The potential significance of nectar-feeding bats as pollinators in mangrove habitats of Peninsular Malaysia

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ABSTRACT

We tested the hypothesis that bats are effective pollinators of mangroves in Malaysia. Bats (*Eonycteris spelaea*) visited flowers of two *Sonneratia* species frequently, and deposited large quantities of conspecific pollen grains on stigmas. The bats are likely to be important pollinators of the two mangrove species.

Abstract in Malay is available with online material.

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). Pollinators often differ in the 'quality' and 'quantity' of the pollination services they provide (Schemske & Horvitz 1984, Fishbein & Venable 1996): quality refers to the amount of pollen transferred per visit and quantity refers to the visitation rate. Bats and hummingbirds for example can show similar visitation rates, although bats are more effective pollinators as they consistently transfer greater amounts of conspecific pollen (Muchhala 2006, 2007). However, potentially high rates of conspecific pollen transfer may be reduced by high levels of interspecific pollen transfer (Muchhala 2008), as bats may visit many plant species (Marshall 1983, Fleming *et al.* 2009, Fleming & Kress 2013). Flower visitation rate has recently been highlighted as a poor proxy for pollination efficiency (King *et al.* 2013), and the number of conspecific pollen grains deposited on a virgin stigma (single-visit deposition, or SVD) is a more reliable measure of pollinator effectiveness (Kandori 2002, Ne'eman *et al.* 2010, Stoepler *et al.* 2012, King *et al.* 2013). In this study, we determine whether bats are effective pollinators of mangrove tree species by quantifying SVDs.

Mangrove apples (*Sonneratia* spp.) are widespread and often important components of mangrove ecosystems in Indo-West Pacific regions (Duke 1992). *Sonneratia* species are important for preventing coastal erosion and tidal damage (Mazda *et al.* 2006). In Malaysia, *Sonneratia* trees are likely to be pollinated by nectarivorous bats that visit their flowers for nectar and pollen to obtain sugar and protein nutrients respectively (Start & Marshall 1976, Marshall 1983, Watzke 2006). However, these flowers are also visited by several nocturnal and crepuscular foragers such as moths, Diptera and Hymenoptera (Watzke 2006). The relative contribution of bats as pollinators to the flowers compared with other visitors was previously unknown. Here, we aim to quantify the potential effectiveness of flower-visiting bats as pollinators of *Sonneratia* trees from the quality and quantity components of pollinator effectiveness. We hypothesized that bats are effective pollinators of *Sonneratia* trees by transferring sufficient conspecific pollen grains (quality component) and we determine the effects of repeated visits to flowers (quantity component) to evalaute whether repeated visits by bats increase the amount of conspecfic pollen deposited relative to heterospecific pollen.

The study was located in Terengganu, northeast Peninsular Malaysia (5°40′ N, 102°43′ E), where *Sonneratia alba, S. caseolaris* and a hybrid between *S. ovata* and *S. alba* (M. Kainuma, pers. comm.) occur. The hybrid (hereafter *Sonneratia* sp.) was rare and was not monitored, though pollen grains from it were distinctive and included in analyses. The *Sonneratia* flowers open only for a single night, and the stamens drop the next morning, features typical of many bat-pollinated flowers.

On 35 nights in 2011 and 2012, we set mist-nets in front of flowering *Sonneratia* trees and monitored them at least hourly between 1900 h (dusk) and 0700 h (sunrise) the next day. Pollen grains were sampled by carefully rubbing the bat's body with cotton wool buds and were preserved in vials containing 75 percent ethanol. Bats were identified to species following keys in Kingston *et al.* (2006) and Francis (2008).

Stigmas were collected between May and November 2012. When measuring SVD, observations were conducted from 1900 h until 2300 h, 2–5 m from the trees under moonlight and dim light from headlamps. When a bat visited a flower, the stigma was removed immediately. For total pollen deposition (TD) on stigmas,

flowers that bloomed on the previous night were examined between 0700 to 0730 h to reduce the possibility of pollen deposition by early morning visitors such as hymenopterans. In total, 37 stigmas (20 *S. caseolaris* and 17 *S. alba*) were collected for SVD and 40 stigmas (20 for both *S. caseolaris* and *S. alba*) for TD determinations. All stigmas were preserved in separate 1.5 ml centrifuge tubes containing 75 percent ethanol.

