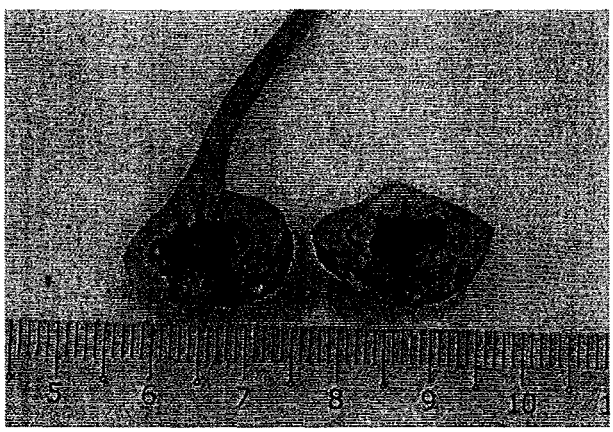


Figure S3.2. The island flying fox (*Pteropus hypomelanus*) observed visiting *Sonneratia alba* flowers for nectar and other floral resources.

a)

b)



c)

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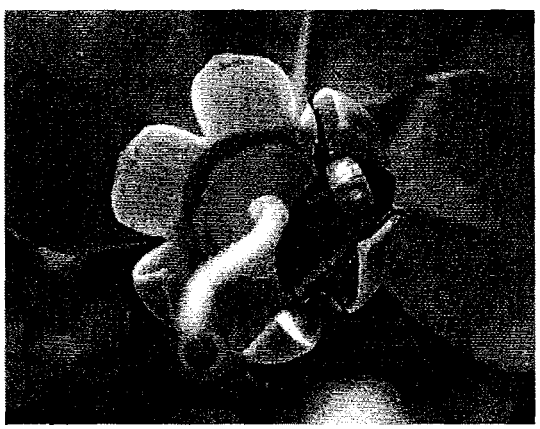


e)

Figure S3.3 Damaged flowers from insect predation on *Sonneratia caseolaris* flowers in a) and b). c) Predation on an ovule observed in an undeveloped flower collected 50 days after pollination. d) A coleopteran visiting *S. caseolaris* to feed on leaves and e) on the flower calyx.

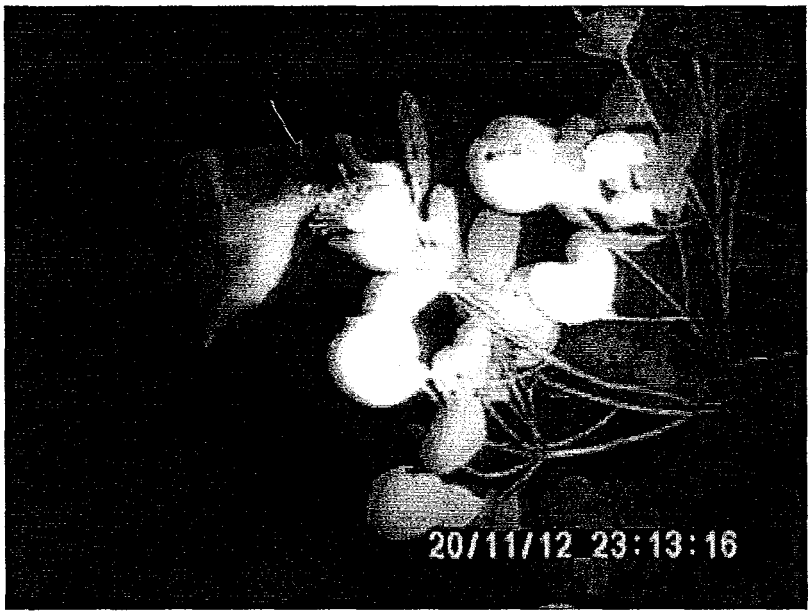
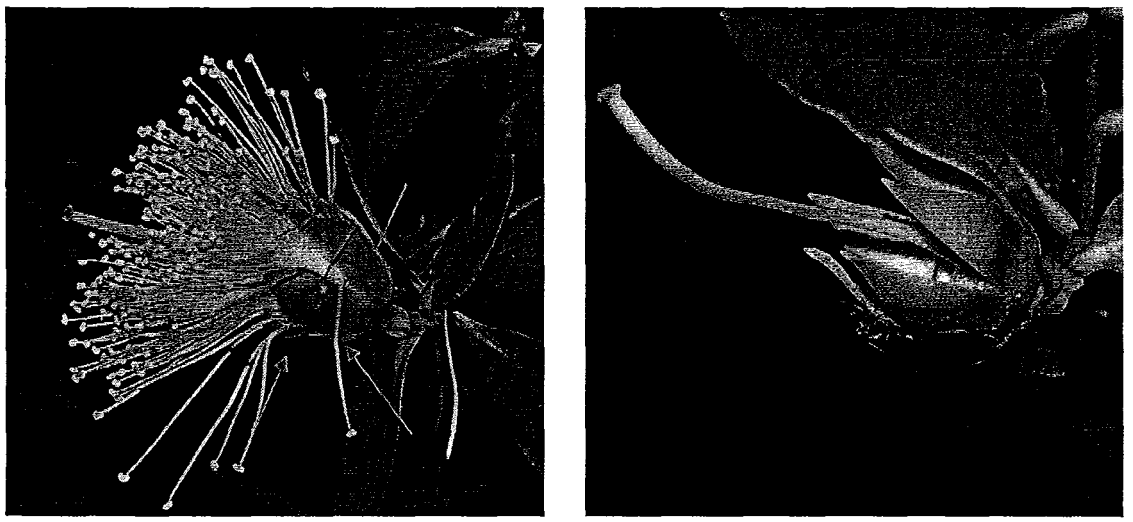
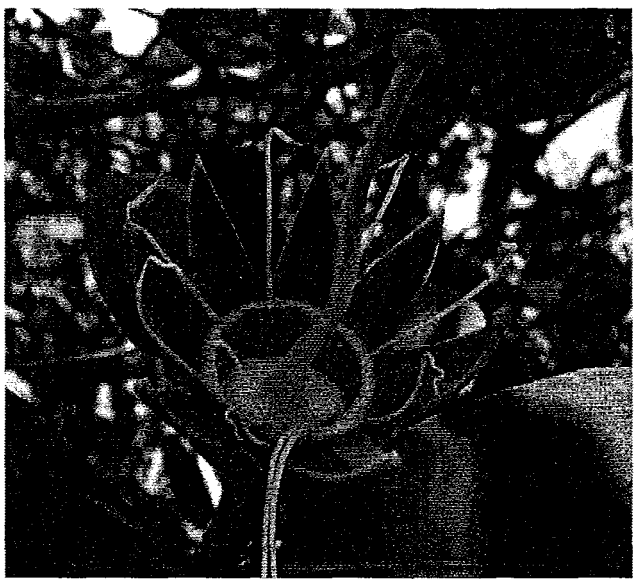


Figure S3.4 Hovering feeding by a bat which contacted anthers only. Arrow indicates stigma.

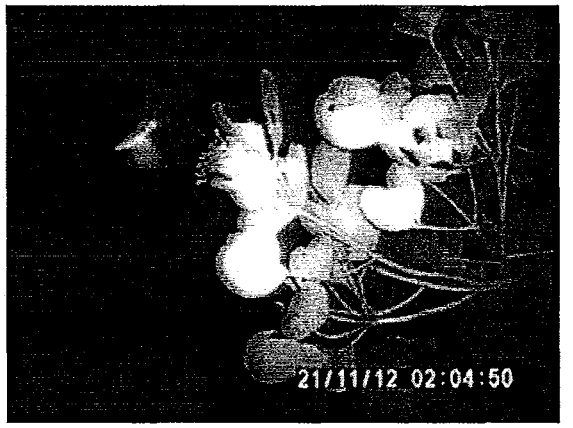


a)

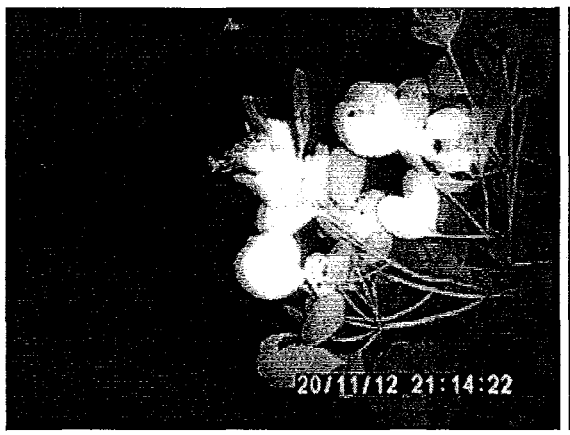


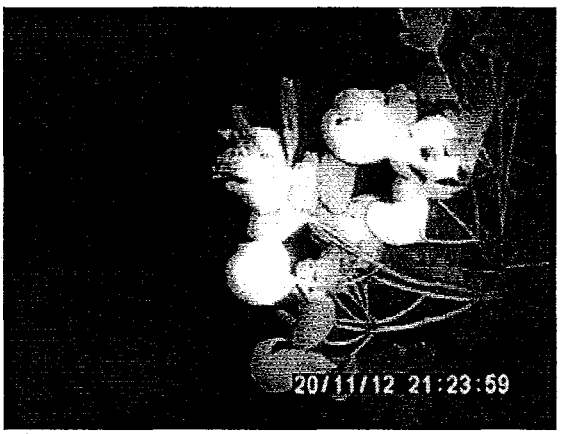
b)

