

ABSTRACT

Title of Dissertation: BAT-PLANT POLLINATION
INTERACTIONS IN SOUTHERN
THAILAND

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The overwhelming majority of flowering plant species depend on animals for pollination, and such pollinators are important for the reproductive success of many economically and environmentally important plant species. Yet pollinators in the Old World tropics are relatively understudied, particularly paleotropical nectarivorous bats (Pteropodidae), and much is unknown about their interactions with night-blooming plant species. To better understand these bat-plant pollination interactions, I conducted fieldwork in southern Thailand for a total of 20 months, spread across three years. I examined the foraging times of pteropodid bat species (Chapter 1), and found that strictly nectarivorous species foraged earlier, and for a shorter duration, than primarily frugivorous species. I also studied year-long foraging patterns of pteropodid bats to determine how different species track floral resources across seasons (Chapter 2). Larger species capable of flying long distances switched diets seasonally to forage on the most abundant floral species, while smaller species

foraged throughout the year on nearby plant species that were low-rewarding but highly reliable. To determine which pteropodid species are potentially important pollinators, I quantified the frequency and effectiveness of their visits to six common bat-pollinated plant taxa for an entire year (Chapter 3). The three strictly nectarivorous species were responsible for almost all pollination, but pollinator importance of each bat species varied across plant species. I further examined the long-term reliability of these pollinators (Chapter 4), and found that pollinator importance values were consistent across the three study years. Lastly, I explored mechanisms that reduce interspecific pollen transfer among bat-pollinated plants, despite having shared pollinators. Using a flight cage experiment, I demonstrated that these plant species deposit pollen on different areas of the bat's body (mechanical partitioning), resulting in greater pollen transfer between conspecific flowers than heterospecific flowers (Chapter 5). Additionally, while I observed ecological and phenological overlap among flowering plant species, pollinators exhibited high floral constancy within a night, resulting in strong ethological separation (Chapter 6). Collectively, these findings illustrate the importance of understudied Old World bat pollinators within a mixed agricultural-forest system, and their strong, interdependent interactions with bat-pollinated plant species within a night, across seasons, and across years.

BAT-PLANT POLLINATION INTERACTIONS IN SOUTHERN THAILAND

by

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Table of Contents

Acknowledgements.....	ii
Table of Contents.....	ivv
List of Tables.....	v
List of Figures.....	vi
Introduction.....	1
Chapter 1: Differences in foraging times between two feeding guilds within Old World fruit bats (Pteropodidae) in southern Thailand.....	6
Chapter 2: Temporally-patchy floral resources shape foraging strategies of generalist and specialist nectarivores.....	24
Chapter 3: Variation in potential pollinator importance within a classic pollination syndrome.....	40
Chapter 4: Are paleotropical, long-lived, resident nectarivorous bats reliable pollinators?.....	61
Chapter 5: Differential pollen placement on an Old World nectar bat increases pollination efficiency.....	72
Chapter 6: Complementary mechanisms limit competition for pollination among paleotropical bat-pollinated plant species across a diverse landscape in southern Thailand.....	92
Appendices.....	114
Bibliography.....	136

List of Tables

TABLE 1. Cumulative capture frequency at bat-visited plant species in southern Thailand for common pteropodid species.....	10
TABLE 2. A general linear model with mixed effects demonstrates the effects of bat feeding guild, food resource type, and time on pteropodid bat capture rates in southern Thailand.....	11
TABLE 3. Spearman's rank correlation coefficients for flowering phenology and bat diet for six common bat-pollinated plant taxa and seven common flower-visiting bat species in southern Thailand	26
TABLE 4. Characteristics of plant species commonly visited by nectar-feeding bats in southern Thailand.....	41

List of Figures

FIGURE 1. Foraging times of pteropodid bat feeding guilds at bat-visited plant species in southern Thailand throughout the night	11
FIGURE 2. Boxplots displaying foraging time distributions of the most commonly netted nectarivorous and frugi-nectarivorous bat species encountered in this study (southern Thailand).....	12
FIGURE 3. Flowering phenologies and number of pollen grains carried by <i>Eonycteris spelaea</i> for five bat-pollinated plant taxa in southern Thailand: <i>Ceiba pentandra</i> , <i>Durio zibethinus</i> , <i>Musa acuminata</i> , <i>Parkia</i> species, and <i>Sonneratia</i> species.....	27
FIGURE 4. Flowering phenologies and number of pollen grains carried by flower-visiting bats in southern Thailand: <i>Macroglossus minimus</i> bats and <i>Sonneratia</i> flowers, <i>M. minimus</i> bats and <i>Musa acuminata</i> flowers, <i>M. sobrinus</i> bats and <i>M. acuminata</i> flowers, <i>Rousettus leschenaulti</i> bats and <i>Durio zibethinus</i> flowers.....	28
FIGURE 5. Plant species visited by a single important pollinator, <i>Eonycteris spelaea</i> , in southern Thailand: <i>Ceiba pentandra</i> , <i>Oroxylum indicum</i> , and <i>Parkia</i> species	45
FIGURE 6. Plant species visited by multiple important pollinators in southern Thailand: <i>Durio zibethinus</i> , <i>Musa acuminata</i> , and <i>Sonneratia</i> species.....	46
FIGURE 7. Pollination networks generated using visitation rates alone versus pollinator importance values of different bat species	48
FIGURE 8. Visitation rates of pteropodid bats in southern Thailand across four bat-pollinated plant species and three years.....	63
FIGURE 9. <i>Eonycteris spelaea</i> , a common nectar bat and important pollinator in Thailand	71
FIGURE 10. Floral designs of plant species pollinated by <i>Eonycteris spelaea</i> in Thailand	72
FIGURE 11. The average amount of pollen transferred by <i>Eonycteris spelaea</i> to female <i>Ceiba pentandra</i> , <i>Durio zibethinus</i> and <i>Musa acuminata</i> flowers by male flowers of different plant species.....	80
FIGURE 12. Back-transformed model estimates of the number of pollen grains placed on different areas of <i>Eonycteris spelaea</i> : the crown of the head, chest, face and ventral side of the wings	81

FIGURE 13. Model predictions of the abundance of bat-pollinated plant species in three broad habitat types within southern Thailand	97
FIGURE 14. Flowering phenologies of inland bat-pollinated plant species in southern Thailand: <i>Ceiba pentandra</i> , <i>Durio zibethinus</i> , <i>Musa acuminata</i> , <i>Oroxylum indicum</i> , <i>Parkia speciosa</i> and <i>P. timoriana</i>	98
FIGURE 15. Flowering phenologies of four <i>Sonneratia</i> mangrove species in southern Thailand: <i>S. alba</i> , <i>S. caseolaris</i> , <i>S. griffithii</i> , and <i>S. ovata</i>	98
FIGURE 16. Pollinator constancy of five nectar-feeding bat taxa in southern Thailand, illustrated by the proportions of different pollen species in the diets of individual bats	100

Introduction

The large majority of plant species worldwide depend on animals for pollination (Bawa 1990; Ollerton et al. 2011). These animal pollinators move gametes between conspecific individuals and are critical for plant reproduction (Darwin 1877; Faegri & van der Pijl 1966; Stebbins 1970). Because pollinators directly impact plant reproductive success, they can be important drivers of floral evolution (Darwin 1877; Faegri & van der Pijl 1966; Stebbins 1970). “Pollination syndromes” (convergently-evolved suites of floral characteristics associated with attracting a specific group of floral visitors) provide clear examples of pollinator-driven selection (Faegri & van der Pijl 1966; Fenster et al. 2004). For example, plant species exhibiting the hummingbird pollination syndrome typically have red flowers with long, narrow corollas and abundant nectar. Because pollination syndromes reflect similar selective pressures imposed by pollinators, even distantly related plant species can share similar traits.

In tropical areas, a diverse array of night-blooming plant species are pollinated by flower-visiting bats. The bat pollination syndrome is characterized by nocturnal anthesis; copious nectar and pollen; and large, sturdy pale-colored flowers or inflorescences (Faegri & van der Pijl 1966; Fleming et al. 2009). These plant species are found in tropical and sub-tropical regions of both hemispheres, where they are visited by resident and migratory bat species. New World bat-pollinated plants are visited by nectar-feeding Phyllostomidae, while their Old World counterparts are pollinated by nectar-feeding Pteropodidae. Most research on bat pollination has been

conducted in the New World tropics, and there is still much that we do not know about Old World bat-plant dynamics. Thus, the goal of this dissertation was to broaden our knowledge of the pollination interactions between nectarivorous pteropodid bats and the night-blooming plant species they visit. This study examined a community of the seven most common pteropodid species in southern Thailand and their main flowering food resources (10 plant species across 6 genera).

Chapter 1, “Differences in foraging times between two feeding guilds within Old World fruit bats (Pteropodidae) in southern Thailand,” has been published in the *Journal of Tropical Ecology* (Stewart et al. 2014). In this chapter, I examined the foraging times of different pteropodid species. Pollinator foraging times have important implications for both the animal (as floral rewards vary over time; Sripoaraya 2005) and the plant (as receptivity to pollination varies over time; Howell & Roth 1981; Groman & Pellmyr 1999). The main finding of this chapter was that strictly nectarivorous species forage earlier and for a shorter duration than frugi-nectarivorous species. These results demonstrate that the family Pteropodidae comprises two functional groups that interact with plant species they visit in very different ways. Additionally, the foraging times of these bat species are strongly influenced by fruit and nectar resource abundance.

In Chapter 2, I studied the foraging patterns of pteropodid bats throughout the year to assess how their diets change in response to fluctuating floral resource availability. The rostrums of pteropodid bats indicate that they span a continuum between specialized nectarivores (long, slender rostrums) to frugi-nectarivorous generalists (short, broad rostrums), and theory predicts that generalists are more likely

to switch food resources than specialists (Abrams 2006; Carnicer et al. 2008). Indeed, the generalist species tracked multiple resources throughout the year and frequently switched diets to forage on the most abundant flowering plant species, while diets of the specialist species remained constant across seasons. These two floral foraging strategies likely reflect the different metabolic constraints and roosting habits of pteropodid bats, and perhaps influence the rates of outcrossing within bat-pollinated plant species.

In Chapter 3, I measured the potential pollinator importance to quantify each bat species' contribution towards pollinating six common plant taxa. Pollinator importance is defined as the product of pollinator visitation rate (the “quantity” component) and pollen transfer effectiveness (the “quality” component) (Stebbins 1970; Fenster et al. 2004; Reynolds et al. 2009). I found that each plant species was pollinated by 1-3 bat species and, in general, the nectar specialists were the main pollinators. This chapter demonstrates the great variability that can occur within a “classic” pollination syndrome, and highlights the importance of measuring pollen transfer effectiveness in addition to visitation rate.

Chapter 4 expands upon the previous chapter by evaluating the constancy of potential pollinator importance across years. Most multi-year pollination studies have examined insect or migratory vertebrate (bat or bird) systems, and have documented high temporal variability (Herrera 1988; Fenster & Dudash 2001; Petanidou et al. 2008). However, the results of this chapter indicate that pteropodid bats are highly reliable pollinators. Pollinator importance values were remarkably constant across the three years examined (2011, 2013, 2014), suggesting that pteropodid bats – as

tropical, long-lived, resident nectarivores – are highly consistent pollinators. These findings are particularly significant given that a number of economically (e.g., durian) and environmentally important (e.g., *Sonneratia* mangroves) plant species, which frequently co-occur in a mixed agriculture forest matrix, all depend on pteropodid bats for pollination.

Chapter 5, “Differential pollen placement on an Old World nectar bat increase pollination efficiency,” has been published in *Annals of Botany* (Stewart & Dudash 2016). In this chapter, I used a flight cage experiment to test whether the flower designs of four common bat-pollinated plant species result in pollen being placed on different areas of the bat’s body, thus minimizing interspecific pollen transfer. These sympatric plant species share pollinators, so any mechanism that reduces incorrect pollen movement would be expected to confer great fitness benefits (Waser 1983; Morales & Traveset 2008). The results of this chapter revealed that different floral designs exhibited by different bat-pollinated species do place pollen on different parts of the bat, and that bats transfer significantly more pollen between conspecific flowers than heterospecific flowers. This study illustrates how even pollinators with broad diets can still be effective pollinators, and demonstrates how multiple plant species can coexist despite sharing pollinators.

Finally, Chapter 6 explores three other mechanisms that can reduce competition for pollination: ecological, phenological, and ethological separation. Transects conducted in three broad habitat types (forest, agriculture, and mangrove) revealed little ecological separation, with the exception of a clear division between mangrove and inland species. Phenological separation also appeared to be very

minimal, as flowering phenologies of the bat-pollinated plant community were not segregated more than expected by random chance. However, most pteropodid bats carried only 1-2 pollen species within a single night, suggesting that high pollinator constancy limits interspecific pollen movement. Together with Chapter 5, these findings suggest that multiple complementary mechanisms act in concert to promote legitimate pollen transfer between conspecific individuals of sympatric flowering bat-pollinated species.

Nectar-feeding bats are important pollinators to many night-blooming plant species. Because they forage at night, their pollination interactions have remained largely unknown, particularly in the understudied Old World tropics. In this dissertation, 20 months of fieldwork spanning three years combined with experimental manipulations have illuminated much about the interactions between pteropodid bats and the flowering plant species they visit. Yet paleotropical studies still lag behind those of temperate and neotropical systems, and the results of this research have led to even more questions. While by no means complete, I hope this dissertation provides a solid basis for further studies examining bat-plant pollination interactions in the Old World tropics.

Chapter 1: Differences in foraging times between two feeding guilds within Old World fruit bats (Pteropodidae) in southern Thailand

ABSTRACT

Bats are important but understudied pollinators in the Paleotropics, and much about their interactions with night-blooming, bat-pollinated plant species is still unknown. We compared visitation times to flowering and fruiting plant resources by nectarivorous bat species (obligate pollinators) and frugi-nectarivorous bat species (facultative pollinators) throughout the night to examine the temporal variability that occurs within Pteropodidae foraging. Timing of pollination is an important determinant of plant reproductive success and more temporally restrictive than fruit dispersal. We netted 179 nectarivorous bats and 209 frugi-nectarivorous bats across 367 total mist-net h at five plant species providing floral resources and six plant species providing fruit resources. We found that all three nectarivorous bat species in southern Thailand forage significantly earlier in the evening (20h30 versus 22h00), and over a significantly shorter time interval (1.73 h versus 3.33 h), than do the five most commonly netted frugi-nectarivorous species. These results indicate that the two feeding guilds may be imposing different selective pressures on bat-pollinated plant species and may comprise different functional groups. We propose that the observed differences in bat foraging times are due to temporal constraints imposed by the rewards of the plant species that they visit.

Key words: chiropterophily, feeding guild, functional group, pollination, pteropodid, temporal variation

INTRODUCTION

Understanding species interactions is a key objective of ecology. Plant-pollinator relationships have long fascinated naturalists (Darwin 1862; Sprengel 1793), yet in spite of over two centuries of research, much of this field remains unexplored given the enormous diversity of pollination interactions found in nature (Faegri & van der Pijl 1966; Willmer 2011). Consequently, ecologists have devised means of classifying and organizing these diverse interactions to better understand them. Pollinators, for example, can be classified into functional groups based on the similar selective pressures they exert on plant species, and independent of their relatedness to one another, as a way of describing their interactions with flowers (Fenster et al. 2004). Insect taxa are commonly classified into different functional groups at the family or genus level (e.g. pollen-collecting bees and oil-collecting bees), but vertebrate pollinators have received less attention and are more often grouped at the level of order, or even class, as is common with nectarivorous birds and bats (Faegri & van der Pijl 1966; Muchhala & Thomson 2010).

While flower-visiting bats have typically been categorized into a single functional group (Faegri & van der Pijl 1966; Muchhala & Thomson 2010), recent studies by Tschapka (2003) and Frick et al. (2013) have investigated the possibility that pollinating bat species differentially affect plant reproductive success, and thus

comprise more than one functional group. These studies showed that a plant's reproductive success can vary greatly depending on whether it is pollinated by a nectar-specialist or an opportunistic visitor from another feeding guild. Furthermore, both studies proposed that the mechanism responsible for these differences was nectaring posture, with perching species delivering more pollen (Frick et al. 2013) or setting more fruit (Tschapka 2003) than hovering species. While there are no similar studies comparing the feeding guilds of pollinating bats in the Paleotropics, we expected different findings from these Neotropical studies since Old World pollinating bats (family Pteropodidae) nearly always land on flowers rather than hover, even nectar-specialist pteropodids (Bumrungsri et al. 2008; Fleming et al. 2009; Marshall 1983).