Identification of pollen was conducted by comparisons with reference material. For each sample, 1 μl of ethanol (from approximately 20 ml ethanol in vials and 1 ml ethanol in centrifuge tubes, thoroughly shaken before extraction for pollen counts) was placed on a glass slide for light microscopy. For each slide, the number of pollen grains for each morphotype (morphologically distinguishable pollen type) was recorded. For each sample, pollen counts comprised 20 replicates of 1 μl samples in ethanol (for pollen deposited on stigmas, the total number of pollen grains was determined based on the ethanol volume). To achieve normality, the numbers of pollen grains carried by bats and deposited on the stigmas were logarithmically transformed (log base 10). All analyses were conducted in IBM SPSS Statistics v.19.0 (Chicago, Illinois, U.S.A). Mean $\pm$ SE are used throughout.

To observe the visitation frequency, we filmed bats visiting *Sonneratia* flowers between March and December 2012 (324 flower-h for *S. caseolaris* and 288 flower-h *S. alba*) using 2–4 night shot surveillance cameras (1/3″ SONY 420 TVL CCTV; Anyon Technology, Muar, Malaysia) simultaneously. The cameras were set up approximately 1 m from flowers with the aid of aluminium poles and connected to a digital video recorder (4 channel Crossfire CF1804; Belco, Taipei, Taiwan) recording between 1900 and 0700 h the next morning.

We caught 137 flower-visiting bats of three species. About 85 percent of bats were cave nectar bats (*Eonycteris spelaea*), which was disproportionately the most frequently captured species ($\chi^2 = 170.69$, df = 2, $P < 0.001$). We also captured 18 lesser short-nosed fruit bats *Cynopterus brachyotis* and a single Geoffroy's rousette (*Rousettus amplexicaudatus*). The bats carried 11 morphotypes of pollen, of which six were identified to species and five to genera. The species recorded were the three *Sonneratia* species (*Sonneratia* sp., *S. alba*, and *S. caseolaris*), *Ceiba pentandra*, *Melaleuca cajuputi* and *Oroxylum indicum*, whereas the five morphotypes identified to genera were *Acacia* sp., *Durio* sp., *Eugenia* sp., *Musa* sp. and *Parkia* sp. Pollen grains from the *Sonneratia* group (including *Sonneratia* sp. and non-viable *Sonneratia* spp.) were the dominant pollen grains collected (Fig. 1). The total number of pollen grains collected from *E. spelaea* was significantly higher than the number collected from *C. brachyotis* ($t = 6.92$, df = 144, $P < 0.001$). *Eonycteris spelaea* carried significantly more conspecific than heterospecific pollen grains (1796.97 $\pm$ 238.92 grains of conspecific and 533.84 $\pm$ 75.16 grains of heterospecific pollen; Wilcoxon signed-rank test, $T = 1490.00$, $P < 0.001$), whereas *C. brachyotis* carried almost equal numbers of con- and heterospecific grains (165.78 $\pm$ 63.61 conspecific and 135.44 $\pm$ 60.55 heterospecific pollen grains; Wilcoxon signed-rank test, $T = 50.00$, $P = 0.12$) (Fig. 2).

For *S. caseolaris* flowers, the number of pollen grains for SVD was significantly fewer than TD ($t = -2.290$, df = 38,
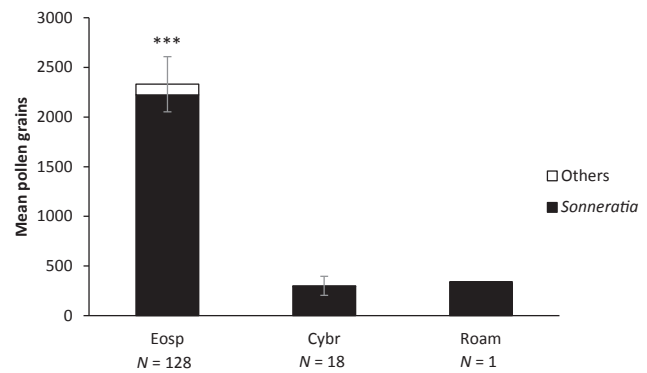