Figure S3.5 Ants visiting a) *Sonneratia caseolaris* and b) *S. alba* flowers.



a) b)



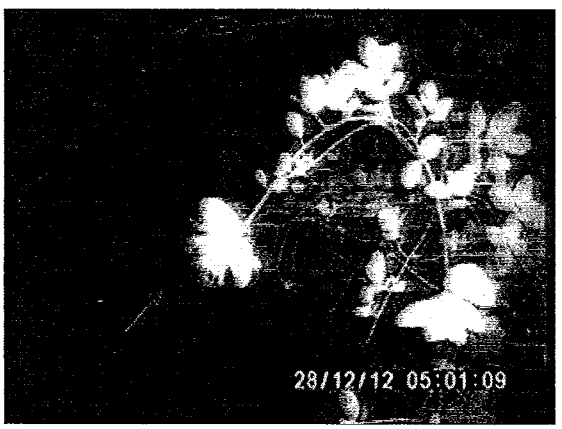


c) d)

Figure S3.6 Feeding by a large moth while making a) a legitimate visit and b) an illegitimate visit. Legitimate visits by a small moth contacting either c) anthers or d) stigmata.

Figure S3.7 Small moths occasionally resting on the calyx between foraging bouts to the anthers.

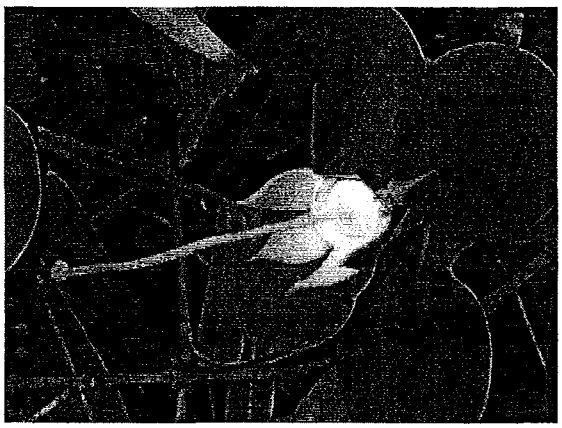
a) b)



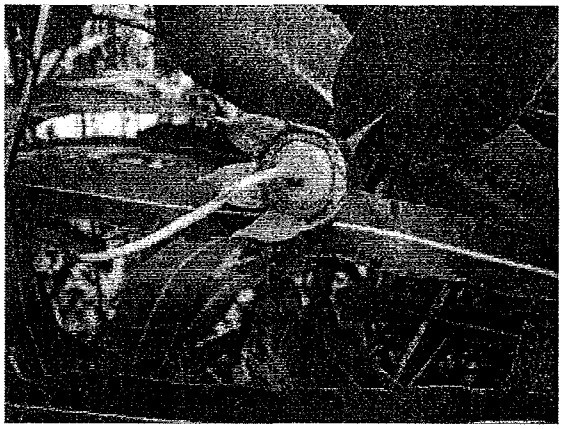
c)

Figure S3.8 Legitimate visits by touching the anthers by a) praying mantis b) bush cricket and c) spider on *Sonneratia* flowers during attempts to catch their prey.

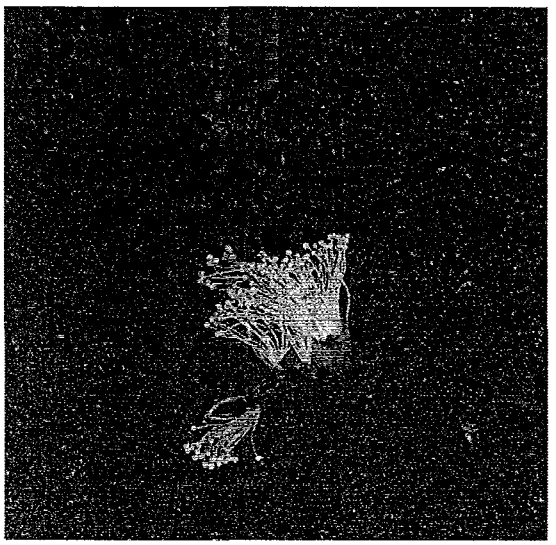
a) b)



c)



d)



e)

Figure S3.9 Predation by rats on *Sonneratia* flowers. a) *Sonneratia alba* flowers and fruits b) Developed fruit of *S. alba* with damaged calyx c) *S. caseolaris* flower d) The ovary of a *S. caseolaris* flower developing into a fruit with a damaged calyx e) Aborted *S. alba* flower after being visited by a rat.

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M., 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Annals of Botany 103, 1579-1588.

Ambruster, W.S., 2012. Evolution and ecological implications of "specialized" pollinator rewards. In Patiny, S. (Ed.), Evolution of Plant-Pollinator Relationships. Cambridge University Press, Cambridge, pp. 44-67.

Andriafidison, D., Andrianaivoarivelo, R.A., Ramilijaona, O.R., Razanahoera, M.R., MacKinnon, J., Jenkins, R.K.B., Racey, P.A., 2006. Nectarivory by endemic Malagasy fruit bats during the dry season. Biotropica 38, 85-90.

Anthony, E.L.P., 1988. Age determination in bats. In Kunz, T.H. (Ed.), Ecological and Behavioral Methods for the Study of Bats. Smithsonian Institution Press, Washington, D.C, pp. 47-58.

Arias-Coyotl, E., Stoner, K.E., Casas, A., 2006. Effectiveness of bats as pollinators of *Stenocereus stellatus* (Cactaceae) in wild, managed in situ, and cultivated populations in La Mixteca Baja, central Mexico. American Journal of Botany 93, 1675–1683.

Arizaga, S., Ezcurra, E., Peters, E., De Arellano, F.R., Vega, E., 2000a. Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. I. Floral biology and pollination mechanisms. American Journal of Botany 87, 1004–1010.

Arizaga, S., Ezcurra, E., Peters, E., De Arellano, F.R., Vega, E., 2000b. Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. II. The role of pollinators. American Journal of Botany 87, 1011-1017.

Armbruster, W.S., Herzig, A.L., 1984. Partitioning and sharing of pollinators by four sympatric species of *Dalechampia* (Euphorbiaceae) in Panama. Annals of the Missouri Botanical Garden 71, 1-16.

Armbruster, W.S., Edwards, M.E., Debevec, E.M., 1994. Floral character displacement generates assemblages structure of western Australian triggerplants (*Stylidium*). Ecology 75, 315-329.

Arroyo, M.T.K., Uslar, P., 1993. Breeding systems in a temperate mediterranean-type climate montane sclerophyllous forest in central Chile. Botanical Journal of the Linnean Society 111, 83-102.

Asian Turtle Trade Working Group, 2000. *Batagur baska*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available at www.iucnredlist.org. (Accessed: 08 February 2014).

Augspurger, C.K., 1980. Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. Evolution 34, 475-488.

Baker, H.G., 1961. The adaptation of flowering plants to nocturnal and crepuscular pollinators. The Quarterly Review of Biology 36, 64-73.

Baker, H.G., Baker, I., Hodges, S.A., 1998. Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. Biotropica 30, 559-586.

Bawa, K.S., 1990. Plant-pollinator interactions in tropical rain forest. Annual Review of Ecology and Systematics 21, 399-422.

Bawa, K.S., Bullock, S.H., Perry, D.R., Coville, R.E., Grayum, M.H., 1985a. Reproductive biology of tropical lowland rain forest trees. II. Pollination systems. American Journal of Botany 72, 346-356.

Bawa, K.S., Perry, D.R., Beach, J.H., 1985b. Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. American Journal of Botany 72, 331-345.

Bell, J.M., Karron, J.D., Mitchell, R.J., 2005. Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. Ecology 86, 762–771.

Bestmann, H.J., Winkler, L., von Helversen, O., 1997. Headspace analysis of volatile flower scent constituents of bats-pollinated plants. Phytochemistry 46, 1169-1172.

Bierzychudek, P., 1981. Pollinator limitation of plant reproductive effort. The American Naturalist 117, 838-840.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313, 351-354.

Bond, W.J., 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions: Biological Sciences 344, 83-90.

Bookman, S.S., 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa.* Ecology 64, 264-273.

Buchmann, S.L., Cane, J.H., 1989. Bees assess pollen returns while sonicating *Solanum* flowers. Oecologia 81, 289-294.

Bullock, S.H., 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. Biotropica 17, 287-301.

Bumrungsri, S., Leelapaibul, W., Racey, P.A., 2007. Resource partitioning in sympatric *Cynopterus* bats in lowland tropical rain forest, Thailand. Biotropica 39, 241–248.

Bumrungsri, S., Harbit, A., Benzie, C., Carmouche, K., Sridith, K., Racey, P., 2008. The pollination ecology of two species of *Parkia* (Mimosaceae) in southern Thailand. Journal of Tropical Ecology 24, 467–475.

