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.

Key words: conspecific pollen grains; E. spelaea; pollen load; pollinator effectiveness; Sonneratia.

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 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 evaluate whether repeated visits by bats increase the amount of conspecific 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 \textit{S. caseolaris} and 17 \textit{S. alba}) were collected for SVD and 40 stigmas (20 for both \textit{S. caseolaris} and \textit{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 ± SE are used throughout.

To observe the visitation frequency, we filmed bats visiting \textit{Sonneratia} flowers between March and December 2012 (324 flower-h for \textit{S. caseolaris} and 288 flower-h \textit{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 (\textit{Eonycteris spelaea}), which was disproportionately the most frequently captured species (\( \chi^2 = 170.69, \text{df} = 2, P < 0.001 \)). We also captured 18 lesser short-nosed fruit bats \textit{Cynopterus brachyotis} and a single Geoffroy’s rousette (\textit{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 \textit{Sonneratia} species (\textit{Sonneratia} sp., \textit{S. alba}, and \textit{S. caseolaris}), \textit{Ceiba pentandra}, \textit{Melalena cajuputi} and \textit{Oroxylum indicum}, whereas the five morphotypes identified to genera were \textit{Acacia} sp., \textit{Durio} sp., \textit{Eugenia} sp., \textit{Musa} sp. and \textit{Parkia} sp. Pollen grains from the \textit{Sonneratia} group (including \textit{Sonneratia} sp. and non-viable \textit{Sonneratia} spp.) were the dominant pollen grains collected (Fig. 1). The total number of pollen grains collected from \textit{E. spelaea} was significantly higher than the number collected from \textit{C. brachyotis} \((t = 6.92, \text{df} = 144, P < 0.001)\). \textit{Eonycteris spelaea} carried significantly more conspecific than heterospecific pollen grains \((1796.97 \pm 238.92 \text{ grains of conspecific and } 533.84 \pm 75.16 \text{ grains of heterospecific pollen; Wilcoxon signed-rank test, } T = 1490.00, P < 0.001)\), whereas \textit{C. brachyotis} carried almost equal numbers of con- and heterospecific grains \((165.78 \pm 63.61 \text{ conspecific and } 135.44 \pm 60.55 \text{ heterospecific pollen grains; Wilcoxon signed-rank test, } T = 50.00, P = 0.12)\) (Fig. 2).

For \textit{S. caseolaris} flowers, the number of pollen grains for SVD was significantly fewer than TD \((t = -2.290, \text{df} = 38, P = 0.036)\).
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), Srithongchuyai et al. (2008) suggested that a single visit by bats to the flowers of Indian trumpet, *O. indica* 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 (Armbuster & 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.
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LITERATURE CITED

ACHARYA, P. R., P. A. RACEY, S. SOTTHIBANDHU, AND S. BUMRUNGSRI. 2015. Feeding behaviour of the dawn bat (Eonycteris spelaea) promoted cross pollination of economically important plants in Southeast Asia. J. Pollini. Natl. 15: 44–50.

ARIAS-COTOTE, E., K. E. STONER, AND A. CASAS. 2006. Effectiveness of bats as pollinators of Stenocereus stellatus (Cactaceae) in wild, manged in situ, and cultivated populations in La Mixteca Baja, central Mexico. Am. J. Bot. 93: 1675–1683.

ARMBRUSTER, W. S., AND A. L. HERRING. 1984. Partitioning and sharing of pollinators by four sympatric species of Dalechampia (Euphorbiaceae) in Panama. Ann. Missouri Bot. Gard. 71: 1–16.

BEARDMORE, M., J. D. KARRON, AND R. J. MITCHELL. 2005. Interspecific competition for pollination by native birds in the understory of a tropical forest, implications for the reproductive success in the bat-pollinated bombacaceous trees. Biotropica 36: 131–133.

CARUSO, C. M., AND M. ALFARO. 2000. Interspecific pollen transfer as a mechanism of competition: Effect of Castilleja linariifolia pollen on seed set of Ipomopsis aggregata. Can. J. Bot. 78: 600–606.

DUCK, N. C. 1992. Mangrove floristics and biogeography. In A. L. Robertson, and D. M. ALONGO (Eds.). Tropical mangrove ecosystems, pp. 63–100. American Geophysical Union, Washington, DC.

FISHEEN, M., AND D. L. VENABLE. 1996. Diversity and temporal change in the effective pollinators of Asclepias curassavica. Ecology 77: 1061–1073.

FISHEEN, M., AND R. WYATT. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in Asclepias uniflora (Caryophyllaceae). Evolution 53: 1723–1733.

FLEMING, T. H., C. GIESSELMAN, AND W. J. KRESS. 2009. The evolution of bat pollination: A phylogenetic perspective. Ann. Bot. 104: 1017–1043.

FLEMING, T. H., AND W. J. KRESS. 2013. The ornaments of life, coevolution and conservation in the tropics. The University of Chicago Press, Chicago, Illinois.