Instead, we wanted to compare the foraging times of pteropodid feeding guilds, since floral visitation time is another mechanism through which pollinators can influence plant reproductive success. Floral visitation time is important since flowers often have a peak receptivity for gamete transfer, so animal species visiting at different times of the night may result in seed set that differ quantitatively and/or qualitatively (Groman & Pellmyr 1999; Howell & Roth 1981). Several studies have documented temporal partitioning in nectarivorous bats (Fischer 1992; Singaravelan & Marimuthu 2004; Thomas & Fenton 1978), and even slight differences in visitation time can have large impacts, particularly since most bat-pollinated flowers in the Paleotropics are only open for one night (Bumrungsri et al. 2008, 2009; Faegri & van der Pijl 1966; Sritongchuay et al. 2008; Willmer 2011). Yet previous studies of foraging times have only compared the visits of two or three bat species at just one

plant species, providing a limited view of this complex system (Bumrungsri et al. 2013; Marshall 1985).

To better understand real-world processes, we sampled the Old World bat community at bat-visited plant species in southern Thailand. Our objective was to examine the nightly foraging times of nectar-specialist bats and frugi-nectarivorous bats to compare temporal variation in their interactions with common bat-visited plant species. We hypothesized that these two groups would forage at different times during the night, given that they rely primarily on different food resources. Consistent differences between the foraging times of nectarivorous and frugi-nectarivorous bat species could indicate that these two feeding guilds are acting as distinct functional groups.

STUDY SITE

This work was conducted in southern Thailand across four provinces (Phatthalung, Satun, Songkhla and Trang) during July–August 2011. The region consists predominantly of lowland tropical rain forest interspersed with urban and agricultural areas. Bat-visited plant species are found in all habitat types. Bats were caught from a representative sample of lowland tropical rain forest, mangrove forest, rubber plantations and mixed fruit orchards. Average temperature, humidity and annual rainfall across southern Thailand are in the range 26.3–28.3°C, 76–84% and 1716–2725 mm, respectively (Thai Meteorological Department, www.tmd.go.th/en).

STUDY SPECIES

Many pteropodid bat species are abundant and important pollinators in southern Thailand (Bumrungsri et al. 2008, 2009; Sritongchuay et al. 2008). Common bat species include *Cynopterus brachyotis* (Muller), *C. horsfieldii* (Gray), *C. sphinx* (Vahl), *Eonycteris spelaea* (Dobson), *Macroglossus minimus* (Geoffroy), *M. sobrinus* (Andersen) and *Megaerops ecaudatus* (Temminck). Species can be categorized into two feeding guilds, nectarivorous and frugi-nectarivorous. Nectar-specialist *Eonycteris* and *Macroglossus* species have the elongated rostrums and tongues characteristic of nectarivores, and feed almost exclusively on floral resources. In contrast, the remaining species have relatively robust rostrums, short tongues, and consume both floral and fruit resources (Bumrungsri et al. 2007, 2013; Francis 2008; Hodgkison et al. 2004a; Marshall 1983, 1985). Most of the pteropodid species in this study predominantly roost in foliage, either solitarily or in small groups (Balasingh et al. 1995; Campbell et al. 2006; Kunz & Fenton 2003), but *E. spelaea* roosts colonially in limestone karst caves (Bumrungsri et al. 2009; Suyanto & Struebig 2007).

A number of common local plant species are known to be pollinated by pteropodid bats and exhibit an array of reproductive flowering strategies throughout the year. With respect to the food resources they provide, they all exhibit the following important floral traits: copious nectar production that peaks early in the evening as well as pollen dehiscence early in the evening (Bumrungsri et al. 2009; Sripaoraya 2005). Self-incompatible, bat-pollinated plant species in this study include *Durio zibethinus*, a big-bang species that can produce over 1000 flowers per tree per night (Bumrungsri et al. 2009; Gould 1978); *Oroxylum indicum* (Bignoniaceae),

which flowers year-round in southern Thailand (Sritongchuay et al. 2010); *Parkia speciosa* (Fabaceae), which exhibits a cornucopia phenology, flowering from April to October; and four *Sonneratia* species (Lythraceae; Bureau of Mangrove Resources Conservation 2009): *S. alba* and *S. ovata* have a multiple bang phenology, *S. caseolaris* flowers year-round and *S. griffithii* is currently under study by the authors (Start 1974; Chapter 2). Finally, *Musa acuminata* (Musaceae), the only self-compatible species (Andersson 1998) investigated here, also flowers continuously throughout the year (Gould 1978; Pillay & Tenkouano 2011; Sripaoraya 2005).

Bat-dispersed plant taxa bearing ripe fruit during the period of data collection included *Ficus* species (Moraceae), *Lansium domesticum* (Meliaceae), *Manilkara zapota* (Sapotaceae), *Nephelium lappaceum* (Sapindaceae), *Sandoricum koetjape* (Meliaceae) and *Syzygium cumini* (Myrtaceae) (Marshall 1985). *Ficus* fruits are a steady-state resource; while individual plants mass fruit, fruiting is not synchronous within or among species, such that fruits are available year-round. All other focal fruiting species are big-bang resources. In southern Thailand, *Ficus* and *S. cumini* are not cultivated, *S. koetjape* is both wild and cultivated, while *L. domesticum*, *M. zapota* and *N. lappaceum* fruits are cultivated.

METHODS

Assessing foraging times

We captured bats from 24 locations in southern Thailand (6°32'–7°46' N, 99°47'–100°16' E), which were all at least 1 km apart. Mist nets (polyester, 38-mm mesh; Avinet Inc., Dryden, NY) were placed near flowering and fruiting individuals of the

aforementioned plant species to catch foraging bats. Sampling effort varied between 1–12 nights per plant species (Table 1), depending on availability; because sample sizes were small for several species, we grouped them into two categories (flowering versus fruiting resources) for analysis. Nets were open from 18h00 (sunset occurred around 18h40) until approximately 03h00 for a total of 33 nights between 1 July and 10 August 2011. Since previous work has demonstrated vertical stratification among fruit bats (Hodgkison et al. 2004b), we placed nets at a range of heights to obtain a representative sampling of foraging bats ($n \geq 6$ nights per height interval of 3 m, up to 12 m). Nets were positioned as close as possible to the flowers of bat-pollinated plants or to the fruits of bat-dispersed plants. Thus, any individuals caught were assumed to have been foraging at the plant species where they were netted. Additionally, placing mist nets close to vegetation made the nets less visible, allowing for relatively high capture success rates.

Nets were checked for bats at least every 30 min. For each netted individual, we recorded species, sex, reproductive status, time of capture, geographic coordinates, habitat type and the plant species at which the bat was netted. Individual bats were identified to species following Francis (2008). We then determined the bat's feeding guild (nectarivore or frugi-nectarivore) based on literature (Bumrungsri et al. 2007, 2013; Francis 2008; Hodgkison et al. 2004a; Marshall 1983, 1985) corroborated with personal observation of foraging choices made in the field (A. Stewart). Male reproductive classes consisted of juvenile and adult (determined from examination of phalangeal epiphyses); female reproductive classes consisted of

juvenile, lactating, pregnant and non-reproductive (all following Kunz & Parsons 2009).

Statistical analysis

To assess whether bat feeding guild (nectarivore versus frugi-nectarivore), food resource type (floral versus fruit), or time influenced pollinator foraging behavior, we used the GLIMMIX procedure in SAS 9.2 (SAS Institute Inc.) to perform a general linear model with mixed effects. In the mixed model, the dependent variable was capture rate (bats per mist-net h), with 1 mist-net h (mnh) defined as one net open for 1 h. The independent variables in the model were bat feeding guild, food resource type, time of night (specified as a class variable) as a random effect within feeding guild estimated using an autoregressive covariance matrix (AR1), and all two-way interactions. Degrees of freedom for all tests were estimated using the Kenward-Roger option. Additional covariates (species within feeding guild, sex, reproductive class) were initially incorporated but subsequently removed due to a lack of model convergence. As two-sample Kolmogorov–Smirnov tests revealed no significant differences in foraging time distributions for these variables, data across species within feeding guild, sex and reproductive class were pooled for all subsequent analyses. The distribution was assumed Poisson with a log link and statistical significance was assumed if $P < 0.05$. Model fit was assessed by examining the raw and Pearson's residuals as well as DFBETA plots. The variability in the foraging periods of nectarivorous and frugi-nectarivorous bats were compared with F-tests using R 2.15.2 (R Development Core Team).

RESULTS

We netted a total of 388 pteropodid bats of ten species (Table 1; Appendix I) over 367 total mnh; 229 mnh at flowering plants ($N = 35$ sites), 138 mnh at fruiting plants ($N = 23$ sites). The mixed model found that bat feeding guild, time, the feeding guild by resource interaction and the feeding guild by time interaction all significantly influenced bat capture rate (Table 2). While food resource type was not significant ($P = 0.19$), it was kept in the model since it was part of a significant two-way interaction. We found no evidence for a resource by time interaction ($P = 0.84$), so this term was removed from the model.

Frugi-nectarivorous bats had significantly higher overall visitation rates to all pooled food resources than nectarivorous bats ($P = 0.01$), but the significant feeding guild by resource interaction ($P = 0.0005$) revealed that visitation rate is dependent on food resource type (Figure 1a; Table 2). Specifically, nectarivorous bats strongly preferred flowering plants, visiting flowering plants (mean \pm SD, 0.68 ± 0.48 bats per mnh) over six times more frequently on average than fruiting plants (0.10 ± 0.15 bats per mnh), and frugi-nectarivorous bats preferred fruiting plants (0.78 ± 1.21 bats per mnh) over flowering plants (0.43 ± 0.57 bats per mnh), as expected.

As a whole, pteropodid bats did not have a constant foraging rate between 18h00 and 03h00 ($P < 0.0001$); instead, their foraging activity rose until 20h00 and then decreased until 03h00 (Figure 1a). This preference for early foraging was significantly stronger in nectarivorous bats than in frugi-nectarivorous bats ($P = 0.01$; Figure 1b); the median foraging time was 20h30 for nectarivorous bats ($N = 178$) and

TABLE 1. Cumulative capture frequency (total number individuals netted / total number nights sampled) at bat-visited plant species in southern Thailand for each pteropodid species included in the analyses. Plant species listed along top (with number of nights in parentheses), from left to right: *Durio zibethinus*, *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, *Ficus* species, *Lansium domesticum*, *Manilkara zapota*, *Nephelium lappaceum*, *Sandoricum koetjape* and *Syzygium cumini*. Bat species listed along left side; asterisks denote nectarivorous species, all others are frugi-nectarivorous.

	Flowering					Fruiting					
	<i>Durio</i> (1)	<i>Musa</i> (12)	<i>Orox.</i> (6)	<i>Park.</i> (12)	<i>Sonn.</i> (4)	<i>Ficus</i> (11)	<i>Lans.</i> (3)	<i>Man.</i> (1)	<i>Nep.</i> (3)	<i>Sand.</i> (4)	<i>Syzy.</i> (1)
* <i>Eonycteris spelaea</i>	0	3.33	2.33	3.92	0	0	0	0	0.33	0.25	0
* <i>Macroglossus minimus</i>	0	1.58	0	0.17	2.75	0.27	0	0	0	0	0
* <i>M. sobrinus</i>	2	2.33	0.17	0.17	0	0.55	0	0	0	0	0
<i>Cynopterus brachyotis</i>	3	0.17	0.17	0.17	0	0.55	0	0	0.33	0.25	0
<i>C. horsfieldii</i>	2	1.25	0.67	0.75	0	2.27	0	0	1.67	2	1
<i>C. sphinx</i>	6	2.83	1.17	0.67	0	3.82	0.33	0	1.33	2.25	0
<i>Megaerops ecaudatus</i>	6	0.17	0	0	0	0.09	0	1	0	0	0
<i>Penthetor lucasi</i>	0	0	0	0	0	0	0	0	0	0.25	0
<i>Rousettus amplexicaudatus</i>	0	0	0	0	0	0	0.33	0	0	0	0
<i>R. leschenaulti</i>	0	0	0	0	0	0	0	1	0	0	0

TABLE 2. A general linear model with mixed effects (GLIMMIX, SAS 9.2) demonstrates the effects of bat feeding guild (nectarivore or frugi-nectarivore), food resource type (floral or fruit), and time on pteropodid bat capture rates in southern Thailand. ndf = numerator degrees of freedom; ddf = denominator degrees of freedom.

	ndf	ddf	<i>F</i>	<i>P</i>
Bat feeding guild	1	77	6.37	0.01
Food resource type	1	49	1.73	0.19
Time	7	350	6.68	<0.0001
Feeding guild × resource	1	49	14.0	0.0005
Feeding guild × time	7	350	2.68	0.01

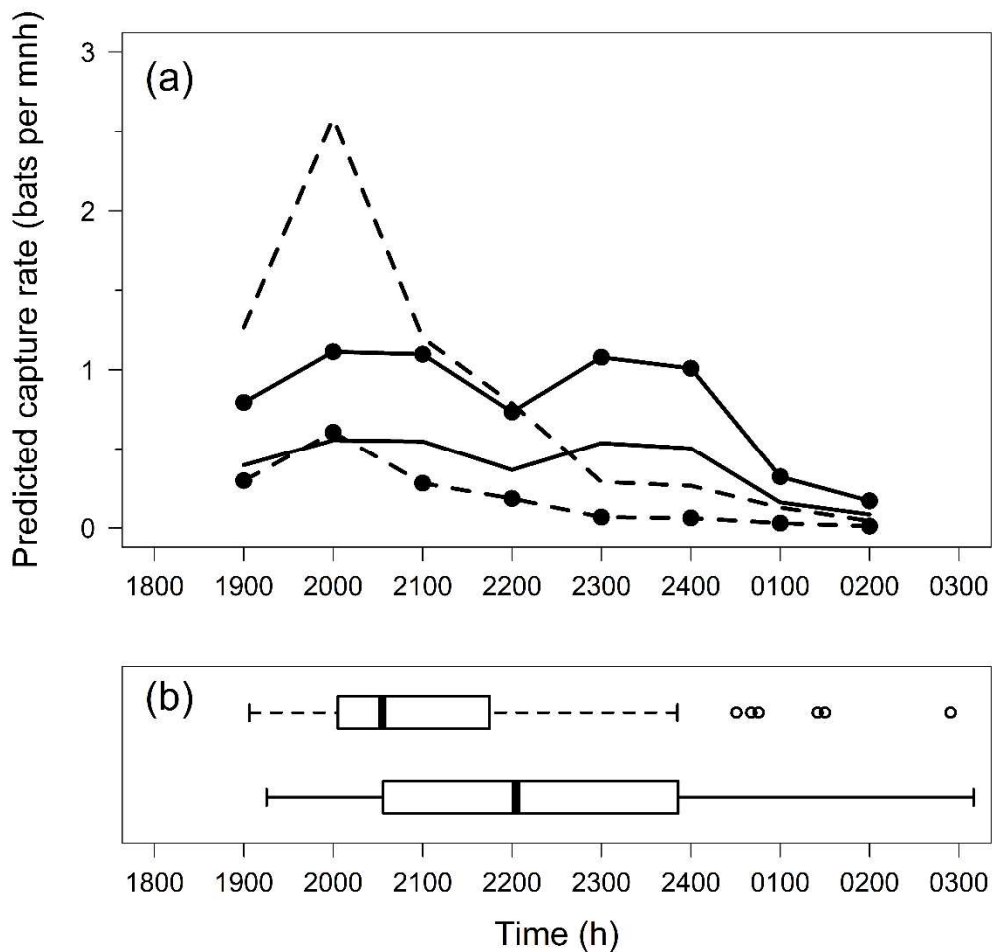


Figure 1. Foraging times of pteropodid bat feeding guilds at bat-visited plant species in southern Thailand throughout the night. Bat capture rates of nectarivores (dashed lines) and frugi-nectarivores (solid lines) at flowering (unmarked) and fruiting (circles embedded within lines) resources predicted from the generalized linear mixed model (a). Boxplots of observed nectarivorous (dashed line, $N = 179$) and frugi-nectarivorous (solid line, $N = 211$) bat nettings in southern Thailand, depicting the quartiles and outliers as defined based on 1.5 times the interquartile range (b).

22h00 for frugi-nectarivorous bats ($N = 211$). Foraging by nectarivorous bats also occurred over a significantly narrower period of time than foraging by frugi-nectarivorous bats (interquartile range, IQR = 1.73 h and 3.33 h, respectively; F-test, $P < 0.0001$; Figure 1b). Comparing the distribution of foraging time by bat species revealed that these results were not driven by any one species alone; all nectarivorous species had earlier and less variable foraging times than the frugi-nectarivorous species (Figure 2).