Bumrungsri, S., Sripaoraya, E., Chongsiri, T., Sridith, K., Racey, P.A., 2009. The pollination ecology of durian (*Durio zibethinus*, Bombacaceae) in southern Thailand. Journal of Tropical Ecology 25, 85–92.

Bumrungsri, S., Lang, D., Harrower, C., Sripaoraya, E., Kitpipit, K., Racey, P.A., 2013. The dawn bat, *Eonycteris spelaea* Dobson (Chriptera: Pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. Acta Chiropterologica 15, 95-104.

Canela, M.B.F., Sazima, M., 2003. *Aechema pectinata*: a hummingbird-dependent bromeliad with inconspicuous flowers from the rainforest of south-eastern Brazil. Annals of Botany 92, 731-737.

Caruso, C.M., Alfaro, M., 2000. Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata.* Canadian Journal of Botany 78, 600-606.

Casper, B.B., 1988. Evidence for selective embryo abortion in *Cryptantha flava.* The American Naturalist 132, 318-326.

Castellanos, M.C., Wilson, P., Thomson, J.D., 2003. Pollen transfer by hummingbirds and bumblebess and the divergence of pollination modes in *Penstemon.* Evolution 57, 2742-2752.

Christian, C.E., 2001. Consequences of a biological invasion reveal the importance of mutualism for plant communities. Nature 413, 635-639.

Clare, E.L., Goerlitz, H.L., Drapeau, V.A., Holderied, M.W., Adams, A.M., Nagel, J., Dumont, E.R., Hebert, P.D.N., Fenton, M.B., 2013. Trophic niche flexibility in *Glossophaga soricina*: how nectar seeker sneaks an insect snack. Funtional Ecology, doi: 10.1111/1365-2435.12192.

Cochard, R., Ranamukhaarachci, S.L., Shivakoti, G.P., Shipin, O.V., Edwards, P.J., Seeland, K.T., 2008. The 2004 tsunami in Acheh and southern Thailand: a review on coastal ecosystems, wave hazards and vulnerability. Perspective in Plant Ecology, Evolution and Systematics 10, 3-40.

Collevatti, R.G., Grattapaglia, D., Hay, J.D., 2001. High resolution microsatellite based analysis of the mating system allows the detection of significant biparental inbreeding in *Caryocar brasiliense*, an endangered tropical tree species. Heredity 86, 60-67.

Collevatti, R.G., Estolano, R., Garci, S.F., Hay, J.D., 2010. Short-distance pollen dispersal and high self-pollination in a bat-pollinated Neotropical tree. Tree Genetics and Genomes 6, 555–564.

Colling, G., Reckinger, C., Matthies, D., 2004. Effects of pollen quantity and quality on reproduction and offspring vigor in the rare plant *Scorzonera humilis* (Asteraceae). American Journal of Botany 91, 1774–1782.

Coombe, B.G., 1976. The development of fleshy fruits. Annual Review of Plant Physiology 27, 507-528.
Corlett, R.T., 1998. Frugivory and seed dispersal by vertebrates in the Oriental (Indomalayan) region. Biological Reviews 73, 413-448.
Corlett, R.T., 2009. Seed dispersal distances and plant migration potential in tropical east Asia. Biotropica 41, 592–598.
Coupland, G.T., Paling, E.I., McGuinness, K.A., 2006. Floral abortion and pollination in four species of tropical mangroves from northern Australia. Aquatic Botany 84, 151–157.
Courts, S.E., 1997. Insectivory in captive Livingstone's and Rodrigues fruit bats *Pteropus livingstonii* and *P. rodricensis* (Chiroptera: Pteropodidae): an adaptation for obtaining protein. Journal of Zoology 242, 404-410.
Courts, S.E., 1998. Dietary strategies of the Old World fruit bats (Megachiroptera, Pteropodidae): how do they obtain sufficient protein. Mammal Review 28: 185-194.
Crist, T.O., Friese, C.F., 1993. The impact of fungi on soil: implications for plants and granivores in a semiarid shrub-steppe. Ecology 74, 2231-2239.
Crome, F.H.J., Irvine, A.K., 1986. "Two Bob each way": the pollination and breeding system of the Australian rain forest tree *Syzygium cormiflorum* (Myrtaceae). Biotropica 18, 115-125.
Cruden, R.W., 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31, 32-46.
Cruden, R.W., 2000. Pollen grains: why so many? Plant Systematics and Evolution 222, 43-165.
Dafni, A., 1992. Pollination Ecology: A Practical Approach. Oxford University Press, Oxford.
Dafni, A., Eisikowitch, D., Ivri, Y., 1987. Nectar flow and pollinators' efficiency in two co-occurring species of *Capparis* (Caparidaceaea) in Israel. Plant Systematics and Evolution 157, 181-186.
Dafni, A., Kevan, P.G., Husband, B.C., 2005. Practical Pollination Biology. Enviroquest Ltd., Cambridge.
Dahdouh-Geubas, F., Jayatissa, L.P., Di Nitto, D., Bosire, J.O., Lo Seen, D., Koedam, N., 2005. How effective were mangroves as a defence against the recent tsunami? Current Biology 15, 443-447.
Danielsen, F., Sorensen, M.K., Olwig, M.F., Selvam, V., Parish, F., Burgess, N.D., Hiralshi, T., Karunagaran, V.M., Rasmussen, M.S., Hansen, L.B., Quarto, A., Suryadiputra, N., 2005. The Asian tsunami: a protective role for coastal vegetation. Science 310, 643.
de Jong, T.J., Waser, N.M., Klinkhamer, P.G.L., 1993. Geitonogamy: the neglected side of selfing. Trends in Ecology and Evolution 8, 321-325.
Del Vaglio, M.A., Nicolaou, H., Bosso, L., Russo, D., 2011. Feeding habits of the Egyptian fruit bat *Rousettus aegyptiacus* on Cyprus Island: a first assessment. Hystrix Italian Journal of Mammalogy 22, 281-289.
Devy, M.S., Davidar, P., 2003. Pollination systems of trees in Kakachi, a mid-elevation wet evergreen forest in western Ghats, India. American Journal of Botany 90, 650-657.
Dick, C.W., Hardy, O.J., Jones, A.F., Petit, R.J., 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Tropical Plant Biology 1, 20-33.
Duke, N.C., 1984. A mangrove hybrid, *Sonneratia x gulngai* (Sonneratiaceae) from north-eastern Australia. Austrobaileya 2, 103-105.
Duke, N.C., 1992. Mangrove floristics and biogeography. In Robertson, A.I., Alongi, D.M. (Eds.), Tropical Mangrove Ecosystems. American Geophysical Union, Washington DC, pp. 63-100.
Duke, N.C., 1994. A mangrove hybrid, *Sonneratia x urama* (Sonneratiaceae) from northern Australia and southern New Guinea. Australian Systematic Botany 7, 521-526.
Duke, N.C., Jackes, B.R., 1987. A systematic revision of the mangrove genus *Sonneratia* (Sonneratiaceae) in Australasia. Blumea 32, 277-302.
Duke, N.C., Ball, M.C., Ellison, J.C., 1998. Factors influencing biodiversity and distributional gradients in mangrove. Global Ecology and Biogeography Letters 7, 27-47.

Dulberger, R., 1981. The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpiniaceae). American Journal of Botany 68, 1350-1360.
Elangovan, V., Marimuthu, G., Kunz, T.H., 2000. Nectar feeding behavior in short nosed fruit bat, *Cynopterus sphinx* (Pteropodidae). Acta Chiropterologica 2, 1-5.
Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24, 217-242.
Engel, C.E., Irwin, R.E., 2003. Linking pollinator visitation rate and pollen receipt. American Journal of Botany 90, 1612-1618.
Etcheverry, A.V., Aleman, M.M., Figueroa-Fleming, T., Lopez-Spahr, D., Gomez, C.A., Yanez, C., Figueroa-Castro, D.M., Ortega-Baes, P., 2012. Pollen:ovule ratio and its relationship with other floral traits in Papilionoideae (Leguminosae): an evaluation with Argentine species. Plant Biology 14, 171-178.
Faegri, K., van der Pijl, L., 1979. The Principles of Pollination Ecology, 3rd edn. Pergamon, Oxford.
FAO, 2007. The World's Mangrove 1980-2005. Food and Agriculture Organization of the United Nations, Rome.
Fenster, C.B., 1991. Gene fow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. Evolution 45, 398-409.
Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D., 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution and Systematics 35, 375-403.
Field, C.B., Osborn, J.G., Hoffman, L.L., Polsenberg, J.F., Ackerly, D.D., Berry, J.A., Bjoerkman, O., Held, A., Matson, P.A. and Mooney, H.A., 1998. Mangrove biodiversity and ecosystem function. Global Ecology and Biogeography Letters 7, 3-14.
Fishbein, M., Venable, D.L., 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. Ecology 77, 1061–1073.
Fishman, L., Wyatt, R., 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 53, 1723-1733.
Flanagan, R.J., Mitchell, R.J., Knutowski, D., Karron, J.D., 2009. Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). American Journal of Botany 96, 809-815.
Fleming, T.H., 1982. Foraging strategies of plant-visiting bats. In Kunz, T.H. (Ed.), Ecology of Bats. Plenum Publishing Corporation, New York, pp. 287-325.
Fleming, T.H., Heithaus, E.R., 1981. Frugivorous bats, seed shadows, and the structure of tropical forests. Biotropica 13, 45-53.
Fleming, T.H., Holland, N.J., 1998. The evolution of obligate pollination mutualisms: senita cactus and senita moth. Oecologia 114, 368-375.
Fleming, T.H., Kress, W.J., 2011. A brief history of fruits and frugivores. Acta Oecologica 37, 521-530.
Fleming, T.H., Kress, W.J., 2013. The Ornaments of Life, Coevolution and Conservation in the Tropics. The University of Chicago Press, Chicago.
Fleming, T.H., Muchhala, N., 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. Journal of Biogeography 35, 764-780.
Fleming, T.H., Sosa, V.J., 1994. Effects of nectarivorous and frugivorous mammals on reproductive success of plants. Journal of Mammalogy 75, 845-851.
Fleming, T.H., Nunez, R.A., da Silveira Lobo Sternberg, L., 1993. Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia 94, 72-75.
Fleming, T.H., Geiselman, C., Kress, W.J., 2009. The evolution of bat pollination: a phylogenetic perspective. Annals of Botany 104, 1017-1043.