FIGURE 1. Number of pollen grains collected from each bat species. Error bars indicate SE. Mean $\pm$ SE are used throughout. Eosp = *Eonycteris spelaea*, Cybr = *Cynopterus brachyotis*, Roam = *Rousettus amplexicaudatus*. N = number of pollen swabs. From the total captures, pollen swabs were collected on 151 occasions, and only four captures were negative for pollen load on bats' bodies at the time of capture (two individuals each of *E. spelaea* and *C. brachyotis*, caught visiting *Sonneratia alba* trees). These four individuals were excluded from further analysis. *Sonneratia* pollen grains consists of *Sonneratia* sp., *S. alba*, *S. caseolaris* and the non-viable *Sonneratia* spp. (the non-viable *Sonneratia* spp. consists of non-viable pollen grains from the three *Sonneratia* species given the uncertainties in identifying the grains to species. The non-viable *Sonneratia* pollen grains were distinguished from the viable grains from their smaller size and look translucent when observed under the light microscope). Others (non *Sonneratia* pollen grains) consists of *Acacia* sp., *Ceiba pentandra*, *Durio* sp., *Eugenia* sp., *Melaleuca cajuputi*, *Musa* sp., *Oroxylum indicum* and *Parkia* sp. From the total pollen grains collected from their bodies, bats carried >90 percent of *Sonneratia* pollen grains at their time of capture. The pollen grains from non-bat-pollinated flowers (*Acacia* sp., *Eugenia* sp., and *M. cajuputi*) represented only about 0.07 percent of the total pollen grains collected from the bats. ***($P < 0.001$) indicates a significant difference in the number of grains carried by *E. spelaea* and and *C. brachyotis*.
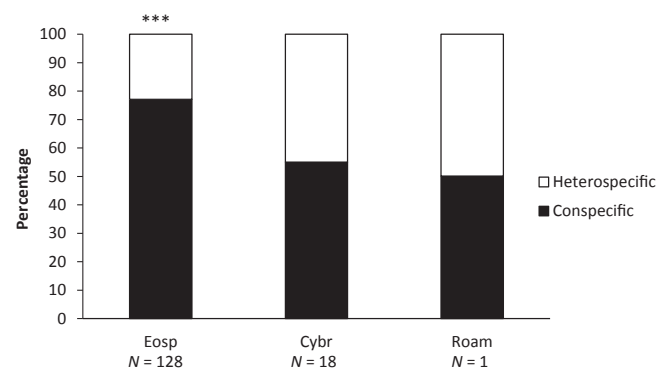

FIGURE 2. Percentages of conspecific and heterospecific pollen grains collected from each bat species. Eosp = *Eonycteris spelaea*, Cybr = *Cynopterus brachyotis*, Roam = *Rousettus amplexicaudatus*, N = number of pollen swabs. *Eonycteris spelaea* carried significantly more conspecific than heterospecific pollen grains (***$P < 0.001$).

$P = 0.028$). The numbers of conspecific and heterospecific pollen grains were not significantly different for SVD ($t = 1.597$, df = 19, $P = 0.127$) and TD ($t = 1.718$, df = 19, $P = 0.102$). The number of conspecific pollen grains was also not significantly different between SVD and TD ($t = 1.939$, df = 38, $P = 0.630$); the number of heterospecific pollen grains however, was significantly higher for TD as compared to SVD ($t = 3.701$, df = 38, $P < 0.001$) (Fig. 3). These results suggest that the increase in pollen deposition over the entire flowering night is largely the consequence of more heterospecific pollen being deposited.

Conversely for *S. alba* flowers, the number of pollen grains for SVD was not significantly different from the number deposited in TD ($t = 0.211$, df = 35, $P = 0.834$). The number of conspecific pollen grains deposited on the stigmas was significantly lower than the heterospecific pollen grains for both SVD ($t = -4.804$, df = 16, $P < 0.001$) and TD ($t = 6.170$, df = 19, $P < 0.001$). The comparisons of pollen types for SVD vs. TD showed no significant difference in the number of conspecific ($t = 0.402$, df = 38, $P = 0.690$) and heterospecific ($t = 0.402$, df = 35, $P = 0.914$) pollen grains. These results suggest that pollen deposition does not increase after the first pollinator visit in *S. alba*, and that pollen is dominated by heterospecific grains both during the first visit and throughout the night.

From 68 observations of bats visiting flowers, 46 were by *E. spelaea* (identified by their long snouts). There were 45 feeding visits by bats at eight *S. caseolaris* flowers during two nights. We recorded 23 feeding visits by bats at nine *S. alba* flowers over five nights.

The bats visited several flower species, as demonstrated by the mixed pollen loads on their bodies, as seen in other studies (Heithaus *et al.* 1975, Muchhala & Jarrin-V 2002, Watzke 2006). However, all bat species predominantly carried *Sonneratia* pollen (*S. alba, S. caseolaris, Sonneratia* sp., and the non-viable *Sonneratia* spp.); so, there was therefore little opportunity for pollen wastage (Law & Lean 1999). Based on the number of conspecific pollen
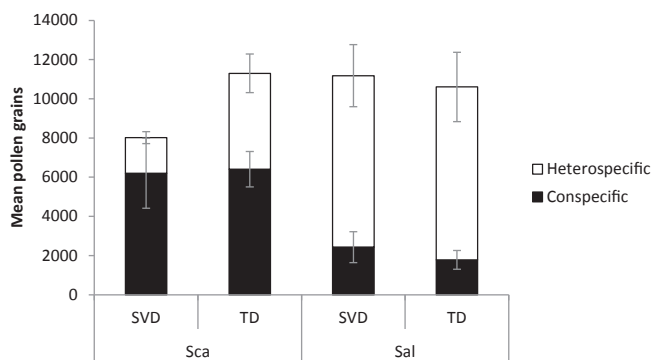