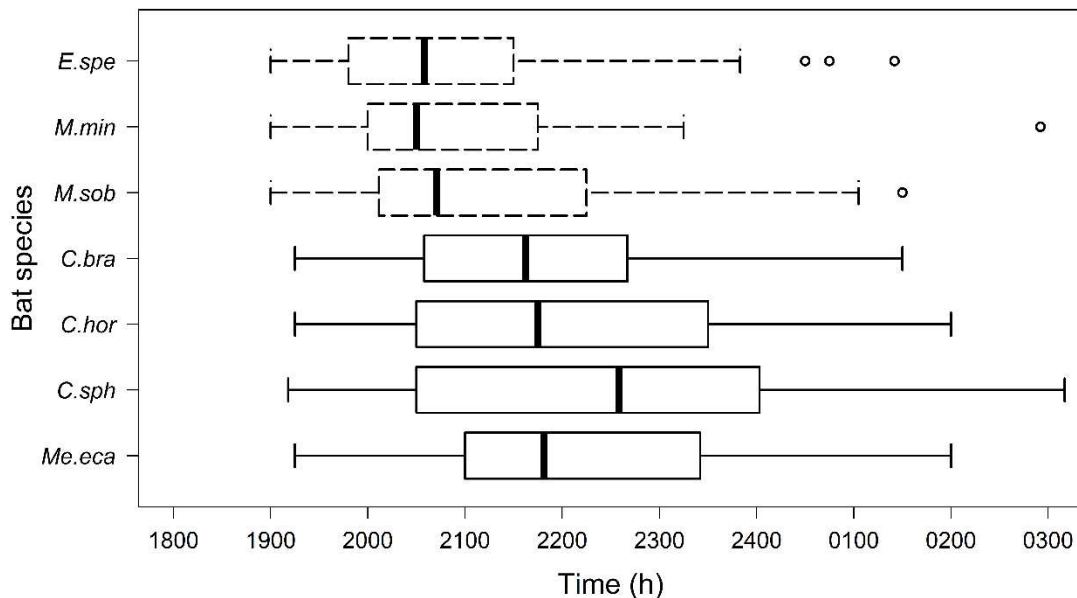


Figure 2. Boxplots displaying foraging time distributions of the most commonly netted nectarivorous (dashed lines) and frugi-nectarivorous (solid lines) bat species encountered in this study (southern Thailand). Species (with number of bats netted in parentheses), top to bottom: *Eonycteris spelaea* = *E.spe* (105), *Macroglossus minimus* = *M.min* (35), *M. sobrinus* = *M.sob* (39), *Cynopterus brachyotis* = *C.bra* (16), *C. horsfieldii* = *C.hor* (69), *C. sphinx* = *C.sph* (111), *Megaerops ecaudatus* = *Me.eca* (10).

DISCUSSION

This study compared differences in the foraging times of Old World nectarivorous versus frugi-nectarivorous bats to examine their interactions with plants that depend on them for pollination and/or seed dispersal. We found that nectarivorous bats foraged significantly earlier in the evening, and over a significantly shorter time period, than frugi-nectarivorous bats. Similar findings have been reported in the Neotropics; a number of studies have shown that frugivorous bat activity occurs throughout most of the night (Aguilar & Marinho-Filho 2004, Castro-Arellano et al. 2009, Pedro & Taddei 2002, Presley et al. 2009; but see Mancina & Castro-Arellano 2013), and La Val (1970) showed that early foraging activity is especially pronounced among nectarivorous bats. These differences between nectarivorous and frugi-nectarivorous bats may result from a number of causes that are not mutually exclusive, including commuting distance between roost and foraging ground, meeting daily energetic requirements, interspecific competition, and/or the temporal resource constraints imposed by the plant species they prefer to visit.

If commuting distance were an important predictor of capture time, we would expect later capture times for bats commuting from rare, patchily distributed roosts (such as caves) than from abundant, uniformly distributed roosts (such as in foliage); cave roosts, on average, are farther from a randomly selected netting site than foliage roosts, and therefore require longer commute times, as was observed by Thomas & Fenton (1978). We would also expect greater variation in the capture times of bats commuting from cave roosts since the closest cave roost may be very near (short commute time) or very far (long commute time) from a randomly selected netting

site, whereas the distance to the closest foliage roost is much less variable. Since nearly 60% of the nectarivorous bats in this study were cave-roosting *E. spelaea* and 94% of frugi-nectarivorous bats in this study belonged to foliage-roosting *Cynopterus* spp., we would thus expect nectarivorous bats to exhibit later and more variable capture times than frugi-nectarivorous bats. We would particularly expect to see early capture times for frugi-nectarivorous bats since previous work has shown that *Cynopterus* individuals typically forage within 500 m of their night roost (Funakoshi & Akbar 1997; Marimuthu et al. 1998) and several *Cynopterus* roosts were observed within the study area. Contrary to these expectations, capture times of nectarivorous bats were earlier and less variable than those of frugi-nectarivorous bats, indicating that commuting distance is not a main predictor of arrival time at fruiting and flowering resources.

Several previous studies have suggested that phytophagous bats must begin foraging early in order to meet high daily energetic requirements, particularly since fruit and nectar are relatively poor food resources and flight is energetically costly (Mancina & Castro-Arellano 2013; Presley et al. 2009). Yet these requirements apply to both nectarivorous and frugi-nectarivorous bat species, which does not directly explain why nectarivorous bat species forage earlier than frugi-nectarivorous bat species. Nor was there a correlation between body size and foraging time (Pearson's correlation coefficient, $r = 0.02$, $P = 0.62$, $n = 416$). It is possible that differences in the nutrient composition of fruit versus floral resources (which include both pollen and nectar), and/or different nutrient requirements of the two feeding guilds, contribute to the observed foraging differences between nectarivorous and frugi-

nectarivorous bat species. Thus, the relationship between pteropodid diet composition, their dietary requirements, and the nutrient content of their food resources should be examined before making any conclusive statements.

Another possible explanation for the observed difference in frugi-nectarivorous and nectarivorous bat capture times is that species forage during different times in order to reduce interspecific competition at shared resources. Previous studies showing temporal partitioning between pairs of pteropodid species have, in fact, suggested that interspecific competition is a driving factor (Fischer 1992; Singaravelan & Marimuthu 2004), and it likely contributes to the differences in foraging times seen in this study. However, interspecific competition alone does not explain why nectarivorous bats foraged earlier than frugi-nectarivorous bats, rather than the reverse trend. Indeed, if interspecific competition were the main cause, we would expect to see temporal partitioning among nectarivorous species throughout the night, rather than observing concentrated nectarivorous bat activity early in the evening. Thus, we propose that differences in the foraging times of frugi-nectarivorous and nectarivorous bat species are explained in large part by the resource constraints imposed by the plant species they visit.

Many plant species have overcome the challenges of immobility by relying on the services of animals to disperse their gametes (pollen) and offspring (seeds) (Barrett & Harder 1996), however, pollination and seed dispersal are precise processes that must occur within specific periods of time. A fruit that is removed from the plant too early will not be fully developed, while an uneaten fruit that drops to the ground beneath its parent plant faces limited dispersal and greater competition

with its parent and siblings (Howe & Smallwood 1982). Likewise, there is an optimal timeframe for pollination; fertilization can only occur when pollen is mature and stigmas are receptive (Faegri & van der Pijl 1966). Thus, most animal-assisted plant species have adaptations that encourage pollinators and seed dispersers to perform their services within the optimal time period. For example, unripe fruits often contain secondary compounds that make them unpalatable (Cipollini & Levey 1997), and flowers typically produce the most nectar when they are receptive to pollination (Cruden et al. 1983; Martén-Rodríguez et al. 2009; Reynolds et al. 2009). This temporal variability in resource quality imposes selective pressures on the foraging times of pollinators and seed-dispersers; animals that forage when nectar and fruit are most palatable and abundant receive the greatest energetic benefits.

However, the temporal constraints that plants impose on pollinators are often more restrictive than those imposed on seed dispersers. This is particularly true among plant species visited by bats. While mature, bat-dispersed fruits are accessible for days, most bat-pollinated flowers are only open for a few hours (Bumrungsri et al. 2009; Faegri & van der Pijl 1966), providing a much narrower window of opportunity for their visitors. For example, *Oroxylum indicum* flowers begin opening around 19h00 and the corollas drop by 02h00 (Sritongchuay et al. 2008). Additionally, nectar production and sugar concentration typically decline over the course of a night (Elangovan et al. 2000; Elmqvist et al. 1992; Sripaoraya 2005; Vikas et al. 2009; A. Stewart, unpubl. data), placing even stronger selection on early foraging. Aguiar & Marinho-Filho (2004) observed that Neotropical phytophagous bat species foraged early in the evening and also suggested declining nectar production throughout the

night as a possible explanation. Since the nectar of a given flower is only available for a few hours, in contrast to a fruit which can be available for days, we might expect the foraging times of nectarivorous bats to be less variable than those of frugi-nectarivorous bats.

Indeed, our data reveal that nightly foraging by nectarivorous bats does occur within a narrower time range than that of frugi-nectarivorous bats. Furthermore, the interquartile range of nectarivorous-bat foraging activity occurs between 20h00 and 21h44, which coincides with peak nectar production times of bat-pollinated plant species reported in other studies (20h00–22h00; Bumrungsri et al. 2008; Elmqvist et al. 1992; Sritongchuay et al. 2008; Vikas et al. 2009; A. Stewart, unpubl. data). The interquartile range of all frugi-nectarivorous bat foraging activity (20h30–23h50) is not only broader, it is also later in the evening when nectar production has declined for many flowering plant species, which reflects their non-obligate reliance on floral resources.

Our results demonstrate that nectarivorous and frugi-nectarivorous bats appear to be acting as two distinct functional groups that are likely to impose different selective pressures on the plant species they visit. We propose that pteropodid bats utilize different foraging strategies that are shaped by the plant species they visit. However, confirming this hypothesis requires observation of bat-visited plant species throughout the year to verify that the results we observed are consistent across all months of the year, since flowering and fruiting seasons vary by plant species. Furthermore, future work should compare the relative contributions of different pteropodid species to the pollination success of the plant species they visit, as

differences in floral foraging times by pollinating bats may translate into important differences in a plant's reproductive success. Given that the timing and duration of anthesis varies among different plant species, the outcomes will likely span a continuum from plant species that are greatly affected by foraging time differences, to species that are barely influenced. By studying the interactions of nectarivorous and frugi-nectarivorous bat species with the plants they visit, we can better understand plant-animal interactions and how these important visitors promote the reproductive success and genetic diversity of many agricultural and native plant species.

Chapter 2: Temporally-patchy floral resources shape foraging strategies of generalist and specialist nectarivores

ABSTRACT

Floral resources are often patchy, both spatially and temporally, which can require long-lived pollinators such as bats to switch resources seasonally. Furthermore, there is great variation in flowering phenologies among plant species, ranging from low-rewarding but highly reliable “steady-state” plants to high-rewarding but ephemeral “big-bang” plants. Theory predicts that generalist foragers should switch resources more readily, tracking resources that are most abundant in the environment, while specialist foragers should remain constant to preferred food resources. We tested this prediction by assessing how the diets of nectarivorous bats change throughout the year in response to seasonal fluctuations in floral resource availability. Over 15 months of fieldwork we (1) recorded the flowering phenologies of six bat-pollinated plant taxa in southern Thailand, and (2) assessed the diets of seven common flower-visiting bat species by sampling and identifying the plant species pollen that they carried. As predicted, the generalist nectarivore (*Eonycteris spelaea*) frequently switched diets to forage on the most abundant floral resources, while the specialist nectarivores (*Macroglossus minimus* and *M. sobrinus*) foraged on one or two steady-state plant species year-round. Furthermore, the larger and faster bat species (*Eonycteris spelaea* and *Rousettus leschenaulti*) were presumably able to fly longer distances in search of high-rewarding big-bang flowers, while the smaller bat species (*Macroglossus minimus* and *M. sobrinus*) appeared to prefer highly reliable steady-

state plant species that require minimal commuting to and from roosting areas. This study demonstrates how the interaction between plant flowering phenology and pollinator diet breadth can shape the frequency and constancy of pollinator visits, as well as the potential extent of gene flow occurring within a patchy floral environment.

Key words: big-bang, chiropterophily, diet switching, nectar bat, phenology, pollination, Pteropodidae, resource availability, steady-state

INTRODUCTION

Plant-pollinator interactions are one of the few types of consumer-resource relationships in which both parties receive a net benefit and actively promote further interaction (Holland et al. 2005; Willmer 2011). Thus, plant-pollinator interactions are inherently different from predator-prey interactions, which have been the focus of optimal foraging theory (Charnov 1976; Perry & Pianka 1997; but see Pyke 1978, Goulson 1999). Foraging pollinators transfer gametes (pollen) between flowering conspecific individuals and are therefore critical to plant reproductive success (Sprengel 1793, 1996; Darwin 1862; Harder & Barrett 1996; Willmer 2011). Plants also promote pollinator fitness by providing food rewards (typically nectar or pollen) and can influence pollinator foraging behavior through variable flowering phenologies (Gentry 1974; Stephenson 1982; Zimmerman 1988; Peters et al. 2013; Stewart et al. 2014).

Gentry (1974) defined several phenological patterns of flowering, including “steady-state” and “big-bang.” Steady-state plant species produce small numbers of flowers over an extended period, while big-bang plant species produce large numbers

of flowers over a very brief period. According to optimal foraging theory, big-bang resources should be more attractive, and pollinators are expected to spend longer amounts of time at big-bang resources (when they are available), than steady-state resources (Stephenson 1982; Pleasants 1989). Consequently, steady-state plant species are predicted to encourage trapline foraging behavior (Gentry 1974; Machado & Vogel 2004) and outcrossing (which is expected to enhance reproductive success; Ohashi & Thomson 2009); in contrast, big-bang species are hypothesized to receive higher loads of self or geitonogamous pollen (Augspurger 1980; Stephenson 1982; Eckert 2000).

On a broader scale, the year-round pattern of flowering phenologies at the community level also influences pollinator behavior. Specifically, spatially- and/or temporally-patchy distributions of resources are more likely to lead to diet switching than homogenous distributions (Fleming 1992; Renton 2001). Since floral abundance tends to be highly variable (Martin & Karr 1986; Pyke 1983; Goulson 1999), we therefore might expect long-lived nectarivores to switch diets frequently. Additionally, theory predicts that generalist foragers should be more likely to switch resources than specialists (Abrams 2006; Carnicer et al. 2008). In other words, generalists should be more likely to track temporal fluctuations in resource abundance, as has been demonstrated in passerine birds (Carnicer et al. 2008) and fish (Correa & Winemiller 2014).

One system in which these predictions can be tested is the diverse community of nectar-feeding bats (Pteropodidae) and bat-pollinated plants in the Old World tropics. Bat-pollinated plants exhibit a range of flowering phenologies, and the

species most commonly visited by nectar bats include both steady-state species (e.g., *Musa acuminata* and *Parkia speciosa*) as well as big-bang species (e.g., *Ceiba pentandra* and *Durio zibethinus*; Stewart & Dudash 2016). Furthermore, plant-visiting bat species span a continuum from specialist nectarivores (which have long, slender rostrums and tongues well-suited to probing flowers for nectar) to generalist nectarivores (which have elongated rostrums, but not as slender as those of nectar-specialists) to generalist frugi-nectarivores (which have short, broad rostrums with powerful jaws for handling fruits) (Start & Marshall 1976; Francis 2008). We can therefore make and test predictions regarding how floral abundance influences bat foraging behavior across different flowering phenologies and bat diets.

To assess how the diets of nectarivorous bats change in response to seasonal fluctuations in floral resource availability, we (1) monitored flowering phenologies of six bat-pollinated plant taxa in southern Thailand and (2) measured the abundance of their respective pollen in the diets of common flower-visiting bat species for 15 continuous months. By comparing flowering phenologies and bat diets on a monthly basis, we could determine which bat species track which plant species. We predicted that generalist nectarivore *Eonycteris spelaea* would switch floral resources across seasons and forage on the most locally abundant plant species in flower. We further predicted that specialist nectarivores *Macroglossus minimus* and *M. sobrinus* would consistently forage on steady-state species throughout the year, regardless of the abundance of other resources. This study demonstrates how pollinator foraging behavior is guided by both pollinator diet breadth and the (seasonally fluctuating) floral resource availability of sympatric plant species.

METHODS

Study site

We conducted our study in southern Thailand, where several species of flower-visiting bats and their food resources are common. Throughout data collection, we rotated among four protected areas (Khao Banthat Wildlife Sanctuary, Khao Pu Khao Ya National Park, Thale Ban National Park, and Ton Nga Chang Wildlife Sanctuary), which are spread across Krabi, Phatthalung, Songkla, and Trang provinces. Common habitat types include secondary forests, mixed fruit orchards, rubber plantations, oil palm plantations, and mangroves (Appendix II). The most common flower-visiting bat species in the area include three nectarivorous species (*E. spelaea*, *M. minimus*, *M. sobrinus*) and four frugi-nectarivorous species (*Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*, and *R. leschenaulti*; Stewart et al. 2014).

Flowering phenology

We noted flowering phenologies of the following bat-pollinated plant species: *Ceiba pentandra* (Malvaceae; kapok or silk-cotton tree), *Durio zibethinus* (Malvaceae; durian), *Musa acuminata* (Musaceae; banana), *Oroxylum indicum* (Bignoniaceae; Indian trumpet flower), *Parkia speciosa* (Fabaceae; petai or sator), *P. timoriana* (Fabaceae; tree bean or riang), and four *Sonneratia* species (Lythraceae; *S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*). Detailed descriptions of the plant study species can be found in Appendix III. Surveys were conducted monthly (for *Sonneratia* species) or biweekly (for all other plant species) between March 2013 and May 2014. We rotated among the four parks for all species except *Sonneratia*, which

only occurred in Thale Ban National Park. During each sampling period, we selected ten random individuals and recorded whether or not they had flowers. This measure provided a standardized method to compare flowering phenologies across plant species, as different species produce different quantities of flowers (and data for *M. acuminata* is binomial, i.e., inflorescence present or absent).