Franceschinelli, E.V., Bawa, K.S., 2000. The effect of ecological factors on the mating system of a south American shrub species (*Helicteres brevispira*). Heredity 84, 116-123.

Francis, C.M., 2008. A Field Guide to the Mammals of South-East Asia. New Holland Publishers Ltd., United Kingdom.

Frick, W.F., Price, R.D., Heady, P.A., Kay, K.M., (2013). Insectivorous bat pollinates columnar cactus more effectively per visit than specialized nectar bat. The American Naturalist 181, 137-144.

Fuchs, E.J., Lobo, J.A., Quesada, M., 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. Conservation Biology 17, 149–157.

Fujita, M.S., Tuttle, M.D., 1991. Flying foxes (Chiroptera: Pteropodidae): threatened animals of key ecological and economic importance. Conservation Biology 5, 455-463.

Gallai, N., Salles, J-M., Settele, J., Vaissiere, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological economics 68, 810-821.

Gentry, A.H., 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6, 64-68.

Ghosh, A., Gupta, S., Maity, S., Das, S., 2008. Study of floral morphology of some Indian mangroves in relation to pollination. Research Journal of Botany 3, 9-16.

Giannini, N.P., Kalko, E.K.V., 2004. Trophic structure in a large assemblage of phyllostomid bats in Panama. Oikos 105, 209-220.

Gleason, S.M., Ewel, K.C., 2002. Organic matter dynamics on the forest floor of a Micronesian mangrove forest: an investigation of species composition shifts. Biotropica 34, 190-198.

Gong, W.K., Ong, J.E., 1990. Plant biomass and nutrient flux in a managed mangrove forest in Malaysia. Estuarine, Coastal and Shelf Science 31, 519-530.

Gonzales, R.S., Ingle, N.R., Lagunzad, D.A., Nakashizuka, T., 2009. Seed dispersal by birds and bats in lowland Philippine forest successional area. Biotropica 41, 452-458.

Goodwillie, C., Kalisz, S., Eckert, C.G., 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annual Review of Ecology, Evolution and Systematics 36, 47-79.

Gould, E., 1978. Foraging behavior of Malaysian nectar-feeding bats. Biotropica 10, 184-193.

Goutham-Bharathi, M.P., Kaliyamoorthy, M., Dam Roy, S., Krishnan, P., George, G., Murugan, C., 2012. *Sonneratia ovata* (Sonneratiaceae)- a new distributional record for India from Andaman and Nicobar Islands. Taiwania 57, 406-409.

Griffin, S.R., Barret, S.C.H., 2002. Factors affecting low seed:ovule ratios in a spring woodland herb, *Trillium grandiflorum* (Melanthiaceae). International Journal of Plant Sciences 163, 581-590.

Harper, C.J., Swartz, S.M., Brainerd, E.L., 2013. Specialized bat tongue is a hemodynamic nectar mop. Proceedings of the National Academy of Sciences 110, 8852-8857.

Harun-or-Rashid, S., Biswas, S.R., Bocker, R., Kruse, M., 2009. Mangrove community recovery potential after catastrophic disturbance in Bangladesh. Forest Ecology and Management 257, 923-930.

Hatcher, B.G., Johannes, R.E., Robertson, A.I., 1989. Review of research relevant to conservation of shallow tropical marine ecosystem. Biological Annual Review 27, 337-414.

Heithaus, E.R., Opler, P.A., Baker, H.G., 1974. Bat activity and pollination of *Bauhinia pauletia*: plant-pollinator coevolution. Ecology 55, 412-419.

Heithaus, E.R., Fleming, T.H., Opler, P.A., 1975. Foraging patterns and resource utilization in seven species of bats in a seasonal tropical forest. Ecology 56, 841-854.

Heizmann, P., Luu, D.T., Dumas, C., 2000. Pollen-stigma adhesion in the Brassicaceae. Annals of Botany 85, 23-27.

Henry, M., Pons, J.M., Cosson, J.F., 2007. Foraging behaviour of a frugivorous bat helps bridge landscape connectivity and ecological processes in a fragmented rainforest. Journal of Animal Ecology 76, 801–813.
Hernindez-Conrique, D., Iniguez-Davalos, L., I., Storz, J., F., 1997. Selective feeding by phyllostomid fruit bats in a subtropical montane cloud forest. Biotropica 29, 376-379.2
Herrera, C.M., 1987. Components of pollinator quality: comparative analysis of a diverse insect assemblage. Oikos 50, 79-90.
Herrera, C.M., 1988a. Variation in mutualisms: the spatiotemporal mosaic of a pollinator assemblage. Biological Journal of the Linnean Society 35, 95-125.
Herrera, C.M., 1988b. The fruiting ecology of *Osyris quadripartita*: individual variation and evolutionary potential. Ecology 69, 233-249.
Herrera, L.G.M., Del Rio, C.M., 1998. Pollen digestion by New World bats: effects of processing time and feeding habits. Ecology 79, 2828–2838.
Herrera, L.G.M., Hobson, K.A., Miron, L.M., Ramirez, N.P., Mendez, G.C., Sanchez-Cordero, V., 2001. Sources of protein in two species of phytophagous bats in a seasonal dry forest: evidence from stable isotope analysis. Journal of Mammalogy 82: 352-361.
Hevly, R.H., 1979. Dietary habits of two nectar and pollen feeding bats in southern Arizona and northern Mexico. Journal of the Arizona-Nevada Academy of Science 14, 13-18.
Hirayama, K., Ishida, K., Tomaru, N., 2005. Effects of pollen shortage and self-pollination on seed production of an endangered tree, *Magnolia stellata*. Annals of Botany 95, 1009–1015.
Hodgkison, R., Balding, S.T., Zubaid, A., Kunz, T.H., 2003. Fruit bats (Chiroptera: Pteropodidae) as seed dispersers and pollinators in a lowland Malaysian rain forest. Biotropica 35, 491-502.
Hogart, P.T. (2007). The Biology of Mangrove and Sea Grasses, 2nd edn. Oxford University Press, Oxford.
Hojjat, S.S., 2011. Effect of seed size on germination and seedling growth of some lentils genotypes (*Lens culinaris* Medik.). International Journal of Agriculture and Crop Science 3, 1-5.
Holland, N.J., Chamberlain, S.A., 2007. Ecological and evolutionary mechanisms for low seed:ovule ratios: need for a pluralistic approach? Ecology 88, 706-715.
Holloway, R.H.P., 2003. Natural History Notes on the River Terrapin *Batagur baska* (gray, 1831) in Cambodia. Research Fellowship Program Report. Wildlife Conservation Society, New York.
Holmquist, K.G., Mitchell, R.J., D, K.J., 2012. Influence of pollinator grooming on pollen-mediated gene dispersal in *Mimulus ringens* (Phrymaceae). Plant Species Biology 27, 77-85.
Holsinger, K.E., Feldman, M.W., Christiansen, F.B., 1984. The evolution of self-fertilization in plants: a population genetic model. The American Naturalist 124, 446-453.
Horner, M.A., Fleming, T.H., Sahley, C.T., 1998. Foraging behaviour and energetics of a nectar-feeding bat, *Leptonycteris curasoae* (Chiroptera: Phyllostomidae). Journal of Zoology 244, 575-586.
Howe, H.F., Smallwood, J., 1982. Ecology of seed dispersal. Annual Review of Ecology and Systematics 13, 201-228.
Howe, H.F., Wesley, L.C., 1988. Ecological Relationships of Plants and Animals. Oxford University Press, New York.
Howell, D.J., 1974. Bats and pollen: physiological aspects of the syndrome of chiropterophily. Comparative Biochemistry and Physiology 48A, 263-276.
Howell, D.J., Hodgkin, N., 1976. Feeding adaptations in the hairs and tongues of nectar-feeding bats. Journal of Morphology 148, 329-336.
Hu, X.W., Wang, Y.R., Wu, Y.P., 2009. Effects of the pericarp on imbibition, seed germination, and seedling establishment in seeds of *Hedysarum scoparium* Fisch. et Mey. Ecological Research 24, 559-564.