FRANCIS, C. M. 2008. A field guide to the mammals of south-east Asia. New Holland Publishers Ltd, London, UK.

HEITHAUS, E. R., T. H. FLEMING, AND P. A. OPLER. 1975. Foraging patterns and resource utilization in seven species of bats in a seasonal tropical forest. Ecology 56: 841–854.

INDOYE, D. W., D. E. GILL, M. R. DUBASH, AND C. B. FENSTER. 1994. A model and lexicon for pollen fate. Am. J. Bot. 81: 1517–1530.

KANDORI, I. 2002. Diverse visitors with various pollinator importance and temporal change in the important pollinators of Geranium thunbergii (Geraniaceae). Ecol. Res. 17: 283–294.

KING, C., G. BALLANTYNE, AND P. G. WILKNER. 2013. Why flower visitation is a poor proxy for pollination: Measuring single-pollen deposition, with implications for pollination networks and conservation. Methods Ecol. Evol. 4: 811–818.

KINGSTON, T., B. L. LIM, AND A. ZURAIL. 2006. Bats of Krau wildlife reserve. Penerbit Universiti Kebangsaan Malaysia, Bangi, Malaysia.

LAW, B. S., AND M. LEAN. 1999. Common blossom bats (Syosseterys australis) as pollinators in fragmentated Australian tropical rainforest. Biol. Conserv. 91: 201–212.

MARSHALL, A. G. 1983. Bats, flowers and fruit: Evolutionary relationships in the Old World. Biol. J. Linn. Soc. 20: 115–135.

MAZA, Y., M. MAGI, Y. IZEDA, T. KUROKAWA, AND T. ASANO. 2006. Wave reduction in mangrove forest dominated by Sonneratia sp. Wetl. Ecol. Manag 14: 365–378.

MUCHHALA, N. 2006. The pollination biology of Bormwietera (Campasmaceae): Specialization and syndromes. Am. J. Bot. 93: 1081–1089.

MUCHHALA, N. 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. Am. Nat. 169: 494–504.

MUCHHALA, N. 2008. Functional significance of interspecific variation in Bormwietera flower morphology: Evidence from nectar bat captures in Ecuador. Biotropica 40: 332–337.

MUCHHALA, N., A. CAZCA, J. C. VEZUTI, AND J. D. THOMSON. 2008. A generalized pollination system in the tropics: Bats, birds and Apfelandra ananthis. Ann. Bot. 103: 1481–1487.

MUCHHALA, N., AND P. PARR. 2002. Flower visitation by bats in cloud forests of western Ecuador. Biotropica 34: 387–395.

NEDMAN, G., A. JURGENS, L. NEWSBROOK-LYDYS, S. G. POTT, AND A. DAFEL. 2010. A framework for comparing pollinator performances: Effectiveness and efficiency. Biol. Rev. 85: 435–451.

NOR ZALIPAH, M. 2014. The role of nectar-feeding bats (Pteropodidae) in pollination ecology of the genus Sonneratia at Setiu Mangrove Areas, Terengganu, Malaysia. PhD dissertation, University of Bristol, Bristol, U.K.

QUESADA, M., K. E. STONER, J. A. LOBO, Y. HERRERIAS-DIEGO, C. PALACIOS-GUERRERO, M. A. MUNGUIA-ROSA, K. A. O.-SALAZAR, AND V. RUIZ-GUERRERO. 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.

QUESADA, M., K. E. STONER, V. RUIZ-GUERRERO, C. PALACIOS-GUERRERO, AND J. A. LOBO. 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.

SCHEMBSKI, D. W., AND C. A. HORVITZ. 1984. Variation among floral visitors in pollination ability: A precondition for mutualism specialization. Science 225: 519–521.

SRITHONGCHUAY, T., S. BUMRUNGSRI, AND E. SRIPAO-RAYA. 2008. The pollination ecology of the late-successional tree, Ormosia indium (Bignoniaceae) in Thailand. J. Trop. Ecol. 24: 477–484.

START, A. N., AND A. G. MARSHALL. 1976. Nectarivorous bats as pollinators of trees in west Malaysia. In J. BURLEY, AND B. T. STYLES (Eds.). Variation, breeding and conservation of tropical forest trees, pp. 141–150. Academic Press, London, U.K.

STOEPLER, T. M., A. EDGE, A. STEEL, R. L. O’QUINN, AND M. FISHEEN. 2012. Differential pollinator effectiveness and importance in a milkweed (Asclepias, Apocynaceae) hybrid zone. Am. J. Bot. 99: 448–458.

WATZKE, S. 2006. Ressourcenutzung und Paarungssystem der Nektarivoren Flughundart Macroglossus minimus (Pteropodidae: Macroglossinae) in West-Malaysia. PhD dissertation, Ludwig-Maximilians-Universität München, Germany.