Bat diet

We tracked floral diets of the three nectarivorous bat species (*E. spelaea*, *M. minimus*, *M. sobrinus*) and four frugi-nectarivorous bat species (*C. brachyotis*, *C. horsfieldii*, *C. sphinx*, *R. leschenaulti*) between March 2013 and May 2014 ($N = 122$ nights). While the frugi-nectarivorous bat species undoubtedly foraged on fruiting resources throughout the year, we focused on flowering resources for this study. Mist-nets were placed in the canopy directly in front of flowers of our plant study species to catch pteropodid bats during their nightly foraging. Floral diets were then inferred from pollen samples collected from the fur of 1,219 netted bats. Each bat was uniformly swabbed with 0.4 mL of solidified fuchsin glycerin gelatin (Beattie 1972) along the face, chest, and ventral side of the wings (for detailed description, see Stewart & Dudash 2016). Pollen grains that adhered to the gelatin were fixed on microscope slides; we then used a compound light microscope to count and identify pollen grains by comparison against a reference collection. Most pollen could be identified to species level, except for *Parkia* and *Sonneratia*, which were identified to genus.

Statistical analysis

We compared the relationship between flowering phenology (percentage of individuals of plant species i in flower, averaged per month) and bat diet (number of pollen grains of plant species i carried by bats, averaged per month) using Spearman's rank correlation coefficient as our data were not normally distributed (Hollander et al. 2013). We assessed correlations for each combination of bat and plant species in which at least 20% of the individuals of bat species j (caught during the flowering period of plant species i) carried pollen of plant species i . We selected a cutoff of 20% because there was a natural divide in our data between bat species that regularly foraged on a given plant species (one in five bats carried pollen) and those that did not ($\leq 15\%$ of bats carried pollen; Table 3).

RESULTS

We were primarily able to examine the relationship between bat diet and plant floral resource availability with the three nectarivorous bat species, as they carried pollen more consistently than the four frugivorous species. Nectarivorous *E. spelaea* was the most frequent bat species encountered, and individuals frequently carried pollen of *C. pentandra*, *D. zibethinus*, *M. acuminata*, *Parkia*, and *Sonneratia* flowers (Table 3).

With *E. spelaea* bats, there was a significant positive correlation between flowering phenology and number of pollen grains carried for *C. pentandra* ($r_s = 0.88$, $P < 0.001$, $n = 120$), *D. zibethinus* ($r_s = 0.70$, $P < 0.01$, $n = 142$), and *M. acuminata* flowers ($r_s = 0.76$, $P < 0.01$, $n = 148$), but not for *Parkia* ($r_s = 0.32$, $P > 0.05$, $n = 278$) or *Sonneratia* flowers ($r_s = 0.04$, $P > 0.05$, $n = 219$) (Table 3; Figure 3). When

TABLE 3. Spearman's rank correlation coefficients (r_s , in bold) for flowering phenology (percentage of individuals in flower) and bat diet (number of pollen grains carried) for six common bat-pollinated plant taxa and seven common flower-visiting bat species in southern Thailand. Correlations were only tested for combinations in which at least 20% of the bat individuals carried pollen of a given plant species, during the flowering season of that plant species. Under the name of each bat species, the total number of netted bats is noted; within each cell, the number and percentage of netted bats found carrying pollen for each plant species (during the flowering period) are indicated in parentheses. Data for *Parkia* spp. (*P. speciosa* and *P. timoriana*) and *Sonneratia* spp. (*S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*) are combined as their pollen could only be identified to the genus level. [NS = not significant, * <0.05, ** <0.01, *** <0.001]

		Plant species					
		<i>Ceiba pentandra</i>	<i>Durio zibethinus</i>	<i>Musa acuminata</i>	<i>Oroxylum indicum</i>	<i>Parkia</i> spp.	<i>Sonneratia</i> spp.
Nectarivorous bat spp.	<i>Eonycteris spelaea</i> (n = 746)	0.88 *** (120 bats, 53%)	0.70 ** (142 bats, 67%)	0.76 ** (148 bats, 20%)	(56 bats, 7%)	0.32 NS (278 bats, 37%)	0.04 NS (219 bats, 29%)
	<i>Macroglossus minimus</i> (n = 115)	(3 bats, 10%)	(1 bat, 7%)	0.22 NS (34 bats, 30%)	(0 bats, 0%)	(6 bats, 5%)	0.11 NS (81 bats, 70%)
	<i>Macroglossus sobrinus</i> (n = 73)	(6 bats, 8%)	(2 bats, 1%)	0.34 NS (68 bats, 93%)	(0 bats, 0%)	(11 bats, 15%)	(8 bats, 11%)
Frugi-nectarivorous bat spp.	<i>Rousettus leschenaulti</i> (n = 81)	(6 bats, 7%)	0.87 *** (26 bats, 80%)	(0 bats, 0%)	(2 bats, 2%)	(8 bats, 10%)	(10 bats, 12%)
	<i>Cynopterus brachyotis</i> (n = 59)	(6 bats, 10%)	(1 bat, 8%)	(0 bats, 0%)	(0 bats, 0%)	(4 bats, 7%)	(6 bats, 6%)
	<i>Cynopterus horsfieldii</i> (n = 101)	(2 bats, 8%)	(3 bats, 5%)	(0 bats, 0%)	(0 bats, 0%)	(7 bats, 7%)	(9 bats, 9%)
	<i>Cynopterus sphinx</i> (n = 44)	(2 bats, 14%)	(2 bats, 3%)	(1 bat, 0.5%)	(0 bats, 0%)	(7 bats, 9%)	(6 bats, 10%)

examining the two nectarivorous *Macroglossus* species and their main food resources, we did not find significant correlations between flowering phenology and number of pollen grains carried (*M. minimus*, *M. acuminata*: $r_s = 0.22$, $P > 0.05$, $n = 34$; *M. minimus*, *Sonneratia* spp.: $r_s = 0.11$, $P > 0.05$, $n = 81$; *M. sobrinus*, *M. acuminata*: $r_s = 0.34$, $P > 0.05$, $n = 68$) (Table 3; Figure 4a-c). However, we did find a significant positive correlation between flowering phenology of *D. zibethinus* and the

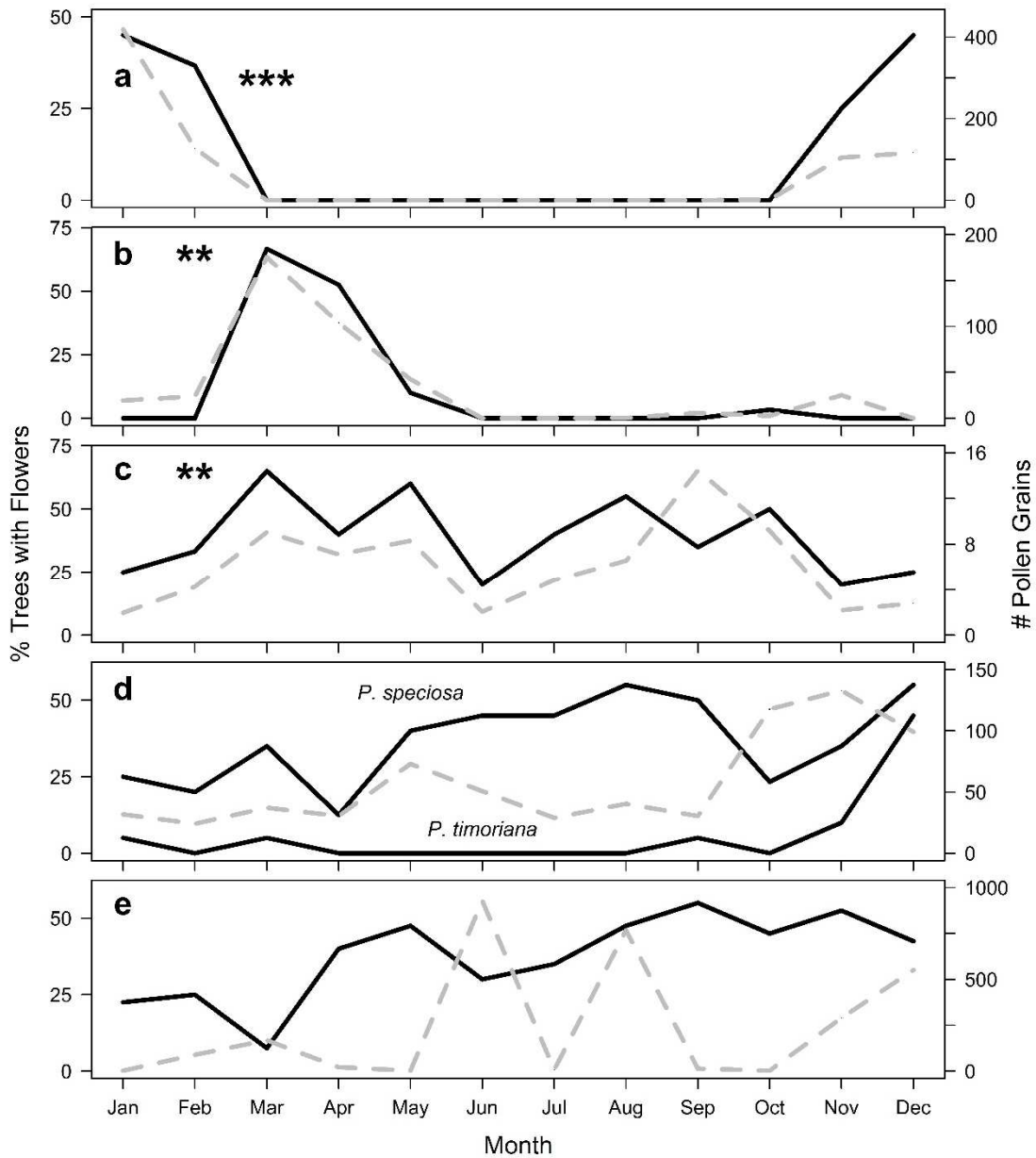


FIGURE 3. Flowering phenology of plant species *i* (solid black lines) and number of pollen grains of plant species *i* (dashed grey lines) carried by *Eonycteris spelaea* for five bat-pollinated plant taxa in southern Thailand: (a) *Ceiba pentandra*, (b) *Durio zibethinus*, (c) *Musa acuminata*, (d) *Parkia* species (*P. speciosa* and *P. timoriana*), and (e) *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata* combined). There is a significant, positive correlation between flowering phenology and number of pollen grains carried for *C. pentandra* ($r_s = 0.88$, $P < 0.001$, $n = 120$), *D. zibethinus* ($r_s = 0.70$, $P < 0.01$, $n = 142$), and *M. acuminata* ($r_s = 0.76$, $P < 0.01$, $n = 148$). Note: y-axes are different, as they are scaled to standardize the large differences in flowering phenology and number of pollen grains carried across plant species.

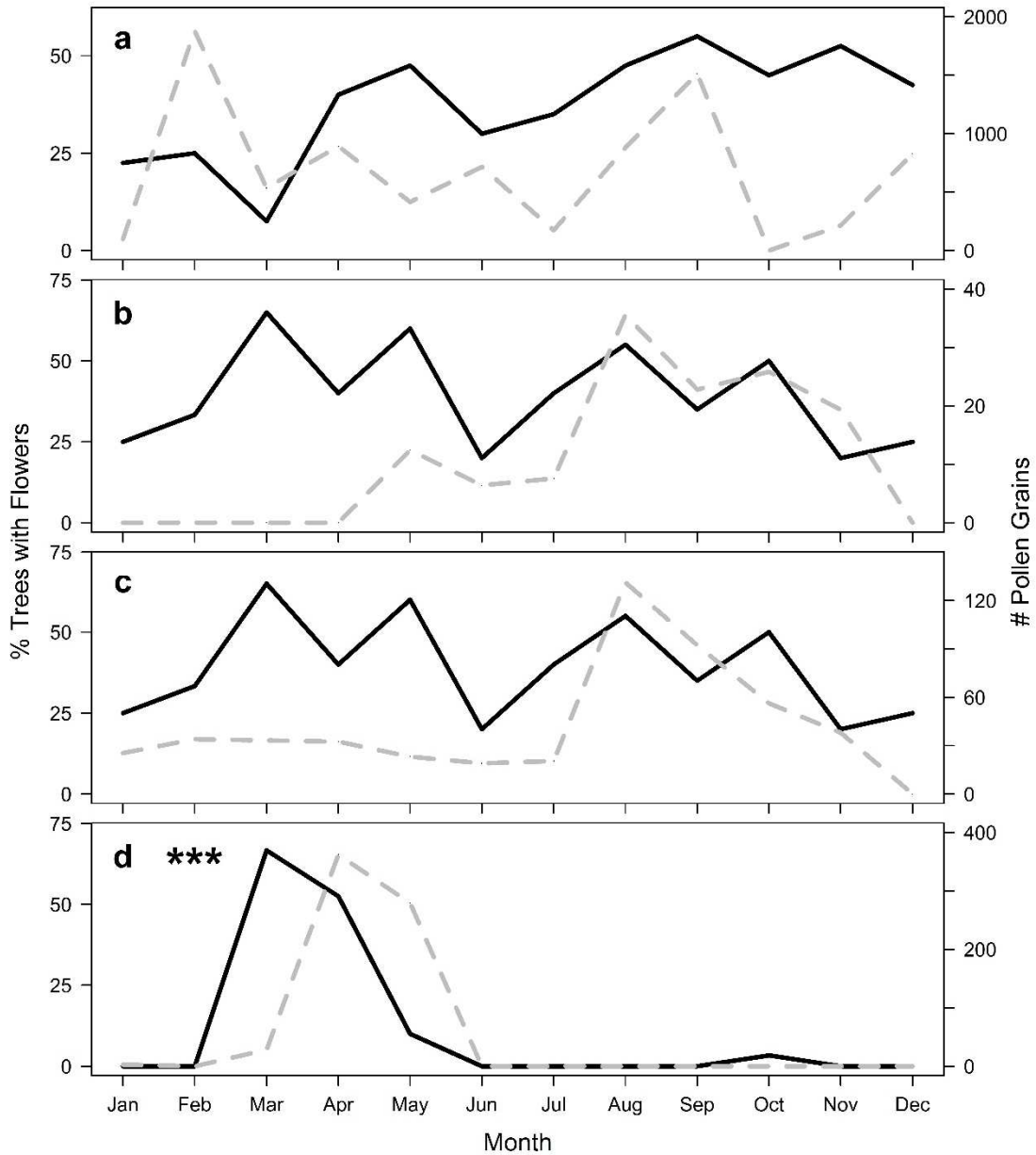


FIGURE 4. Flowering phenology of plant species *i* (solid black lines) and number of pollen grains of plant species *i* (dashed grey lines) carried by flower-visiting bats in southern Thailand: (a) *Macroglossus minimus* bats and *Sonneratia* flowers (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata* combined), (b) *M. minimus* bats and *Musa acuminata* flowers, (c) *M. sobrinus* bats and *M. acuminata* flowers, (d) *Rousettus leschenaulti* bats and *Durio zibethinus* flowers. There is a significant, positive correlation between flowering phenology of *D. zibethinus* and number of *D. zibethinus* pollen grains carried by *R. leschenaulti* ($r_s = 0.87$, $P < 0.001$, $n = 26$). Note: y-axes are different, as they are scaled to standardize the large differences in flowering phenology and number of pollen grains carried across plant species.

amount of *D. zibethinus* pollen carried by frugivorous *R. leschenaulti* ($r_s = 0.87$, $P < 0.001$, $n = 26$) (Table 3; Figure 4d). Frugivorous *Cynopterus* bats rarely carried pollen, and sample sizes were insufficient to test for correlations between floral resource availability and *Cynopterus* diets (Table 3).

DISCUSSION

Within the family Pteropodidae, we observed two floral foraging strategies: frequent switching of resources (between big-bang and steady-state flowering plant species), and year-round use of one or two steady-state flowering plant species. This year-long study on seasonal foraging patterns complements earlier work on the nightly foraging patterns of pteropodid bats (Stewart et al. 2014). Collectively, these findings shed further light on bat-plant pollination interactions, as foraging behavior can have large impacts on both plant and pollinator. For example, pollinators are expected to forage in ways that optimize energy intake, but this can vary by pollinator size (Von Helversen & Winter 2003; Greenleaf et al. 2007) and metabolic needs (Schmitt 1980). Furthermore, pollinator foraging greatly influences plant reproductive success (Kunin 1993; Harder & Barrett 1996; Willmer 2011) and gene flow (Schmitt 1980; Loveless & Hamrick 1984).