Hulbert, A.H., Hosoi, S.A., Temeles, E.J., Ewald, P.W., 1996. Mobility of *Impatiens capensis* flowers: effect on pollen deposition and hummingbird foraging. Oecologia 105, 243-246.

Husband, B.C., Schemske, D.W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54-70.

Ibarra-Cerdena, C.N., Iniguez-Davalos, L.I., Sanchez-Cordero, V., 2005. Pollination ecology of *Stenocereus queretaroensis* (Cactaceae), a chiropterophilous columnar cactus, in a tropical dry forest of Mexico. American Journal of Botany 92, 503-509.

Inouye, D.W., 1980. The terminology of floral larceny. Ecology 61, 1251-1253.

Inouye, D.W., Gill, D.E., Dudash, M.R., Fenster, C.B., 1994. A model and lexicon for pollen fate. American Journal of Botany 81, 1517-1530.

Islam, S.S., Azad, M.A.K., Kabir, J., Hossain, M.A.T., 2012. Financial analysis of keora (*Sonneratia apetala*) plantations in Bangladesh. Open Journal of Statistics 2, 124-130.

Janzen, D.H., 1970. Herbivores and the number of tree species in tropical forests. The American Naturalist 104, 501-528.

Janzen, D.H., 1977. A note on optimal mate selection by plants. The American Naturalist 111, 365-371.

Jiny Varghese, K., Belzik, N., Nisha, A.R., Resiya, S., Resmi, S., Silvipriya, K.S., 2010. Pharmacognostical and phytochemical studies of a mangrove (*Sonneratia caseolaris*) from Kochi of Kerala state in India. Journal of Pharmacy Research 3, 2625-2627.

Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. Trend in Ecology and Evolutions 15, 140-143.

Jurgens, A., Witt, T., Gottsberger, G., 2002. Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: correlation with breeding system, pollination, life form, style number and sexual system. Sexual Plant Reproduction 14, 279-289.

Kamaruzaman, J., Dahalan, T., 2008. Managing sustainable mangrove forests in Peninsular Malaysia. Journal of Sustainable Management 1, 88-96.

Kathiresan, K., Bingham, B.L., 2001. Biology of mangroves and mangrove ecosystems. Advance in Marine Biology 40, 81-251.

Kathiresan, K., Rajendran, N., 2005. Coastal mangrove forests mitigated tsunami. Estuaries, Coastal and Shelf Science 65, 601-606.

Kearns, C.A., Inouye, D.W., 1997. Pollinators, flowering plants, and conservation biology. Bioscience 47, 297-307.

Kearns, C.A., Inouye, D.W., Waser, N.M., 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 28, 83-112.

Kelly, J.K., Rasch, A., Kalisz, S., 2002. A method to estimate pollen viability from pollen size variation. American Journal of Botany 89, 1021-1023.

King, C., Ballantyne, G., Willmer, P.G., 2013. Why flower visitation is a poor proxy for pollination: measuring single-pollen deposition, with implications for pollination networks and conservation. Methods in Ecology and Evolution 4, 811-818.

Kingston, T., Lim, B.L., Zubaid, A., 2006. Bats of Krau Wildlife Reserve. Penerbit Universiti Kebangsaan Malaysia, Bangi.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274, 303-313.

Knudsen, J.T., Ericksson, R., Gershenzon, J., Stahl, B., 2006. Diversity and distribution of floral scent. The Botanical Review 72, 1-120.

Korine, C., Izhaki, I., Arada, Z., 1999. Is the Egyptian fruit-bat *Rousettus aegyptiacus* a pest in Israel? An analysis of the bat's diet and implications for its conservation. Biological Conservation 88, 301-306.

Kress, J.W., 1985. Bat pollination of an old world *Heliconia*. Biotropica 17, 167-179.

Kunz, T.H., Diaz, C.A., 1995. Folivory in fruit-eating bats, with new evidence from *Artibeus jamaicensis* (Chiroptera: Phyllostomidae). Biotropica 27, 106-120.

Kunz, T.H., de Torrez, E.B., Bauer, D., Lobova, T., Fleming, T.H., 2011. Ecosystem services provided by bats. Annals of the New York Academy of Science 1223, 1-38.

Langley, C.M., 1996. Search image: selective attention to specific visual features of prey. Journal of Experimental Psychology: Animal Behaviour Processes 22, 152-163.
Law, B.S., 1992a. Physiological factors affecting pollen use by Queensland blossom bats (*Syconyctcris australis*). Functional Ecology 6, 257-264.
Law, B.S., 1992b. The maintenance nitrogen requirements of the Queensland blossom bat (*Syconycteris australis*) on a sugar/pollen diet: is nitrogen a limiting resource? Physiological Zoology 65, 634-648.
Law, B.S., Lean, M., 1999. Common blossom bats (*Syconycteris australis*) as pollinators in fragmented Australian tropical rainforest. Biological Conservation 91, 201-212.
Lee, T.D., 1984. Patterns of fruit maturations: a gametophyte competition hypothesis. The American Naturalist 123, 427-432.
Leigh, E.G.J., Davidar, P., Dick, C.W., Puyravaud, J.P., Terborgh, J., ter Steege, H., Wright, S.J., 2004. Why do some tropical forests have so many species of trees? Biotropica 36, 447–473.
Levin, D.A., Berube, D.E., 1972. *Phlox* and *Colias*: the efficiency of a pollination system. Evolution 26, 242-250.
Levri, M.A., 1998. The effect of timing of pollination on the mating system and fitness of *Kalmia latifolia* (Ericaceae). American Journal of Botany 85, 1626-1630.
Lewis, R.R.I., 2000. Ecologically based goal setting in mangrove forest and tidal marsh restoration. Ecological Engeneering 15, 191-198.
Li, H., Chen, G., 2009. Genetic variation within the endangered mangrove species *Sonneratia paracaseolaris* (Sonneratiaceae) in China detected by inter-simple sequence repeats analysis. Biochemical, Biosystematics and Ecology 37, 260-265.
Lim, B.L., 1970. Food habits and breeding cycle of the Malaysian fruit-eating bat, *Cynopterus brachyotis*. Journal of Mammalogy 51, 174-177.
Liu, A.-Z., Li, D.-Z., Wang, H., Kress, W.J., 2002. Ornithophilous and chiropterophilous pollination in *Musa itinerans* (Musaceae), a pioneer species in tropical rain forests of Yunnan, southwestern China. Biotropica 34, 254-260.
Lobo, J.A., Quesada, M., Stoner, K.E., 2005. Effects of pollination by bats on the mating system of *Ceiba pentandra* (Bombacaceae) populations in two tropical life zones in Costa Rica. American Journal of Botany 92, 370-376.
Lopez, J., Rodriguez-Riano, T., Ortega-Olivencia, A., Devesa, J.A., Ruiz, T., 1999. Pollination mechanism and pollen-ovule ratios in some *Genisteae* (*Fabaceae*) from southwestern Europe. Plant Systematic and Evolution 216, 23-47.
Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15, 65-95.
Lowry, J.B., 1989. Green-leaf fractionation by fruit bats: is this feeding behaviour a unique nutritional strategy for herbivores? Australian Wildlife Research 16, 203-236.
Machado, I.C.S., Sazima, I., Sazima, M., 1998. Bat pollination of the terrestrial herb *Irlbachia alata* (Gentianaceae) in northeastern Brazil. Plant Systematics and Evolution 209, 231-237.
Mancina, C.A., Balseiro, F., Herrera, L.G.M., 2005. Pollen digestion by nectarivorous and frugivorous Antillean bats. Mammalian Biology 70, 282-290.
Mao, L., Batten, D.J., Fujiki, T., Li, Z., Dai, L., Weng, C., 2012. Key to mangrove pollen and spores of southern China: an aid to palynological interpretation of quaternary deposits in the South China Sea. Review of Palaeobotany and Palynology 176–177, 41-67.
Marques, M.C.M., Fischer, E., 2009. Effect of bats on seed distribution and germination of *Calophyllum brasiliense* (Clusiaceae). Ecotropica 15, 1-6.
Marr, D.L., Leebens-Mack, J., Elms, L., Pellmyr, O., 2000. Pollen dispersal in *Yucca filamentosa* (Agavaceae): the paradox of self-pollination behaviour by *Tegeticula yuccasella* (Prodoxidae). American Journal of Botany 87, 670-677.
Marshall, A.G., 1983. Bats, flowers and fruit: evolutionary relationships in the Old World. Biological Journal of the Linnean Society 20, 115-135.

Martinell, M.C., Dotterl, S., Blanche, C., Rovira, A., Masso, S., Bosch, M., 2010. Nocturnal pollination of the endemic *Silene sennenii* (Caryophyllaceae): an endangered mutualism? Plant Ecology 211, 203-208.

Matallana, G., Wendt, T., Araujo, D.S.D., Scarano, F.R., 2005. High abundance of dioecious plants in a tropical coastal vegetation. American Journal of Botany 92, 1513-1519.

Mayfield, M., Waser, N.M., Price, M., 2001. Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. Annals of Botany 88, 591-596.

Mazda, Y., Magi, M., Ikeda, Y., Kurokawa, T., Asano, T., 2006. Wave reduction in mangrove forest dominated by *Sonneratia* sp. Wetlands Ecology and Management 14, 365-378.