FIGURE 3. Number of conspecific and heterospecific pollen grains on stigmas of the *Sonneratia* flowers from single-visit deposition (SVD) by bats and total pollen deposition (TD) after the blooming night. Non-viable pollen grains from all three *Sonneratia* species in the study area were classified as 'heterospecific pollen' on the basis of non-viable pollen contributes to reproductive interference by clogging the stigmas of the flowers. Sca = *S. caseolaris*, Sal = *S. alba*. Error bars indicate SE. Mean ± SE are used throughout.

grains collected while visiting *Sonneratia* flowers, *E. spelaea* is likely to be a more important pollinator of *Sonneratia* species than *C. brachyotis*. Start and Marshall (1976) and Watzke (2006) recorded *Sonneratia* spp. (*S. caseolaris* in the case of Watzke 2006) as the most common pollen on *E. spelaea*.

All the stigmas collected after the first bat visited the flowers (SVD) were positive for pollen grains, indicating that the bats effectively transferred pollen from their bodies to the stigmas while visiting the flowers. Moreover, pollen comprised numerous conspecific grains, supporting our hypothesis that bats contribute an important quality component of pollinator effectiveness. However, we cannot exclude the possibility that some of the pollen came from the same flower, resulting in self-pollination.

For the quantity component, contrasting results were recorded for the two *Sonneratia* species. For *S. caseolaris*, higher visitation by bats to the flowers was recorded (1–18 [$N = 8$], with two flowers receiving 13 and 18 feeding visits), consistent with the higher number of pollen grains in TD than in SVD. For *S. alba* flowers, however, SVD and TD were very similar in accordance with the low visitation rate to the flowers (between 1–6 visits [$N = 9$], with eight flowers receiving 1–3 feeding visits only). Pollen load was higher for TD than SVD in *S. caseolaris*, which received frequent visits from bats over the night, but not for *S. alba* which received few. Therefore, the relatively higher TD in *S. caseolaris* is probably partly due to repeated pollinator visits to the same flowers (Quesada *et al.* 2004). On their first visit to the *S. caseolaris* flowers, bats deposited similar quantities of conspecific and heterospecific pollen grains onto the stigmas; however, multiple visits by bats to the flowers throughout the blooming night resulted in more heterospecific pollen being deposited onto the stigmas. For both species, however, the number of conspecific pollen grains from SVD was sufficient to fertilize all the ovules in the flowers (estimated in Nor Zalipah 2014). Furthermore, Nor Zalipah (2014) reported that the number of seeds/fruit for *S. caseolaris* was 623.19 ± 60.62 ($N = 37$ fruits) and only 50.77 ± 3.71 ($N = 13$ fruits) for *S. alba*. Therefore, a single visit by bats may be sufficient to initiate fruit set. This is in contrast to the quantity component of pollinator effectiveness, in which multiple visits to the same flowers reduced the quality of bats as pollinating agents as they may deposit relatively more heterospecific pollen consequently (Muchhala *et al.* 2008).

Even though high visitation rates may sometimes indicate the effectiveness of pollinators (Quesada *et al.* 2003, Arias-Coyotl *et al.* 2006), 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. Repeated visits to the same flowers may also result in stigma blockage by transfer of foreign pollen, subsequently reducing the reproductive success of the plant (Armbruster & Herzig 1984, Fishman & Wyatt 1999, Caruso & Alfaro 2000, Bell *et al.* 2005) by reducing the chances of subsequently deposited conspecific pollen to fertilize the ovules. Multiple visits to the same flower or plant also might result in geitonogamous crosses and set no fruit in self-incompatible plants (Quesada *et al.* 2004, Arias-Coyotl *et al.* 2006). Recently, Acharya *et al.* (2015) reported *E. spelaea* as effective pollinators of durian (*Durio zibethinus*) and bitter bean (*Parkia speciosa*) in southern Thailand, from their high conspecific pollen deposition

during SVD, and also from their high visitation frequency to the flowers. Even though they did not report the effect of multiple visits on pollen deposition, pollination of these two species by bats is especially important because they promote cross-pollination by visiting multiple conspecific trees throughout their feeding night.

Therefore, our study indicates that high visitation frequency does not necessarily confirm the effectiveness of bats as pollinators: indeed, being the first visitors to flowers may be more important in determining their effectiveness as pollinators (King et al. 2013). Therefore, based on the number of conspecific pollen grains in SVD, bats are likely to be effective pollinators of the two mangrove species, S. alba and S. caseolaris, in Malaysia.

ACKNOWLEDGMENTS

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