As predicted, generalist nectarivore *Eonycteris spelaea* had the broadest diet and switched between big-bang and steady-state plant species (Table 3; Figure 3). In particular, *E. spelaea* foraged heavily on *C. pentandra* and *D. zibethinus* when these big-bang species were in flower; *C. pentandra* pollen was by far the most abundant pollen species collected during peak *C. pentandra* flowering (January), and *D.*

zibethinus pollen was the most abundant species collected during peak *D. zibethinus* flowering (March; Figure 3a-b). *Eonycteris spelaea* also appeared to track *M. acuminata* availability year-round, however, this steady-state flower resource is not used as heavily, as we collected only small quantities of *M. acuminata* pollen per bat (Figure 3c). Two other steady-state plant taxa, *Parkia* and *Sonneratia*, also contributed greatly to the diet of *E. spelaea* at various times throughout the year, but not always when the flowers of these species were most abundant (Figure 3d-e). Consistent with theory (Abrams 2006; Carnicer et al. 2008), the most generalist nectarivore (*E. spelaea*) was the most likely to switch floral resources and to forage on the flowering plant species that were locally abundant.

The only other bat species to forage at a big-bang floral resource was *R. leschenaulti*. This species is primarily frugivorous (Francis 2008), but foraged heavily on *D. zibethinus* during its flowering season (Figure 4d). Interestingly, *R. leschenaulti* bats do not appear to forage on *C. pentandra* flowers to the same extent they do *D. zibethinus* flowers, despite the fact that both are big-bang plant species belonging to the same family (Malvaceae) with similar floral designs (dense clusters of white, five-petaled flowers). Similar findings were reported in India, where *R. leschenaulti* was also an uncommon visitor to *C. pentandra* flowers (Singaravelan & Marimuthu 2004). As *C. pentandra* trees tend to grow singly or in small clusters, they may be less attractive than the high numbers of *D. zibethinus* trees found in fruit orchards. Additionally, *R. leschenaulti* may forage on other, more preferred, fruiting resources during November through February when *C. pentandra* is in flower. As has been noted with many frugivorous bat species (Heithaus et al. 1975; Fleming & Heithaus

1986; Hodgkison et al. 2004; Stewart et al. 2014), *R. leschenaulti* bats likely forage on fruits for most of the year, but supplement their diet with nectar when preferred floral resources are abundant, such as during mass flowering of *D. zibethinus*.

Unlike *E. spelaea* and *R. leschenaulti*, the two smaller-bodied nectarivorous *Macroglossus* species had specialized diets and never utilized big-bang floral resources. Instead, they foraged on steady-state flowering plant species year-round, with *M. sobrinus* predominantly carrying *M. acuminata* pollen, and *M. minimus* carrying both *M. acuminata* and *Sonneratia* pollen (Figure 4a-c). Both *M. acuminata* and *Sonneratia* typically occur in dense stands, which likely makes them more attractive than the other steady-state flowering plant species (e.g., *O. indicum* and *P. speciosa*), which often grow singly or in groups of less than five trees (A. Stewart, pers. obs.). Von Helversen & Winter (2003) demonstrated that large neotropical bats can fly farther, fly faster, and survive longer on fat reserves than small bats, which led them to hypothesize that large bats can search widely for high-rewarding floral patches, while small bats should choose the less risky option of foraging at low-rewarding but highly reliable floral resources.

This hypothesis on foraging strategy has held true in the neotropics (Heithaus et al. 1975; Morrison 1978; Fleming & Heithaus 1986), and also fits our paleotropical system. In our study area, tiny *Macroglossus* bats (18 – 23 g; Appendix IV) live near their preferred food resources, which flower year-round, and therefore spend little time and energy commuting. In contrast, large *E. spelaea* (53 – 59 g; Appendix IV) and *R. leschenaulti* (73 – 89 g; Appendix IV) cover long distances as they fly between

their cave roosts and foraging grounds (Start & Marshall 1976; Acharya et al. 2015) and are able to search for high-rewarding big-bang flowering plant species.

The three *Cynopterus* species did not regularly carry pollen of either big-bang or steady-state plant species, reflecting their primarily frugivorous diet (Bumrungsri et al. 2007; Stewart et al. 2014). Other scholars have predicted that mass-flowering plants should attract a large number of opportunistic visitors (Baker 1973; Augspurger 1980). However, *Cynopterus* individuals rarely carried pollen of big-bang species, and they were most likely to carry pollen of *Parkia* and *Sonneratia* (Table 3). The floral foraging choices of these primarily frugivorous bats (which have broad muzzles and short tongues; Francis 2008; Stewart et al. 2014) may be constrained by floral morphology. Among *C. pentandra*, *D. zibethinus*, *M. acuminata*, and *O. indicum* flowers, the nectar is difficult to reach as it is recessed within the corolla tube. In contrast, the nectar of *Parkia* and *Sonneratia* flowers is much more accessible; *Parkia* nectar beads along the surface of the inflorescence (Stewart & Dudash 2016), and *Sonneratia* nectar gathers in the cup of the calyx. In India, however, *C. sphinx* has been observed visiting the flowers of *Musa paradisiaca* (Elangovan et al. 2000), *C. pentandra* (Singaravelan & Marimuthu 2004), and *O. indicum* (Vikas 2009). Possibly, *E. spelaea* is the better competitor for floral resources and displaces *Cynopterus* bats from foraging at flowers in Thailand (where *E. spelaea* is abundant), while in India (outside the range of *E. spelaea*), *Cynopterus* are able to take greater advantage of floral resources. Given the small numbers of *Cynopterus* individuals carrying pollen in our study area, these bats presumably

concentrate on fruit resources throughout the year, consistent with previous work (Elangovan et al. 1999, Bumrungsri et al. 2007).

Steady-state and big-bang flowering plant species are both important for pollinators, but the interaction between plant and pollinator is inherently different for these two extreme resource types. While steady-state flowering plant species offer fairly small rewards, they are highly reliable and are important for sustaining pollinator populations (Baker 1963; Gentry 1974; Peters et al. 2013). Furthermore, steady-state flowering is generally predicted to promote outcrossing, since the limited floral displays have small rewards and pollinators must visit multiple plants (often spatially separated) in order to meet their energetic requirements (Gentry 1974; Ohashi & Thomson 2009). Conversely, big-bang flowering plant species are predicted to receive a higher proportion of self (or geitonogamous) pollen since pollinators are expected to spend a longer amount of time on a single plant (Augspurger 1980; Stephenson 1982). High loads of self or geitonogamous pollen may impose fitness costs for both self-compatible and self-incompatible species (Stephenson 1982; Dudash 1991; Eckert 2000), as even self-compatible species can suffer when there is substantial inbreeding depression (e.g., Dudash 1990; Husband & Schemske 1996).

Previous work suggests that big-bang, self-incompatible *D. zibethinus* may receive high loads of self or geitonogamous pollen, as *E. spelaea* has been observed concentrating on a subset of *D. zibethinus* trees within a foraging bout (Acharya et al. 2015). However, *E. spelaea* are also capable of transferring pollen over long distances, and Acharya et al. (in press) observed *E. spelaea* flying eight kilometers

between isolated *D. zibethinus* patches. In contrast, the much smaller *Macroglossus* bats foraging on steady-state resources likely move more frequently between flowers of different plants, but they also fly shorter distances overall, and thus gene flow is probably limited to a smaller geographic area. Thus, for bat-pollinated plants in southern Thailand, there may be a trade-off between the percentage of outcrossed flowers and the average outcrossing distance. Webb and Bawa (1983) reported similar findings in Costa Rica, where a hummingbird-pollinated plant experienced greater outcrossing distances, but higher loads of self pollen, than a butterfly-pollinated plant. However, this pattern was not observed in a study of bees and butterflies (Schmitt 1980), so there is likely wide variation among different systems and warrants further study.

Our study illustrates two distinctive floral foraging strategies within the nectar-feeding Pteropodidae. These differences in foraging strategy appear to contribute to the broad diet of *E. spelaea* and the specialized diets of *Macroglossus* bats, and are also consistent with the hypothesis that generalist foragers are more likely to switch diets than specialist foragers throughout seasons. Our results demonstrate that some pteropodid species have the ability to forage opportunistically, and this strategy may be important for their successful long-term persistence in the mixed-use agricultural-forest environment of southern Thailand. Furthermore, plant species flowering phenology (big-bang versus steady-state) likely has important consequences on the rates of outcrossing and the average distance of gene flow. Further research is needed to investigate how the interaction of bat foraging behavior and flowering phenology affect the genetic diversity of bat-pollinated plant species.

Chapter 3: Variation in potential pollinator importance within a classic pollination syndrome

ABSTRACT

Pollinators directly influence plant reproductive success, and therefore can be important drivers of floral evolution. The selective pressures that pollinators exert on floral design can result in “pollination syndromes,” such as the pale-colored, night-blooming flowers typical of bat-pollinated plants. Yet many flower-visiting animals differ in their contributions towards pollination success, and not all visitors are true pollinators. To identify the major drivers of floral evolution within the bat pollination syndrome, we quantified potential pollinator importance of seven nectarivorous bat species in southern Thailand. Pollinator importance was calculated as the product of bat visitation rate (obtained from mist-netting data) and pollen transfer efficiency (estimated from bat pollen loads). Furthermore, we examined variation within this bat pollination syndrome by comparing differences in pollinator importance across six bat-pollinated plant taxa. We found that pollinator importance varied by both bat species and plant species. In general, the nectar-specialist bat species were more important pollinators, yet the order of importance differed across our study plant species. Additionally, pollinator importance was dictated much more by pollen transfer effectiveness than visitation rate. Our results illustrate how floral visitors differentially contribute towards pollination success, and highlight the variability that can occur even within a “classic” pollination syndrome.

Key words: chiropterophily, pollen transfer efficiency, Pteropodidae, Thailand, visitation rate

INTRODUCTION

Immobility is one of the greatest challenges that plants encounter for reproduction. One way that many angiosperms have overcome this obstacle is by using animal pollinators to transfer gametes (pollen) between conspecific individuals (Darwin 1877; Faegri & van der Pijl 1966; Stebbins 1970; Raven et al. 2005). Pollinators are especially critical for plant species that are self-incompatible, as well as plant species occurring in highly diverse communities at low abundances, as is common in the tropics (Bawa 1990). In fact, it has been estimated that over 90% of plant species in tropical communities are animal-pollinated (Bawa 1990; Ollerton et al. 2011).

Since pollinators directly impact plant reproductive success through pollen transfer, they can substantially influence floral evolution. Transitions to animal pollination are positively correlated with increased diversification rates (Armbruster & Muchhala 2009; Kay & Sargent 2009), and pollination syndromes provide strong evidence for pollinator-driven selection on flowers (Fenster et al. 2004, 2015; Rosas-Guerrero et al. 2014). For example, the bat pollination syndrome reflects floral traits selected by bat pollinators, including nocturnal anthesis; copious nectar and pollen; and pale, robust flowers that are easily visible at night (Faegri and van der Pijl 1966; Fleming et al. 2009).

However, it is important to distinguish between floral visitors and true pollinators. An animal species that visits flowers but transfers little or no pollen is not

a pollinator (Inouye 1980; Inouye et al. 1994) and is not a major driver of floral evolution (Fenster et al. 2004). Thus, pollinator importance (the product of visitation frequency and pollen transfer effectiveness) is a more accurate metric than visitation rate alone when assessing the true pollinators of a plant species (Stebbins 1970; Schemske & Horvitz 1984; Fenster et al. 2004; Reynolds et al. 2009).

Bat pollination has been known for over a hundred years (e.g., Moseley 1879; Faegri and van der Pijl 1966), yet efforts to distinguish visitors from pollinators lag behind studies of insect-pollinated systems (Motten et al. 1981; Armbruster et al. 1989; Pettersson 1991; Olsen 1997). Several studies in the Old World tropics have observed bats visiting flowers (Baker & Harris 1959; Gould 1978; Itino et al. 1991; Elangovan et al. 2000; Winkelmann et al. 2003), but did not investigate pollen transfer effectiveness. Additionally, while some studies have quantified pollen transfer (Srithongchuay et al. 2008; Acharya et al. 2015) or demonstrated fruit set (Nathan et al. 2005; Bumrungsri et al. 2008, 2009), we still lack information about the relative pollination contributions of different bat species. This knowledge is fundamental for understanding how diverse bat species promote the reproductive success of many ecologically- and economically-important plant species that rely on bats for pollination (Bumrungsri et al. 2013).

To determine which paleotropical bat species are major pollinators, we quantified pollinator importance from floral visitation rates and bat pollen loads collected in southern Thailand. Our field data encompassed seven nectar-feeding bat species and six night-blooming plant taxa, which allowed us to explore the variation among interactions within this bat pollination syndrome. Additionally, we compared

pollination networks generated from visitation rates alone versus pollinator importance (visitation rate x pollen transfer effectiveness) values to examine the breadth and magnitude of bat-plant interactions within these communities. We predicted that nectar-specialist bat species would have higher visitation rates, transfer greater pollen loads, and thus be more important pollinators than primarily frugivorous bat species. We also hypothesized that pollinator importance values would vary by plant species, with different nectar bat species specializing on separate plant resources. This study illustrates how contributions towards pollination success can vary extensively, even by seemingly-similar floral visitors, within a classic pollination syndrome.

METHODS

Study site

We collected data in southern Thailand across four provinces (Phatthalung, Satun, Songkhla and Trang) from March 2013 through August 2014. The region consists predominantly of lowland tropical rain forest interspersed with urban and agricultural areas. The major agricultural practices are rubber plantations, oil palm plantations, and mixed fruit orchards (Appendix II). Commonly cultivated fruits of economic importance to local communities (Bumrungsri et al. 2013) include durian (*Durio zibethinus*), banana (*Musa acuminata*), petai (*Parkia speciosa*), langsat (*Lansium domesticum*), rambutan (*Nephelium lappaceum*), and mangosteen (*Garcinia mangostana*).

Bat study species

All flower-visiting bat species in the Old World tropics belong to the family Pteropodidae. We focused on three nectar-specialist species (*Eonycteris spelaea*, *Macroglossus minimus*, and *M. sobrinus*) and four primarily frugivorous species (*Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*, and *Rousettus leschenaulti*) (Stewart et al. 2014). While we occasionally caught other pteropodid species (*Megaerops ecaudatus*, *Penthetor lucasi*, *R. amplexicaudatus*), sample sizes were too small to accurately estimate visitation rates or pollen loads.

The nectar-specialist bats exhibit long yet narrow muzzles and tongues, which are characteristic of nectarivores (Freeman 1995), and relatively weak jaws with small teeth, reflecting the lack of fruit in their diets (Francis 2008). *Eonycteris spelaea* forages broadly on the nectar of many bat-pollinated plant species (Start & Marshall 1976; Marshall 1985; Bumrungsri et al. 2013). In contrast, *Macroglossus sobrinus* is reported to specialize on banana flowers (*Musa acuminata*; Start & Marshall 1976; Marshall 1983; Itino et al. 1991), while *M. minimus* feeds on both mangrove flowers (*Sonneratia* spp.; Start & Marshall 1976; Marshall 1983) and banana flowers (*M. acuminata*; Start & Marshall 1976; Winkelmann et al. 2003).

The four primarily frugivorous bat species have powerful jaws and well-developed molars equipped for handling fruit (Francis 2008). While *Cynopterus* species have short, broad muzzles, *R. leschenaulti* has a relatively long muzzle similar to nectar-specialist *E. spelaea* (Francis 2008), leading us to hypothesize that *R. leschenaulti* relies on floral resources to a greater degree than *Cynopterus* bats. Previous studies indicate that *Cynopterus* species and *R. leschenaulti* primarily forage

on fruits such as figs (*Ficus* spp.), neem (*Azadirachta indica*), sapodilla (*Manilkara zapota*), and turkey berry (*Solanum torvum*), but also visit the flowers of banana plants (*M. acuminata*) and kapok trees (*Ceiba pentandra*) (Bumrungsri et al. 2007; Marshall 1985; Sudhakaran & Doss 2012).

Plant study species

For this study, we chose plant taxa that are prominent in the diets of pteropodid bats (Start & Marshall 1976; Marshall 1983; Bumrungsri et al. 2013). Additionally, the selected plant species all exhibit classic traits of the bat pollination syndrome, including pale flowers, nocturnal anthesis, evening pollen dehiscence, and abundant nectar (Faegri & van der Pijl 1966). Flowers of these plant species open for a single night, with corollas and stamens dropping by morning (Faegri & van der Pijl 1966; Itino et al. 1991; Srithongchuay et al. 2008; Bumrungsri et al. 2009). Our six study plant taxa (Table 4) were *Ceiba pentandra*, *Durio zibethinus*, *Musa acuminata*, *Oroxylum indicum*, two species of *Parkia* (*P. speciosa* and *P. timoriana*), and four species of *Sonneratia* (*S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*) (see Appendix III for species details). Pollinator importance was determined at the genus level for *Parkia* and *Sonneratia* since congeneric flowers are nearly identical in design and their pollen could not be differentiated from one another.

TABLE 4. Characteristics of plant species commonly visited by nectar-feeding bats in southern Thailand.