Meehan, H.J., McConkey, K.R., Drake, D.R., 2002. Potential disruptions to seed dispersal mutualisms in Tonga, western Polynesia. Journal of Biogeography 29, 695-712.

Mione, T., Anderson, G.J., 1992. Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum* (Solanaceae). American Journal of Botany 79, 279-287.

Mohd Lokman, H., Sulong, I., 2001. Mangroves of Terengganu. Kolej Universiti Sains dan Teknologi Malaysia and Forestry Department Peninsular Malaysia, Kuala Lumpur.

Molina-Freaner, F., Cervantes-Salas, M., Morales-Romero, D., Buchmann, S., Fleming, T.F., 2003. Does the pollinator abundance hypothesis explain geographic variation in the breeding system of *Pachycereus pringlei*? International Journal of Plant Sciences 164, 383-393.

Moll, D., Moll, E.O., 2004. The Ecology, Exploitation, and Conservation of River Terrapin. Oxford University Press, New York.

Momose, K., Yumoto, T., Nagamitsu, T., Kato, M., Nagamasu, H., Sakai, S., Harrison, R.D., Itioka, T., Hamid, A.A., Inoue, T., 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. American Journal of Botany 85, 1477–1501.

Motten, A.F., 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. Ecological Monographs 56, 21-42.

Motten, A.F., Stone, J.L., 2000. Heritability of stigma position and the effect of anther-stigma separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). American Journal of Botany 87, 339-347.

Mqokeli, B.R., Downs, C.T., 2013. Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*). Zoomorphology 132, 111–119.

Muchhala, N., 2003. Exploring boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). Oecologia 134, 373-380.

Muchhala, N., 2006. The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. American Journal of Botany 93, 1081-1089.

Muchhala, N., 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. The American Naturalist 169, 494–504.

Muchhala, N., 2008. Functional significance of interspecific variation in *Burmeistera* flower morphology: evidence from nectar bat captures in Ecuador. Biotropica 40, 332–337.

Muchhala, N., Jarrin-V, P., 2002. Flower visitation by bats in cloud forests of western Ecuador. Biotropica 34, 387-395.

Muchhala, N., Potts, M.D., 2007. Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. Philosophical Transactions of the Royal Society B 274, 2731–2737.

Muchhala, N., Caiza, A., Vizuete, J.C., Thomson, J.D., 2008. A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*. Annals of Botany 103, 1481–1487.

Murcia, C., Feinsinger, P., 1996. Interspecific pollen loss by hummingbirds visiting flower mixture: effects of floral architecture. Ecology 77, 550-560.

Muscarella, R., Fleming, T.H., 2007. The role of frugivorous bats in tropical forest succession. Biological Review 82, 573-590.
Nakamoto, A., Kinjo, K., Izawa, M., 2009. The role of Orii's flying-fox (*Pteropus dasymallus inopinatus*) as a pollinator and a seed disperser on Okinawa-jima Island, the Ryukyu Archipelago. Japan Ecological Research 24, 405-414.
Nakisah, M.A., Fauziah, A.H., 2003. Setiu Wetlands, Tranquility Amidst Plenty. Kolej Universiti Sains dan Teknologi Malaysia, Kuala Terengganu.
Naranjo, M.E., Rengifo, C., Soriano, P.J., 2003. Effect of ingestion by bats and birds on seed germination of *Stenocereus griseus* and *Subpilocereus repandus* (Cactaceae). Journal of Tropical Ecology 19, 19-25.
Nason, J.D., Herre, E.A., Hamrick, J.L., 1998. The breeding structure of a tropical keystone plant resource. Nature 391, 685-687.
Nassar, J.M., Ramirez, N., Linares, O., 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. American Journal of Botany 84, 918–927.
Nassar, J.M., Hamrick, J.L., Fleming, T.H., 2003. Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). American Journal of Botany 90, 1628-1637.
Nathan, P.T., Raghuram, H., Elangovan, V., Karuppudurai, T., Marimuthu, G., 2005. Bat pollination of kapok tree, *Ceiba pentandra*. Current Science 88, 1679-1681.
Nathan, P.T., Karuppudurai, T., Raghuram, H., Marimuthu, G., 2009. Bat foraging strategies and pollination of *Madhuca latifolia* (Sapotaceae) in southern India. Acta Chiropterologica 11, 435–441.
Neal, P.R., Anderson, G.J., 2004. Does the 'old bag' make a good wind bag?: comparison of four fabrics commonly used as exclusion bags in studies of pollination and reproductive biology. Annals of Botany 93, 603-607.
Ne'eman, G., Jurgens, A., Newstrom-Llyods, L., Potts, S.G., Dafni, A., 2010. A framework for comparing pollinator performances: effectiveness and efficiency. Biological Review 85, 435-451.
Nelson, S.L., Kunz, T.H., Humphrey, S.R., 2005. Folivory in fruit bats: leaves provide a natural source of calcium. Journal of Chemical Ecology 31, 1683-1691.
Nielsen, M.G. (2000). Distribution of the ant (Hymenoptera: Formicidae) fauna in the canopy of the mangrove tree *Sonneratia alba* J. Smith in northern Australia. Australian Journal of Entomology 39, 275-279.
Niesenbaum, R.A., 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). American Journal of Botany 86, 261-268.
Nilsson, L.A., Johnsson, L., Ralison, L., Randrianjohany, E., 1987. Angraecoids orchids and hawkmoths in central Madagascar: specialized pollination systems and generalist foragers. Biotropica 19, 310-318.
Noor Azura, S., 2013. Eksport durian beku meningkat RM24 juta (in Malay language). *Sinar Harian*. 14 April 2013. Available at http://www.doa.gov.my/c/document_library/get_file?uuid=b3ff5ea1-8df3-4241-a1f0-204ac24c9dac&groupId=38371 (Accessed: 24 June 2013).
Nowak, R.M., 1994. Walker's Bats of the World. Johns Hopkins University Press, Baltimore.
Nunes-Silva, P., Hnrcir, M., Shipp, L., Imperatriz-Fonseca, V.L., Kevan, P.G., 2013. The behaviour of *Bombus impatiens* (Apidae, Bombini) on tomato (*Lycopersicon esculentum* Mill., Solanaceae) flowers: pollination and reward perception. Journal of Pollination Ecology 11, 33-40.
Nyhagen, D.F., Turnbull, S.D., Olesen, J.M., Jones, C.G., 2005. An investigation into the role of the Mauritian flying fox, *Pteropus niger*, in forest regeneration. Biological Conservation 122. 491-497.
Obeso, J.R., 2004. A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. Perspectives in Plant Ecology, Evolution and Systematics 6, 217-225.

Ollerton, J., Alarcon, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C., Rottenberry, J., 2009. A global test of the pollination syndrome hypothesis. Annals of Botany 103, 1471-1480.

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326.

Ortega, E., Dicenta, F., Egea, J., 2007. Rain effect on pollen–stigma adhesion and fertilization in almond. Scientia Horticulturae 112, 345-348.

Osborn, J.G. and Polsenberg, J.F., 1996. Meeting of the mangrovellers: the interface of biodiversity and ecosystem function. Trends in Ecology and Evolution 11, 354-356.

Palmer, M., Travis, J., Antonovics, J., 1989. Temporal mechanisms influencing gender expression and pollen flow within a self-incompatible perennial, *Amianthium muscaetoxicum* (Liliaceae). Oecologia 78, 231-236.

Pandit, S., Choudhury, B.C., 2001. Factors affecting pollinator visitation and reproductive success in *Sonneratia caseolaris* and *Aegiceras corniculatum* in a mangrove forest in India. Journal of Tropical Ecology 17, 431-447.

Parry-Jones, K., Augee, M.L., 1991. Food selection by grey-headed flying foxes *Pteropus poliocephalus* occupying a summer colony site near Gosford, New South Wales. Wildlife Research 18, 111-124.

Pasquet, R.S., Peltier, A., Hufford, M.B., Knudsen, J.T., Herren, H.R., Gepts, P., 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. Proceeding of the National Academy of Sciences 105, 3456–13461.

Pellmyr, O., Huth, C.J., 1994. Evolution stability of mutualism between yuccas and yucca moths. Nature 372, 257-260.

Pellmyr, O., Thompson, J.N., Brown, J.M., Harrison, R.G., 1996. Evolution of pollination and mutualism in the yucca moth lineage. The American Naturalist 148, 827-847.

Perfectti, F., Gomez, J.M., Bosch, J., 2009. The functional consequences of diversity in plant-pollinator interactions. Oikos 118, 1430-1440.

Perret, M., Chautems, A., Spichiger, R., Peixoto., M., Savolainen, V., 2001. Nectar sugar composition in relation to pollination syndrome in Sinnigieae (Gesneriaceae). Annals of Botany 87, 267-273.

Petit, S., 2011. Effects of mixed-species pollen load on fruits, seeds, and seedlings of two sympatric columnar cactus species. Ecological Research 26, 461–469.

Pettersson, S., Knudsen, J.T., 2001. Floral scent and nectar production in *Parkia biglobosa* Jacq. (Leguminosae: Mimosoideae). Botanical Journal of the Linnean Society 135, 97-106.