Plant Species	Common Name(s)	Reproductive Morphology	Self-compatible?	# Ovules per Flower	# Flowers/ Tree/Night	Flowering Season	Documented Visitors
<i>Ceiba pentandra</i>	silk-cotton, kapok	hermaphroditic ^a	yes ^a	>200 ^a	1,000's ^a	Nov–Feb ^b	<i>C. sphinx</i> ^c , <i>R. leschenaulti</i> ^c
<i>Durio zibethinus</i>	durian	hermaphroditic ^d	no ^d	25-35 ^e	1,000's ^d	Mar–Apr ^{b,d}	<i>E. spelaea</i> ^d
<i>Musa acuminata</i>	banana	temporally dioecious ^f	yes ^g	300-1,500 ^h	15 – 40 ⁱ	year-round ^{b,f,j}	<i>Cynopterus</i> ^j , <i>E. spelaea</i> ^{j,k} , <i>Macroglossus</i> ^{ij,k}
<i>Oroxylum indicum</i>	Indian trumpet flower	hermaphroditic ^l	no ^l	~300 ^l	1-40 ^{j,l}	year-round ^{b,l}	<i>C. sphinx</i> ^m , <i>E. spelaea</i> ^l
<i>Parkia speciosa</i>	petai, sator	hermaphroditic ⁿ	no ⁿ	16-19 ⁿ	10-70 ⁿ	year-round ^{b,n}	<i>Cynopterus</i> ^{n,o} , <i>E. spelaea</i> ^{j,n}
<i>Parkia timoriana</i>	bean tree, riang	hermaphroditic ⁿ	no ⁿ	16-19 ⁿ	10's – 100's ⁿ	Dec–Jan ^{b,n}	<i>E. spelaea</i> ⁿ
<i>Sonneratia</i> spp.	NA	hermaphroditic ^p	unknown	~220 ^q	10's – 1,000's ^r	year-round ^{b,r}	<i>M. minimus</i> ^r

^a Gribel et al. 1999; ^b Stewart & Dudash, in prep.; ^c Singaravelan & Marimuthu 2004; ^d Bumrungsri et al. 2009; ^e Kozai et al. 2014; ^f Pillay & Tenkouano 2012; ^g Andersson 1998; ^h Fortescue & Turner 2005; ⁱ Itino et al. 1991; ^j Gould 1978; ^k Nur 1976; ^l Srithongchuay et al. 2008; ^m Vikas et al. 2009; ⁿ Bumrungsri et al. 2008; ^o Vanlalngaha 2014; ^p Bureau of Mangrove Resources Conservation 2009; ^q Primack et al. 1981; ^r Start & Marshall 1976

Data collection

Pollinator importance consists of two components: visitation frequency and pollen transfer effectiveness (Stebbins 1970; Schemske & Horvitz 1984; Fenster et al. 2004). We determined bat visitation rates to our plant study species by mist-netting at a novel location each night ($N=193$ nights, March 2013 – August 2014). When netting in *Sonneratia* mangroves, we were limited to areas with boardwalks, but rotated among different sections within and across sampling periods. For all plant species, we selected individuals with mature flower buds (i.e., anthesis would occur that evening) and rigged nets as close to flowers as possible, several hours before the evening emergence of nectarivorous bats. Since nets were placed directly in front of flowers, only bats attempting to forage at the flowers were caught (A. Stewart, pers. obs.). We used a slingshot to place guidelines in the canopy (4 – 20 m), thereby creating a pulley system to raise and lower mist-nets. Nets were checked every half hour until around 0200 h since bat activity is minimal after this time (Sripaoraya 2005; Stewart et al. 2014). We calculated nightly visitation rate (number of bats caught per hour) for each bat species at each focal plant species.

To estimate pollen transfer effectiveness, we used pollen load data from 1,211 netted bats. Individuals were tagged following Balasingh et al. (1992) to prevent repeat sampling. We uniformly dabbed fuchsin glycerin gelatin (Beattie 1972) along the bat's fur to pick up pollen from each bat's head, chest, and ventral side of the wings. To use a consistent amount of gel for each bat, solidified glycerin gelatin was dispensed from a syringe in increments of 0.1 mL per sample (four samples collected per bat). Each sample was then fixed on a slide (Beattie 1972), and

a compound light microscope was used to count and identify all pollen grains by comparison against a reference collection. Unknown pollen taxa were also recorded, but were very rare (Appendix V). Pollinator importance (PI) for each bat species at each focal plant species was calculated as the product of visitation rate (of bat species i at plant species j) and pollen load (of pollen species j on bat species i).

We verified that bat pollen load is an accurate metric for pollen transfer effectiveness within our study system by examining the correlation between pollinator pollen load (acquired after individual bats visited male flowers) and the number of pollen grains subsequently deposited on stigmas of conspecific female flowers (linear model, $F_{1,34} = 38.9$, $P < 0.0001$, $r^2 = 0.53$). These data were collected under controlled settings utilizing three of the focal bat species during flight cage trials (see Appendix VI for detailed methods and results).

Statistical analysis

For visitation frequency and pollen loads, we compared differences among bat species by estimating bootstrapped 95% confidence intervals (95% CI) following Reynolds and Fenster (2008). This bootstrapping method was preferable over other models since it sampled from the actual distribution of our data (Haukoos & Lewis 2005) and other distributions such as Gaussian, Poisson, and negative binomial did not accurately approximate the residuals.

This bootstrapping technique can also be used to estimate the means and confidence intervals of parameters that are the product of two or more variables (following Reynolds & Fenster 2008), such as our measure of pollinator importance.

Briefly, we resampled visitation rate and pollen load 10,000 times, and calculated the mean visitation rate and mean pollen load for each iteration. The bootstrapped means of visitation rate and pollen load were multiplied to generate 10,000 values of pollinator importance. We calculated the mean of these 10,000 values, sorted them in ascending order, and took the 250th and 9,750th observations as estimates of the 95% confidence interval. Bat species with non-overlapping 95% CI are significantly different ($P < 0.05$). All simulations were performed in R 3.1.1 (R Development Core Team).

Pollination networks

To examine the diversity and strength of pollination interactions within our study area, we generated a pollination network with the R package “bipartite” (Dormann et al. 2008) using pollinator importance values (visitation rate x pollen transfer effectiveness, see above). Given that visitation rate is commonly used to infer pollination contributions, we also created a network using this metric to determine if visitation rate alone accurately depicts pollination interactions within our study system. To assess the similarity of these two networks, we compared the connectance (number of links out of all possible links), mean number of shared partners (calculated for each pair of species), and mean niche overlap (calculated for each pair of species using Pianka’s index) (Dormann et al. 2008). For all three measures, larger values denote greater generalization within the system, while smaller values indicate that interactions are more specialized. We used t-tests to compare the number of shared partners and the amount of niche overlap for each network.

RESULTS

While we detected some variation in bat pollinator importance among the different plant species, *E. spelaea* was consistently an important pollinator (Figure 5, 6). For three plant species (*C. pentandra*, *O. indicum*, and *Parkia*), *E. spelaea* was the only bat species to reliably visit and carry conspecific pollen loads (Figure 5).

Consequently, these three plant taxa are dependent on a single pollinator species within our study area (Figure 5).

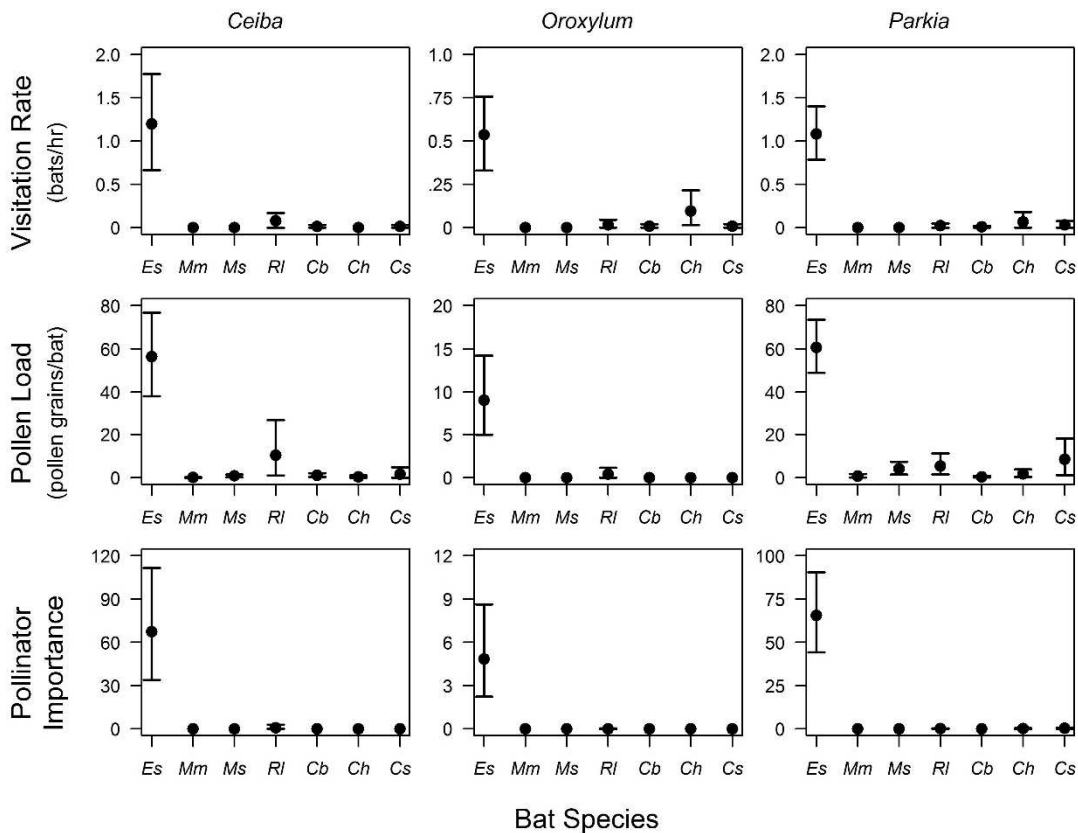


FIGURE 5. Plant species visited by a single important pollinator, *Eonycteris spelaea*, in southern Thailand: *Ceiba pentandra* (left column), *Oroxylum indicum* (middle column), and *Parkia* spp. (right column; *P. speciosa* and *P. timoriana* combined). Bootstrapped means and 95% confidence intervals were generated for visitation rates (top row) and pollen loads (middle row). Pollinator importance values (bottom row) were determined from the product of bootstrapped visitation rates and bat pollen loads (10,000 iterations). Bat species with non-overlapping 95% confidence intervals are significantly different. Bat species along x-axes, left to right: *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Rousettus leschenaulti*, *Cynopterus brachyotis*, *C. horsfieldii*, and *C. sphinx*. Note different scales for y-axes.

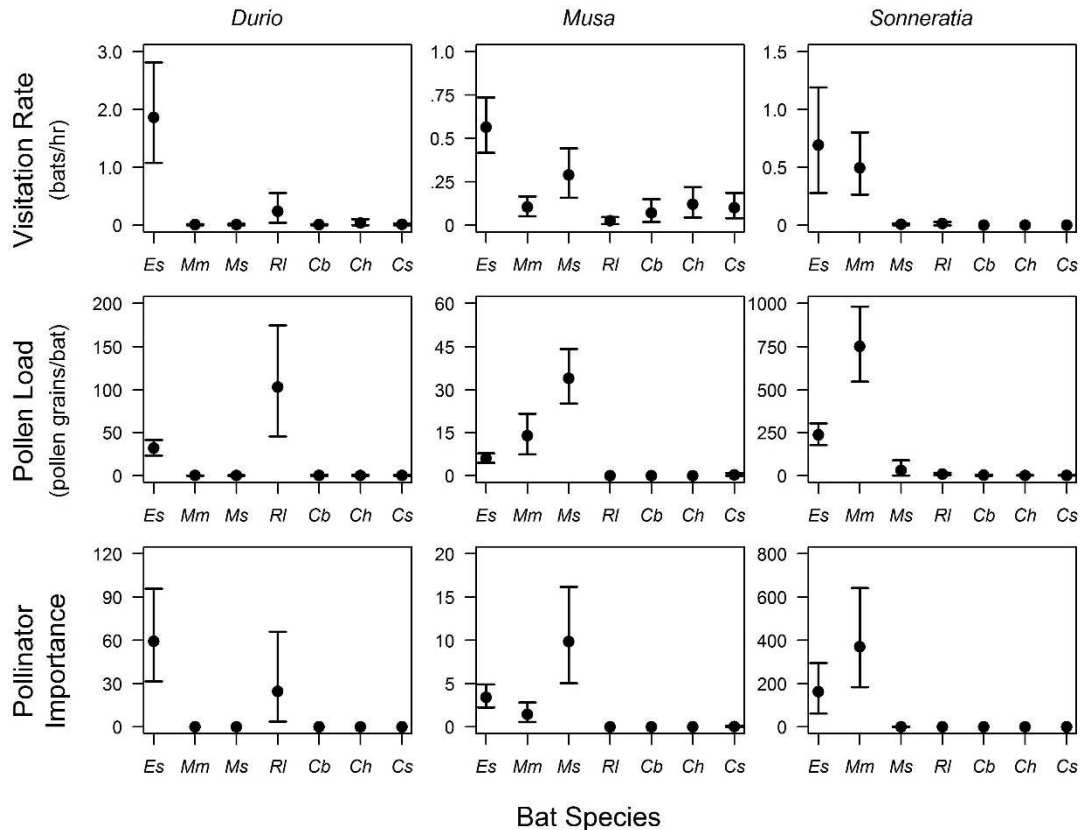


FIGURE 6. Plant species visited by multiple important pollinators in southern Thailand: *Durio zibethinus* (left column), *Musa acuminata* (middle column), and *Sonneratia* spp. (right column; *S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata* combined). Bootstrapped means and 95% confidence intervals were generated for visitation rates (top row) and pollen loads (middle row). Pollinator importance values (bottom row) were determined from the product of bootstrapped visitation rates and bat pollen loads (10,000 iterations). Bat species with non-overlapping 95% confidence intervals are significantly different. Bat species along x-axes, left to right: *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Rousettus leschenaulti*, *Cynopterus brachyotis*, *C. horsfieldii*, and *C. sphinx*. Note different scales for y-axes.

The remaining three plant taxa have multiple important pollinators (Figure 6). We found that *E. spelaea* and *R. leschenaulti* were significantly more important pollinators of *D. zibethinus* than the remaining five bat species (Figure 6). When examining the separate components of pollinator importance, we found that *E. spelaea* visited *D. zibethinus* significantly more often than all other bat species, but *R. leschenaulti* carried significantly more *D. zibethinus* pollen. The most important pollinators of *M. acuminata* were *E. spelaea*, *M. minimus*, and *M. sobrinus* (Figure 6).

While *E. spelaea* was the most frequent visitor to *M. acuminata*, both *M. minimus* and *M. sobrinus* carried significantly more *M. acuminata* pollen. For the *Sonneratia* species, *M. minimus* and *E. spelaea* were equally important pollinators, and significantly more important than all other bat species (Figure 6). Both *E. spelaea* and *M. minimus* were equally frequent visitors to *Sonneratia*, and both visited significantly more often than the remaining bat species. In terms of pollen load, *M. minimus* carried significantly more *Sonneratia* pollen than all other bat species.

Pollination networks generated using visitation rates alone versus pollinator importance values differed in several key traits (Figure 7). While all seven bat species appeared in the visitation network, *C. brachyotis* was absent from the pollinator importance network since it had an importance value of zero for all plant species studied. Connectance was 0.76 for the visitation network and 0.53 for the pollinator importance network, indicating greater specialization in the importance network. The mean number of bat species shared by pairs of plant species was significantly greater in the visitation network (4.1 species) than in the importance network (1.8 species) ($t = 5.8$, $df = 22$, $P < 0.001$). Similarly, the mean number of plant species shared by pairs of bat species was greater in the visitation network (3.5 species) than in the importance network (1.1 species) ($t = 6.3$, $df = 39$, $P < 0.001$). Additionally, niche overlap between pairs of species was significantly less in the pollinator importance network than in the visitation network for both bat species (0.21 vs. 0.58; $t = 2.7$, $df = 36$, $P = 0.01$) and plant species (0.59 vs. 0.89; $t = 3.6$, $df = 16$, $P = 0.002$). Pianka's index values near 0 indicate little niche overlap and values near 1 indicate large niche overlap (Dormann et al. 2008).