Pettersson, S., Ervik, F., Knudsen, J.T., 2004. Floral scent of bat-pollinated species: west Africa vs. the New World. Biological Journal of the Linnean Society 82, 161-168.

Phua, P.B., Corlett, R., 1989. Seed dispersal by the lesser short-nosed fruit bat (*Cynopterus brachyotis*, Pteropodidae, Megachiroptera). Malayan Nature Journal 42, 251-256.

Picot, M., Jenkins, R.K.B., Ramilijaona, O., Racey, P.A., Carriere, S.M., 2007. The feeding ecology of *Eidolon dupreanum* (Pteropodidae) in eastern Madagascar. African Journal of Ecology 45, 645-650.

Pina, H.H., Montana, C., Mandujano, M.C., 2007. Fruit abortion in the Chihuahuan-dessert endemic cactus *Opuntia microdasys*. Plant Ecology 193, 305-313.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25, 345-353.

Primack, R.B., Duke, N.C., Tomlinson, P.B., 1981. Floral morphology in relation to pollination ecology in five Queensland coastal plants. Austrobaileya 1, 346-355.

Primavera, J.H., Esteban, J.M.A., 2008. A review of mangrove rehabilitation in the Phillipines: successess, failure and future prospects. Wetlands Ecology and Management 16, 345-358.

Proctor, M., Yeo, P., Lack, A., 1996. The Natural History of Pollination. Timber Press, Portland.

Putz, F.E., Chan, H.T., 1986. Tree growth, dynamics, and productivity in a mature mangrove forest in Malaysia. Forest Ecology and Management 17, 211-230.

Qiu, S., Zhou, R.C., Li, Y.Q., Havanond, S., Jaengjai, C., Shi, S.H., 2008. Molecular evidence for natural hybridization between *Sonneratia alba* and *S. griffithii*. Journal of Systematics and Evolution 46, 391-395.

Quesada, M., Fuchs, E.J., Lobo, J.A., 2001. Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree *Pachira quinata* (Bombacaceae). American Journal of Botany 88, 2113-2118.

Quesada, M., Stoner, K.E., Rosas-Guerrero, V., Palasios-Guevara, C., Lobo, J.A., 2003. Effect of habitat disruption on the activity of nectarivorous bats in a dry tropical forest, implications for the reproductive success of the Neotropical tree *Ceiba grandiflora*. Oecologia 135, 400-406.

Quesada, M., Stoner, K.E., Lobo, J.A., Herrerías-Diego, Y., Palacios-Guevara, C., Munguía-Rosas, M.A., O.-Salazar, K.A., Rosas-Guerrero, V., 2004. Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated bombacaceous trees. Biotropica 36, 131-138.

Rajamani, L., Aminah, A., Zubaid, A., Tan, K.H., Kunz, T.H., 1999. Chemical composition of leaves consumed by the lesser dog-faced fruit bat, *Cynopterus brachyotis*, in Peninsular Malaysia. Acta Chiropterologica 1, 209-214.

Reiter, J., Tomaschewski, I., 2003. Chemical composition of leaves consumed by *Ptenochirus jagori*. Mammalian Biology 68, 112-115.

Ren, H., Lu, H., Shen, W., Huang, C., Guo, Q., Li, Z., Jian, S., 2009. *Sonneratia apetala* Buch. Ham in the mangrove ecosystems of China. Ecological Engineering 35, 1243-1248.

Ricklefs, R.E., 2004. A comprehensive framework for global patterns in biodiversity. Ecology Letters 7, 1-15.

Robertson, A.W., Trass, A., Ladley, J.J., Kelly, D., 2006. Assessing the benefits of frugivory for seed germination: the importance of the deinhibition effect. Functional Ecology 20, 58-66.

Roulston, T.H., Crane, J.H., 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution 222, 187-209.

Ruby, J., Nathan, P.T., Balasingh, J., Kunz, T.H., 2000. Chemical composition of fruits and leaves eaten by short-nosed fruit bat, *Cynopterus sphinx*. Journal of Chemical Ecology 26, 2826-2841.

Salmo III, S.G., Fernando, E.S., Peras, J.R., Sukardjo, S., Miyagi, T., Ellison, J., Koedam, N.E., Wang, Y., Primavera, J., Jin Eong, O., Wan-Hong Yong, J., Ngoc Nam, V., 2010. *Sonneratia ovata*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available at www.iucnredlist.org. (Accessed: 30 April 2014).

Sazima, M., Sazima, I., 1978. Bat pollination of the passion flower, *Passiflora mucronata*, in southeastern Brazil. Biotropica 10, 100-109.

Sazima, M., Sazima, I., Buzato, S., 1994. Nectar by day and night: *Simphocampylus sulfurous* (Lobeliaceae) pollinated by hummingbirds and bats. Plant Systematics and Evolution 191, 237-246.

Sazima, M., Buzato, S., Sazima, I., 1999. Bat-pollinated flower assemblages and bat visitors at two atlantic forest sites in Brazil. Annals of Botany 83, 705-7012.

Schemske, D.W., Horvitz, C.C., 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225, 519-521.

Schulke, B., Waser, N.M., 2001. Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. Oecologia 127, 239-245.

Schupp, E.W., 1993. Quantity, quality and the effectiveness of seed dispersal by animals. Vegetatio 107/108, 15-29.

Sekercioglu, C.H., 2006. Increasing awareness of avian ecological function. Trends in Ecology and Evolution 21, 464-471.

Seltzer, C.E., Ndangalasi, H.J., Cordeiro, N.J., 2013. Seed dispersal in the dark: shedding light on the role of fruit bats in Africa. Biotropica 45, 450-456.

Shahrul Anuar, M.S., Nor Zalipah, M., Nurul 'Ain, E., Ibrahim, J., Mark Rayan, D., Ganesan, M., Nazri, A., Mohd Hussain, Y., 2005. Survey of mammals and other vertebrate fauna of Matang mangroves swamp forest. In Shaharuddin, M.I., Azahar, M., Razani, U., Kamaruzaman, A.B., Lim, K.L., Suhaili, R., Jalil, M.S., Latiff, A. (Eds.), Sustainable Management of Matang Mangroves: 100 Years and Beyond. Forestry Department of Peninsular Malaysia, Kuala Lumpur, pp. 319-323.

Shahrul Anuar, M.S., Nurul 'Ain, E., Nor Zalipah, M., Mark Rayan, D., Ganesan, M., 2006. Mammals and other vertebrates fauna survey in Balik Pulau and Pantai Acheh. In Mashhor, M., Mohd Yunus, Z. (Eds.), Ecological of Mangrove Forest – A Case Study of Balik Pulau and Pantai Acheh. Publisher of Science University of Malaysia, Pulau Pinang, pp. 33-72.

Shilton, L.A., Altringham, J.D., Compton, S.G., Whittaker, R.J., 1999. Old World fruit bats can be long-distance seed dispersers through extended retention of viable seeds in the gut. Proceedings of the Royal Society of London B 266, 219-223.

Silander, J.A., Primack, R.B., 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). American Midland Naturalist 100, 213-216.

Simon, R., Holderied, M.W., Koch, C.U., von Helversen, O., 2011. Floral acoustics: conspicuous echoes of a dish-shaped leaf attract bat pollinators. Science 333, 631-633.

Singaravelan, N., Marimuthu, G., 2004. Nectar feeding and pollen carrying from *Ceiba pentandra* by Pteropodid bats. Journal of Mammalogy 85, 1-7.

Singer, R.B., Breier, T.B., Flach, A., Faria-Singer, R., 2006. The pollination mechanism of *Habenaria pleiophylla* Hoehne and Schlechter (Orchidaceae: Orchinidae). Functional Ecosystems and Communities 1, 10-14.

Slauson, L.A., 2000. Pollination biology of two chiropterophilous agaves in Arizona. American Journal of Botany 87, 825-836.

Snow, A.A., 1982. Pollination intensity and potential seed set in *Passiflora vitifolia.* Oecologia 55, 231-237.

Solomon Raju, A.J., Karyamsetty, H.J., 2008. Reproductive ecology of mangrove trees *Ceriops decandra* (Griff.) Ding Hou and *Ceriops tagal* (Perr.) C.B. Robinson (Rhizophoraceae). Acta Botanica Croatica 67, 201-208.

Spears, E.E.J., 1983. A direct measure of pollinator effectiveness. Oecologia 57, 196-199.

Srithongchuay, T., Bumrungsri, S., Sripao-raya, E., 2008. The pollination ecology of the late-successional tree, *Oroxylum indicum* (Bignoniaceae) in Thailand. Journal of Tropical Ecology 24, 477–484.

Stanton, M.L., 1984. Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. Ecology 65, 1105-1112.

Start, A.N., Marshall, A.G., 1976. Nectarivorous bats as pollinators of trees in west Malaysia. In Burley, J., Styles, B.T. (Eds.), Variation, Breeding and Conservation of Tropical Forest Trees. Academic Press, London, pp. 141-150.

Stead, A.D., Roberts, I.N., Dickinson, H.G., 1979. Pollen-pistil interaction in *Brassica oleracea:* events prior to pollen germination. Planta 146, 211-216.

Stebbins, G.L., 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. Annual Review of Ecology and Systematics 1, 307-326.

Stephenson, A.G., 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12, 53-279.

Stephenson, A.G., 1982. When does outcrossing occur in mass flowering plant? Evolution 36, 762-767.

Stephenson, A.G., Winsor, J.A., 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. Evolution 40, 453-458.

Stone, J.L., 1996. Components of pollinations effectiveness in *Psychotria suerrensis*, a tropical distylous shrub. Oecologia 107, 504-512.

Stone, G.N., Willmer, P., Rowe, J.A., 1998. Partitioning of pollinators during flowering in an African acacia community. Ecology 79, 2808-2827.

Sutherland, S., 1986. Pattern of fruit-set: what controls fruit-flower ratios in plants? Evolution 40, 117-128.

Tan, K.H., Zubaid, A., Kunz, T.H., 1998. Food habits of *Cynopterus brachyotis* (Muller) (Chiroptera: Pteropodidae) in Peninsular Malaysia. Journal of Tropical Ecology 14, 299–307.

Tan, K.H., Zubaid, A., Kunz, T.H., 1999. Fruit dispersal by the lesser dog-faced fruit bat, *Cynopterus brachyotis*. Malayan Nature Journal 53, 57 - 62.

Tang, Z.H., Cao, M., Sheng, L.X., Ma, X.F., Walsh, A., Zhang, S.Y., 2008. Seed dispersal of *Morus macroura* (Moraceae) by two frugivorous bats in Xishuangbanna, SW China. Biotropica 40, 127–131.

Tang, Z.H., Xu, J.L., Flanders, J., Ding, X.M., Ma, X.F., Sheng, L., Cao, M., 2012. Seed dispersal of *Syzygium oblatum* (Myrtaceae) by two species of fruit bat (*Cynopterus sphinx* and *Rousettus leschenaulti*) in south west China. Journal of Tropical Ecology 28, 255 261.

Thiele, J., Winter, Y., 2005. Hierarchical strategy for relocating food targets in flower bats: spatial memory versus cue-directed search. Animal Behaviour 69, 315–327.

Thomas, D.W., 1984. Fruit intake and energy budgets of frugivorous bats. Physiological Zoology 57, 457-467.

Thomas, D.W., 1988. Analysis of diets of plant-visiting bats. In Kunz, T.H. (Ed.), Ecological and Behavioral Methods for the Study of Bats. Smithsonian Institution Press, Washington, D.C, pp. 211-220.

Thomson, J., 2003. When is it mutualism? The American Naturalist 162, S1-S9.

Tokeshi, M., Yoko-O, M., Pahlano Daud, J.R., Domits, M., (2007). *Hypolycaena erylus* feeding on mangrove apple and attended by *Oecophylla* weaver ants, in north Sulawesi, Indonesia. Tropical Lepidoptera 17, 35-36.

Tomlinson, P.B., 1986. The Botany of Mangroves. Cambridge University Press, Cambridge.

Traveset, A., 1998. Effect of seed passage through vertebrate frugivores' guts on germination: a review. Perspectives in Plant Ecology, Evolution and Systematics 1, 151–190.

Tschapka, M., 2003. Pollination of the understorey palm *Calyptrogyne ghiesbreghtiana* by hovering and perching bats. Biological Journal of the Linnean Society 80, 281–288.

Tschapka, M., 2004. Energy density patterns of nectar resources permit coexistence within a guild of Neotropical flower-visiting bats. Journal of Zoology 263, 7–21.

Tschapka, M., von Helversen, O., 2007. Phenology, nectar production and visitation behaviour of bats on the flowers of the bromeliad *Werauhia gladioliflora* in a Costa Rican lowland rain forest. Journal of Tropical Ecology 23, 385-395.

Valiente-Banuet, A., Arizmendi, M.C., Rojas-Martinez, A., Dominguez-Canseco, L., 1996. Ecological relationships between columnar cacti and nectar-feeding bats in Mexico. Journal of Tropical Ecology, 12, 103-119.

Valiente-Banuet, A., Rojas-Martínez, A., Arizmendi, M.C., Davila, P., 1997. Pollination biology of two columnar cacti (*Neobuxbaumia mezcalaensis* and *Neobuxbaumia macrocephala*) in the Tehuacan Valley, central Mexico. American Journal of Botany 84, 452-452.

von Helversen, O., Reyer, H.U., 1984. Nectar intake and energy expenditure in a plant bat. Oecologia 63, 178-184.

von Helversen, D., von Helversen, O., 1999. Acoustic guide in bat-pollinated flower. Nature 398, 759-760.

von Helversen, O., Winter, Y., 2003. Glossophagine bats and their flowers: costs and benefits for plants and pollinators. In Kunz, T.H., Fenton, M.B. (Eds.), Bat Ecology. University of Chicago Press, London, pp. 349-397.

von Helversen, O., Winkler, L., Bestmann, H.J., 2000. Sulphur-containing "perfumes" attract flower-visiting bats. Journal of Comparative Physiology A 186, 143-153.

Wahizatul Afzan, A., Roziah, G., Nor Zalipah, M., 2012. Importance of carpenter bee, *Xylocopa varipuncta* (Hymenoptera: Apidae) as pollination agent for mangrove community of Setiu wetlands, Terengganu, Malaysia. Sains Malaysiana 41, 1057-1062.

Wan Jusoh, W.F.A., Hashim, N.R., Ibrahim, Z.Z., 2010. Distribution and abundance of *Pteroptyx* fireflies in Rembau-Linggi estuary, Peninsular Malaysia. EnvironmentAsia 3, 56-60.

Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J., 1996. Generalization in pollination systems, and why it matters. Ecology 77, 1043-1060.

Watzke, S., 2006. Ressourcennutzung und Paarungssystem der Nektarivoren Flughundart *Macroglossus minimus* (Pteropodidae: Macroglossinae) in West-Malaysia. PhD. thesis, Ludwig-Maximilians-Universität München, Munich.

Willmer, P., 2011. Pollination and Floral Ecology. Princeton University Press, New Jersey.

Winsor, J.A., Davis, L.E., Stephenson, A.G., 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. The American Naturalist 129, 643-656.

Wright, S.J., 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. Oecologia 130, 1-14.

Wrobel, A., Eklund, P., Bobrowska-Hagerstrand, M., Hagerstrand, H., 2010. Lignans and norlignans inhibit multidrug resistance protein 1(MRPI/ABCC1)-mediated transport. Anticancer Research 30, 4423-4428.

Wu, S.B., Wen, Y., Li, X.W., Zhao, Y., Zhao, Z., Hu, J.F., 2009. Chemical constituents from the fruits of *Sonneratia caseolaris* and *Sonneratia ovata* (Sonneratiaceae). Biochemical Systematics and Ecology 37, 1-5.

Xin, K., Zhou, Q., Arndt, S.K., Yang, X., 2013. Invasive capacity of the mangrove *Sonneratia apetala* in Hainan Island, China. Journal of Tropical Forest Science 25, 70-78.

York, H.A., Billings, S.A., 2009. Stable-isotope analysis of diets of short-tailed fruit bats (Chiroptera: Phyllostomidae: *Carollia*). Journal of Mammalogy 90, 1469–1477.

Young, H.J., 1988. Differential importance of beetle species pollinating *Dieffenbachia longispatha* (Araceae). Ecology 69, 832-844.

Young, H.J., Dunning, D.W., von Hasseln, K.W., 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). American Journal of Botany 94, 1267-1271.

Zazali, M., 2013. Banana business stays bountiful. *The Star.* 22 February 2013 (Online). Available at http://www.thestar.com.my/News/Community/2013/02/22/Banana-business-stays-bountiful.aspx (Accessed: 24 June 2013).

Zentall, T.R., 2005. Selective and divided attention in animals. Behavioural Processes 69, 1-15.

Zhang, Y.M., Tan, N.H., Yang, Y.B., Lu, Y., Cao, P., Wu, Y.S., 2005. Norlignans from *Sequoia sempervirens*. Chemistry and Biodiversity 2, 497-505.

Zhou, R., Shi, S., Wu, C.I., 2005. Molecular criteria for determining new hybrid species- an application to the *Sonneratia* hybrids. Molecular Phylogenetics and Evolution 35, 595-601.

Zhou, R., Zeng, K., Wu, W., Chen, X., Yang, Z., Shi, S., Wu, C.I., 2007. Population genetics of speciation in nonmodel organisms: I. Ancestral polymorphism in mangroves. Molecular Biology and Evolution 24, 2746-2754.

Zhou, R., Gong, X., Boufford, D., Wu, C.I., Shi, S., 2008. Testing a hypothesis of unidirectional hybridization in plants: observations on *Sonneratia*, *Bruguiera* and *Ligularia*. BMC Evolutionary Biology 8, 149-157.

Zhou, R., Qiu, S., Zhang, M., Guo, M., Chen, S., Shi, S., 2010. *Sonneratia ovata* Backer- a genetically depauperate mangrove species. Biochemical Systematics and Ecology 38, 697-701.

Ziehe, M., Roberds, J.H., 1989. Inbreeding depression due to over dominance in partially self-fertilizing plant populations. Genetics 21, 861-868.