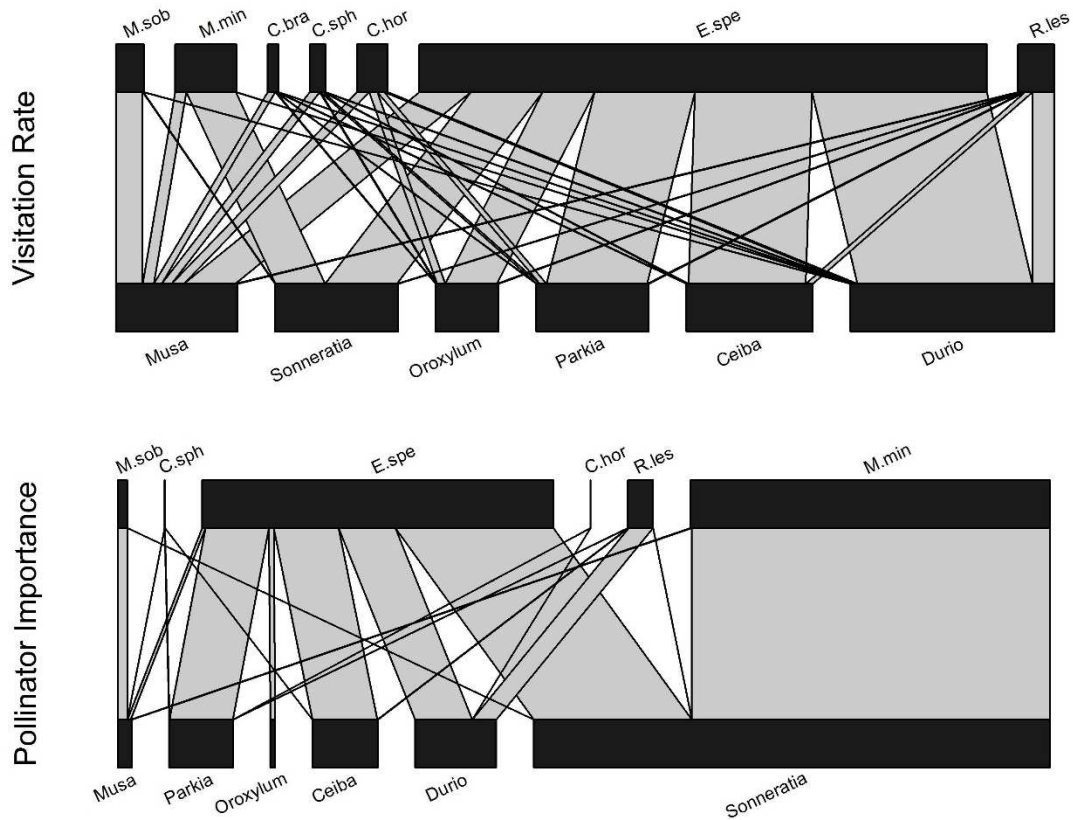


FIGURE 7. Pollination networks generated using visitation rates alone (top) versus pollinator importance values (bottom) of different bat species. Pollinator importance here is the product of visitation rate and pollen load. In each plot, bat species are listed along the top and plant species along the bottom. The widths of the bars connecting bat and plant species are proportional to the strength of the interaction. Bat species: *C.bra*, *Cynopterus brachyotis*; *C.hor*, *Cynopterus horsfieldii*; *C.sph*, *Cynopterus sphinx*; *E.spe*, *Eonycteris spelaea*; *M.min*, *Macroglossus minimus*; *M.sob*, *Macroglossus sobrinus*; *R.les*, *Rousettus leschenaulti*. Plant species: *Ceiba*, *C. pentandra*; *Durio*, *D. zibethinus*; *Musa*, *M. acuminata*; *Oroxylum*, *O. indicum*; *Parkia*, *P. speciosa* and *P. timoriana*; *Sonneratia*, *S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*. Note that species names are ordered to minimize overlap among connecting bars, and therefore differ between the two networks.

DISCUSSION

As predicted, potential pollinator importance varied by both bat species and plant species within our paleotropical study system. The nectar-specialist bat species, in general, were more important pollinators than the primarily frugivorous bat species as expected. We also found that bat pollen loads (i.e., potential pollen transfer efficiency) accounted for more variation in pollinator importance than did visitation

rate, and we further propose that bat pollen load size is correlated with floral design, specifically, stamen number and exertion. Because differences in bat pollen load dictated pollinator importance in our study system, using visitation frequency alone did not provide an accurate representation of which bat species were the important pollinators. Overall, our study illustrates how floral visitors differentially contribute towards pollination success, and highlights the variability that can occur even within a “classic” pollination syndrome.

Pteropodid bats as pollinators

For all six plant species examined, *E. spelaea* was an important pollinator due to both the “quantity” and “quality” components of pollinator importance. This cave-roosting bat species is very abundant in Southeast Asia (Start & Marshall 1976; Stewart et al. 2014) and it was the most frequent visitor to all of our study plants. Indeed, we caught seven times more individuals of *E. spelaea* than any other bat species (Appendix IV). Pollen loads on *E. spelaea* were also very high (Figure 5, 6) as this species is exclusively nectarivorous (Bumrungsri et al. 2013).

The two other nectar-specialist bat species were also important pollinators; *M. minimus* for *Sonneratia* flowers and *M. sobrinus* for *M. acuminata* flowers. Even though we caught fewer *Macroglossus* individuals than *E. spelaea* (Appendix IV), the *Macroglossus* species exhibited high floral constancy and were regular visitors to their preferred plant species. *Macroglossus sobrinus* is a tree-roosting species that lives near and specializes on *M. acuminata* flowers (Start & Marshall 1976). In contrast, *M. minimus* roosts in mangroves along the coast and specializes on

Sonneratia flowers (Start & Marshall 1976), although they expand inland and forage on *M. acuminata* flowers in areas where *M. sobrinus* is absent (Winkelmann et al. 2003). Given that each *Macroglossus* species specializes on a single flower species, pollen loads for their preferred plant species are significantly greater than those found on generalist *E. spelaea* (Figure 6).

Of the four frugi-nectarivorous bats species that we studied, only *R. leschenaulti* was found to be an important pollinator. This finding is consistent with their different morphologies; the muzzle of *R. leschenaulti* is considerably longer and narrower than those of *Cynopterus* (Francis 2008), suggesting a greater reliance on floral resources. The three *Cynopterus* species never visited flowers often enough, or carried substantial enough pollen loads, to be considered true pollinators of the plants in our study area. *Rousettus leschenaulti*, however, was an important pollinator of *D. zibethinus* due to the high pollen loads that individuals carried (Figure 6). Given the economic importance of durian in Southeast Asia (Bumrungsri et al. 2009), it would be useful in the future to compare temporal variation in pollinator importance of *E. spelaea* and *R. leschenaulti*, both within a season and across years.

Variation in pollinator importance

Potential pollinator importance values of the main pollinator, *E. spelaea*, varied nearly 80-fold across our plant study species (PI values were lowest for *O. indicum* and highest for *Sonneratia*). This immense variation is due primarily to differences in pollen loads, which varied 75-fold, rather than differences in visitation rate, which only varied 3-fold. All of our plant study species produce copious pollen,

consistent with the bat pollination syndrome (Faegri & van der Pijl 1966; Muchhala & Thomson 2010), so these differences in pollen loads are likely due to how and where pollen is placed on the bat, rather than total pollen production. In general, the number of pollen grains placed on a bat increased with stamen number and stamen exertion, reflecting the degree of specialization in floral design (Raven et al. 2005).

Specifically, the generalized brush-like morphology of *Sonneratia* flowers have highly exposed, abundant stamens (i.e., polyandrous) and deposit pollen over a large area of the bat, resulting in high pollen loads (hundreds of pollen grains; Figure 6). In contrast, the specialized, bilaterally symmetrical, tubular morphologies of *M. acuminata* and *O. indicum* flowers have only 4-5 stamens (i.e., oligandrous) which are recessed within the corolla and deposit pollen on very precise locations on the bat, resulting in small pollen loads (< 45 pollen grains; Figure 5, 6). The floral designs of *C. pentandra* and *D. zibethinus* fall along the middle of the generalized-specialized spectrum, resulting in intermediate pollen loads (40-150 pollen grains; Figure 5, 6). They produce clusters of single flowers exhibiting radial symmetry (characteristic of generalized flowers; Fenster et al. 2004; Sargent 2004), yet are oligandrous (a characteristic of specialized flowers; Stebbins 1951). Thus, differences in pollen load appear to reflect different strategies for achieving successful pollination: flowers can have fewer anthers and place pollen precisely, have more anthers and deposit pollen broadly, or some intermediate combination.

Bat pollen loads, our measure of pollen transfer efficiency, also varied greatly among bat species visiting the same flower species. Work on flower-visiting insects indicates that larger species are often more effective pollinators since they are more

likely to contact floral reproductive structures (Armbruster 1985; Kandori 2002; Sahli & Conner 2007; Schmidt-Adam et al. 2009). However, this pattern does not appear in our system since the landing behavior of bats on flowers generally ensures substantial contact between both parties, and even the smallest bat species are large enough to contact stigmas and anthers with every visit (Baker and Harris 1959; Itino et al. 1991; Bumrungsri et al. 2008; 2009; Acharya et al. 2015; A. Stewart, pers. obs.).

Furthermore, the number of pollen grains carried on a bat's body was not correlated with bat body size (Pearson's correlation coefficient, $r = -0.09$, $P = 0.28$, $n = 1,227$ bats). Instead, variation in pollen loads carried by bat species may be due to differences in how bats interact with flowers (Tschapka 2003; Frick et al. 2013), or differences in grooming behavior (Thomson 1986; Flanagan et al. 2009). Thus, more behavioral work is necessary to identify the cause of differential pollen loads carried across bat species.

Our pollinator importance estimates reflect an average across the entire year, yet the relative importance of different visitors likely varies both temporally (Kandori 2002; Wiggam & Ferguson 2005) and spatially (Fenster & Dudash 2001; Moeller 2005; Reynolds et al. 2009). Pantropical *C. pentandra* clearly demonstrates the spatial variation that can occur, as its flowers are visited by different nectar-feeding bat species worldwide (Baker & Harris 1959; Gribel et al. 1999; Singaravelan & Marimuthu 2004; Lobo et al. 2005). Temporal variation within our system is probably not as prominent as in insect-pollinated systems (Kandori 2002; Wiggam & Ferguson 2005), given that bats are long-lived and their population sizes fluctuate less than most insect populations. Temporal changes, however, may occur with diet shifts. For

example, the importance of *E. spelaea* may decrease for steady-state plants (e.g., *M. acuminata* and *O. indicum*) during months when big-bang plants (e.g., *C. pentandra* and *D. zibethinus*) are flowering. Examining visitation rates and pollen loads at a finer resolution could illuminate how potential pollinator importance varies spatially and temporally.

Caveats when using pollinator importance to predict fertilization success

Our measure of pollinator importance used pollinator pollen load as an estimate of pollen transfer effectiveness. While prior studies have noted that pollinator pollen loads may not reflect the amount of pollen deposited on floral stigmas (Kandori 2002; Sahli & Conner 2007), we observed a strong correlation between the two measures (Appendix VI), consistent with an experiment using neotropical nectar bats (Muchhala & Thomson 2010). Although we tested the pollen transfer effectiveness of only three of the bat-pollinated plant species owing to their availability, this strong relationship likely holds for other plants species as well. Notably, bat fur has proven effective at transferring pollen grains of both neotropical (Muchhala & Thomson 2010) and paleotropical plant species (Appendix VI). In our current study, the pollen loads on bats were possibly underestimated, as pollen may have fallen off while handling the bats. Thus, our measures of potential pollinator importance should be considered as conservative estimates.

While our measure of pollinator importance does not directly extrapolate to fertilization success, prior work suggests pteropodid bats deposit enough pollen grains per visit to fertilize most ovules in a flower. For example, a number of studies

(Srithongchuay et al. 2008; Acharya et al. 2015; Stewart & Dudash 2016) have shown that the number of pollen grains deposited per stigma per visit matches or exceeds the number of ovules per flower in *C. pentandra* (~208 pollen grains per visit, ~220 ovules), *D. zibethinus* (~50 pollen grains per visit, 25 – 35 ovules), and *O. indicum* flowers (>1,000 pollen grains per visit, ~300 ovules). Studies of fertilization success in Old World bat-pollinated plant species have confirmed the pollination effectiveness of *E. spelaea* (Srithongchuay et al. 2008; Bumrungsri et al. 2009; Acharya et al. 2015). However, our estimates of pollinator importance also reveal that the contributions of *M. minimus*, *M. sobrinus*, and *R. leschenaulti* should be further investigated.

Pollination networks within a community

Most studies examining flower-visiting insects report that pollinator importance values are dictated by visitation rates, rather than pollen transfer effectiveness (Motten et al. 1981; Pettersson 1991; Olsen 1997; Wiggam & Ferguson 2005; Sahli & Conner 2007; but see Schemske and Horvitz 1984; Armbruster et al. 1989). However, in our study system, pollinator importance is driven primarily by pollen transfer effectiveness, i.e., bat pollen loads. Thus, the pollination networks generated from visitation rate data versus pollinator importance values present very different results. The visitation network shows considerable overlap, indicating that species are functionally redundant. In contrast, the pollinator importance network reveals that plant-pollinator interactions are fairly specialized, with only one or two bat species pollinating each plant species (Figure 7). For example, the three

Cynopterus species appear to pollinate several plants according to the visitation network, yet their impact is negligible in the importance network. These findings confirm that pollinator effectiveness is more important than visitation rate in determining the true pollinators within our study system (Fenster et al. 2004).

Conclusions

This study has demonstrated the great variation in potential pollinator importance occurring within the paleotropical bat pollination syndrome. Pollinator importance was influenced more by pollen transfer effectiveness than by visitation rate, which differs dramatically from most insect-pollinated systems (Motten et al. 1981; Pettersson 1991; Olsen 1997; Wiggam & Ferguson 2005; Sahli & Conner 2007). Specifically, we confirm that two nectarivorous species (*M. minimus* and *M. sobrinus*) specialize on specific plant species, while another nectarivorous species (*E. spelaea*) broadly pollinates a number of night-blooming plant species. We thus provide evidence for the prediction that specialist nectarivores contribute more to plant reproductive success than opportunistic or generalist visitors, which has received mixed support in other systems (Motten et al. 1981; Tschapka 2003; Larsson 2005; Moeller 2005; Frick et al. 2013). Ultimately, plant-pollinator interactions can vary greatly, even within a pollination syndrome, and broad generalizations fail to capture the variability that occurs in nature. It is therefore informative to measure both the visitation frequency and pollen transfer effectiveness of flower-visiting animals when assessing plant-pollinator interactions in nature.

Chapter 4: Are paleotropical, long-lived, resident nectarivorous bats reliable pollinators?

ABSTRACT

Multi-year studies can provide valuable information on the temporal variability or stability of important plant-pollinator interactions. To date, the majority of long-term pollination studies have focused on insect, migratory bat, and migratory bird pollinators. Such studies report temporally variable pollination interactions across years, which is unsurprising given that the abundances of insects and migratory animals can fluctuate dramatically. In contrast, we might expect the abundances of tropical, long-lived, resident nectarivorous bat species to be more stable, allowing them to be more consistent pollinators across years. Here we focus on the understudied nectarivorous bats (Pteropodidae) that pollinate many night-blooming native and agricultural plant species in the Old World tropics. We explored the hypothesis that pteropodid bats are reliable pollinators by quantifying their interactions with four bat-pollinated plant species across three years in southern Thailand. Estimates of pollinator importance values (the product of visitation rate and pollen transfer effectiveness) varied little across the three years, suggesting that Old World nectar bats are reliable pollinators. Our results suggest the need for additional multi-year studies of these important pollinators, particularly given their importance in crop production and the maintenance of ecosystem biodiversity within a mixed agricultural-forest landscape.

Key words: bat pollination, *Musa acuminata*, nectar bat, Old World, *Oroxylum indicum*, *Parkia speciosa*, pollinator importance, Pteropodidae, *Sonneratia*, temporal variation

INTRODUCTION

Pollination systems span a continuum from highly specialized to very generalized (Robertson 1928; Faegri & van der Pijl 1966; Johnson & Steiner 2000; Waser & Ollerton 2006). Such wide variation has stimulated discussion over the prevalence of specialized versus generalized systems (Waser et al. 1996; Fenster et al. 2004), the factors that promote each type of system (Gómez & Zamora 2006; Armbruster & Muchhala 2009; Muchhala et al. 2010), and the consequences of having specialized versus generalized pollination systems (Aizen et al. 2002; Gómez et al. 2007). When assessing pollination systems, it is particularly important to distinguish true pollinators from other floral visitors that do not transfer pollen (Inouye 1980; Fenster et al. 2004). Thus, pollinator importance (the product of visitation rate and pollen transfer effectiveness) can provide a more accurate assessment of the pollinating community than visitation rate alone (Stebbins 1970; Schemske & Horvitz 1984; Fenster et al. 2004).

Numerous authors have stressed the importance of multi-year studies, as a pollinator's contribution to plant reproductive success can vary dramatically across years (Pleasants & Waser 1985; Herrera 1988; Sahley 1996; Fenster & Dudash 2001; Fleming et al. 2001; Alarcón et al. 2008; Reynolds et al. 2009; but see Gibson et al. 2006; Sahli & Conner 2007). For example, Petanidou et al. (2008) found that many

plant species appear to have specialized pollination systems in one year, yet are visited by different pollinators across years, such that they exhibit generalized pollination systems at the multi-year scale. It is not particularly surprising that most studies document wide temporal variation in pollination interactions, given that such studies have focused on insect, migratory bat, and migratory bird pollinators. Insect populations are known to be highly variable, fluctuating from summer to winter in temperate areas (Hails 1982; Wolda 1988) and from wet to dry season in tropical areas (Wolda 1978, 1988; Denlinger 1980; Pinhiero et al. 2002). Migratory bats (Sahley 1996; Valiente-Banuet et al. 1996; Fleming et al. 2001; Molina-Freaner & Eguiarte 2003) and birds (Pleasants & Waser 1985; Fenster & Dudash 2001) can also be unreliable pollinators, particularly when the arrival dates of these migrating species do not coincide with the plant's blooming period (Pleasants & Waser 1985). Noticeably absent from the literature are multi-year studies of tropical, long-lived, resident pollinators, such as Old World flower-visiting bats (Pteropodidae).

Are pteropodid bats consistent pollinators across years? Several factors suggest that they are highly reliable pollinators. (1) Flowers of Old World bat-pollinated plant species open for a single night, and thus are not accessible to diurnal flower visitors (Bumrungsri et al. 2009; Faegri & van der Pijl 1966). (2) Nectar production peaks when nectar-specialist bat species are most active in their foraging (Stewart et al. 2014). (3) There is a poor morphological fit between large bat-pollinated flowers and small flower-visiting insects, such that flowers visited by nocturnal insects do not produce fruit (Bumrungsri et al. 2008, 2009). (4) Genetic self-incompatibility (Bumrungsri et al. 2008, 2009; Srithongchuay et al. 2008)

precludes a reproductive assurance strategy found in many self-compatible species (Fenster & Martén-Rodríguez 2007). This suite of circumstances indicates that Old World bat-pollinated plants have not evolved a back-up system of secondary pollinators or autogamous selfing. We explored the hypothesis that pteropodid bats are reliable pollinators by comparing estimates of pollinator importance values for four bat-pollinated plant species across three years in southern Thailand.

METHODS

Study species

The most common flower-visiting bat species in our study area (Phatthalung, Satun, Songkhla, and Trang provinces, Thailand) include three nectar-specialist bat species (*Eonycteris spelaea*, *Macroglossus minimus*, and *M. sobrinus*) and four primarily frugivorous bat species (*Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*, and *Rousettus leschenaulti*) (Stewart et al. 2014). The nectar-specialist species have long muzzles and tongues (which are characteristic of nectarivores; Freeman 1995), and forage almost exclusively on floral resources (Marshall 1985; Bumrungsri et al. 2013; Stewart et al. 2014). In contrast, the primarily frugivorous species have powerful jaws and well-developed molars (Francis 2008); while they mainly forage on fruits, they have also been observed foraging at flowers (Marshall 1985; Bumrungsri et al. 2007; Stewart et al. 2014).

We focused on four bat-pollinated plant taxa for this study: *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, and *Sonneratia* species. These species flowered consistently during our study period (May-August), and are also the major food

resources for nectar-feeding bats during this period (Bumrungsri et al. 2013; see also Chapter 2). While other plant species also flower during this period, we rarely found their pollen on the bats' bodies (Appendix V), suggesting that pteropodid bats are not regular visitors to their flowers. *Musa acuminata* (Musaceae; banana) is a temporally dioecious (Andersson 1998) herbaceous plant species ubiquitous throughout southeast Asia (Itino et al. 1991). Each shoot produces a single inflorescence that displays 15-40 flowers per night for multiple weeks (Itino et al. 1991), and flowering individuals can be found year-round (Gould 1978). While cultivated bananas are parthenocarpic, wild plants require pollination to set fruit (Andersson 1998). *Oroxylum indicum* (Bignoniaceae; Indian trumpet flower) is a self-incompatible tree species found throughout much of Asia (Srithongchuay et al. 2008). Flowers are hermaphroditic; only a few open per night, but flowering trees can be found year-round (Srithongchuay et al. 2010). *Parkia speciosa* (Fabaceae; petai or sator) is a self-incompatible tree species that can have up to 70 capitula open per night (Bumrungsri et al. 2008). Capitula contain 2,500-4,000 flowers, and inflorescences are either hermaphroditic or functionally staminate (Bumrungsri et al. 2008). *Sonneratia* (Lythraceae) is a paleotropical mangrove genus with hermaphroditic flowers (Tomlinson 1994) and flowering tends to occur in flushes (Start & Marshall 1976). Four species are commonly found in our study area (*S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*; Bureau of Mangrove Resources Conservation 2009). (See Appendix III for detailed descriptions of plant study species.)

Data collection

In order to estimate pollinator importance, we collected data on bat visitation rates and pollen loads across four provinces in southern Thailand (Phatthalung, Satun, Songkhla and Trang). We mist-netted at our plant study species for 18 nights in 2011, 27 nights in 2013, and 20 nights in 2014 during May-August of each study year (but we did not mist-net at *Sonneratia* trees in 2011). Mist-nets were placed in the canopy as close as possible to open flowers, and visitation rates for each bat species were determined from the number of bats netted per hour. We also collected pollen loads from each netted bat to estimate pollen transfer effectiveness. Each bat was uniformly swabbed along the head, chest, and ventral side of the wings with fuchsin glycerin gelatin, and pollen grains adhering to the gel were then fixed on a slide (Beattie 1972; Stewart & Dudash 2016). A compound light microscope was used to count and identify all pollen grains by comparison against a reference collection. Pollinator importance for each bat species at each focal plant species was calculated as the product of visitation rate (of bat species i at plant species j) and pollen load (of pollen species j on bat species i) (Moeller 2005; Reynolds et al. 2009; see also Chapter 3).

Statistical analysis

We compared differences in pollinator importance by estimating bootstrapped means and 95% confidence intervals (95% CI) following Reynolds and Fenster (2008). Briefly, we resampled visitation rate and pollen load 10,000 times, and calculated the mean visitation rate and mean pollen load for each iteration. We then multiplied the bootstrapped means of visitation rate and pollen load to generate

10,000 values of pollinator importance. We calculated the mean of these 10,000 values, sorted them numerically, and took the 250th and 9,750th observations as estimates of the 95% confidence interval. Bat species with non-overlapping 95% CI are significantly different ($P < 0.05$). All simulations were performed in R 3.1.1 (R Development Core Team).

RESULTS

In general, there was little variation in pollinator importance values across years (Figure 8; see Appendix VII for variation in visitation rates and pollen loads across years). *Macroglossus sobrinus* was an important pollinator of wild banana (*M. acuminata*) across all three years. *Eonycteris spelaea* and *M. minimus* contributed to some pollination of *M. acuminata* in 2011, but were not important in 2013 and 2014. For *O. indicum*, *E. spelaea* was the most important pollinator across all three years, but importance values varied and were highest in 2011 and lowest in 2014. On the other hand, *E. spelaea* was a consistently important pollinator across all three years for *P. speciosa*. Finally, *Sonneratia* had two main pollinators across the two years that we sampled, *M. minimus* and *E. spelaea*. The four primarily frugivorous bat species (*C. brachyotis*, *C. horsfieldii*, *C. sphinx*, and *R. leschenaulti*; Stewart et al. 2014) either never visited the study plant species and/or never carried pollen of the study plant species during the study period, and thus have pollinator importance values of zero across all three years.

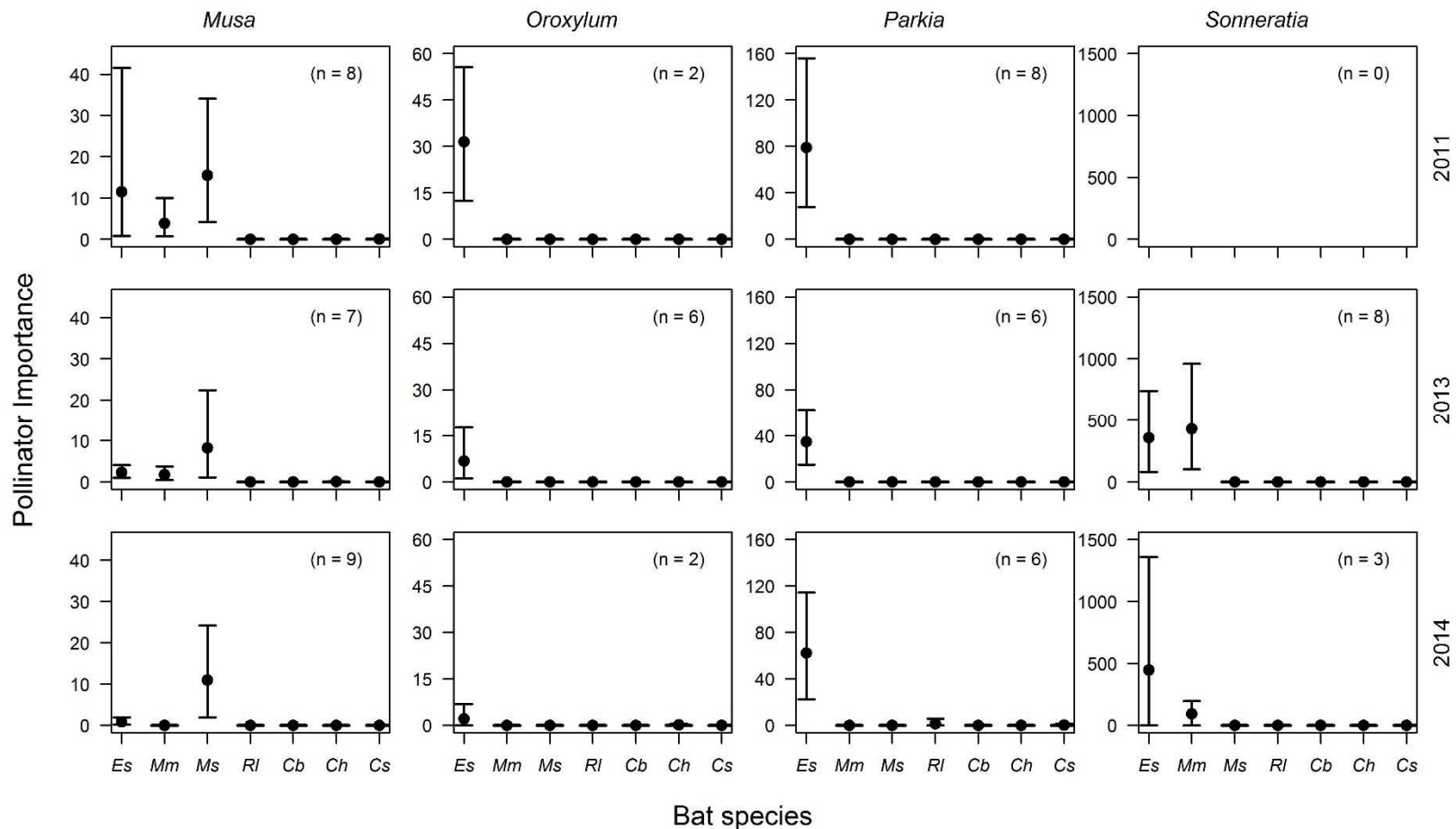


FIGURE 8. Pollinator importance (visitation rate x bat pollen load; bootstrapped means and 95% confidence intervals) of pteropodid bats in southern Thailand across four bat-pollinated plant species and three years. Bat species with non-overlapping 95% confidence intervals are significantly different. Sample sizes in parentheses in the top right corner of each plot indicate the number of nights mist-netted. Plant species in columns (left to right): *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*). Data was not collected at *Sonneratia* trees in 2011. Bat species along x-axes (left to right): *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Rousettus leschenaulti*, *Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*.

DISCUSSION

The large majority of flowering plant species depend on animals for reproductive success (Ollerton et al. 2011), yet these pollination interactions can vary greatly both temporally and spatially (Herrera 1988; Fenster & Dudash 2001; Alarcón et al. 2008; Petanidou et al. 2008; Dupont et al. 2009; Reynolds et al. 2009). Thus, to fully understand a plants species' pollination system and its long-term resiliency, it is necessary to collect visitation and pollen transfer data across multiple years and locations. Most multi-year studies have examined insect, migratory bat, and migratory bird pollinators, which typically demonstrate high temporal variability (Pleasants & Waser 1985; Herrera 1988; Fenster & Dudash 2001; Fleming et al. 2001; Petanidou et al. 2008; but see Gibson et al. 2006; Sahli & Connor 2007). However, the results of this study suggest that tropical, long-lived, resident nectarivorous bats can be reliable pollinators to their perennial, long-lived host plant species across multiple years (Figure 8).

This constancy is seen most clearly with *M. sobrinus* at wild banana plants (*M. acuminata*) and with *E. spelaea* at *P. speciosa* trees, where pollinator importance values were remarkably similar across all three years. The mangrove genus *Sonneratia* had two main pollinators (*M. minimus* and *E. spelaea*), which also had consistent importance values across years. Results for *O. indicum* were most variable; while *E. spelaea* was consistently the most important pollinator, importance values varied broadly across the three years, although this variation may be due in part to the low numbers of nights mist-netted (2 nights each in 2011 and 2014).

While most multi-year studies of bat pollination have examined migratory nectar bats, their findings are consistent with our prediction that resident bats are more reliable pollinators than migratory bats (Valiente-Banuet et al. 1996; Fleming et al. 2001; Molina-Freaner & Eguiarte 2003; Rivera-Marchand & Ackerman 2006). Indeed, multiple authors have postulated that plants occurring within the range of resident nectar bats are highly specialized for bat pollination, while plants that are only visited by migrating bats tend to exhibit generalized pollination systems that include diurnal bird and insect pollinators (Valiente-Banuet et al. 1996; Molina-Freaner & Eguiarte 2003; Rivera-Marchand & Ackerman 2006). Fleming et al. (2001) found that the abundance of a neotropical migratory bat species varied throughout the 8-year study, and that diurnal pollinators were more reliable. Another study of a cactus species in Peru found that the only bat pollinator was a rare endemic species, and when bat abundance was low, hummingbirds and diurnal insects were the major pollinators (Sahley 1996). All of these studies report variable bat abundance and secondary diurnal pollinators. However, in our study area, nectarivorous bats are year-round residents (bat capture rates are consistent across months, see Appendix VIII for details) and bat-pollinated plant species appear not to have evolved a back-up system of diurnal pollinators, given that their flowers last for a single night and drop before dawn.

Furthermore, paleotropical bat-pollinated plant species are predominantly self-incompatible (Bumrungsri et al. 2008, 2009; Srithongchuay et al. 2008), which precludes autogamous selfing as a reproductive assurance mechanism. Fenster and Martén-Rodríguez (2007) found that many animal-pollinated plant species also

exhibited delayed autogamous selfing. They proposed that autogamy ensures reproductive success when plants face variable pollinator activity (Kalisz & Vogler 2003; Kalisz et al. 2004). Conversely, consistent pollinator activity may relax selective pressures that favor autogamy (Moeller 2006). If bat pollinators are highly consistent across years, paleotropical bat-pollinated plant species may not experience large fitness costs despite lacking a reproductive assurance mechanism such as autogamous selfing.

Our findings in this Old World system suggest that pteropodid bats are reliable pollinators across years for the plant species flowering in southern Thailand during the study period. While we predict that tropical, long-lived, resident pollinators (including both bat and bird species) are inherently more reliable than temperate, short-lived, and/or migratory pollinators, this phenomenon may be unique to bats. Ramirez (2004) found that diurnally-pollinated plants are usually polyphilous, while nocturnally-pollinated plants are mostly monophilous or oligophilous. This finding was attributed to the low diversity of nocturnal pollinators relative to diurnal pollinators. Thus, even tropical, long-lived, resident bird pollinators may be unreliable if competition with other diurnal floral visitors shapes their foraging behavior and visitation rates. Further work on resident nectarivorous bats and birds (in both the New and Old World tropics) will help determine if these long-lived, non-migratory pollinators are indeed highly reliable across years. Such studies are particularly valuable given the number of economically (e.g., agave, durian) and environmentally (e.g., *Sonneratia* mangroves) important plant species dependent on these pollinators (Kunz et al. 2011; Bumrungsri et al. 2013).

Chapter 5: Differential pollen placement on an Old World nectar bat increases pollination efficiency

ABSTRACT

Plant species that share pollinators are potentially subject to non-adaptive interspecific pollen transfer, resulting in reduced reproductive success. Mechanisms that increase pollination efficiency between conspecific individuals are therefore highly beneficial. Many nocturnally flowering plant species in Thailand are pollinated by the nectar bat *Eonycteris spelaea* (Pteropodidae). This study tested the hypothesis that plant species within a community reduce interspecific pollen movement by placing pollen on different areas of the bat's body. Using flight cage trials, pollen transfer by *E. spelaea* was compared between conspecific versus heterospecific flowers across four bat-pollinated plant genera. Pollen from four locations on the bat's body was also quantified to determine if pollen placement varies by plant species. It was found that *E. spelaea* transfers significantly more pollen between conspecific than heterospecific flowers, and that diverse floral designs produce significantly different patterns of pollen deposition on *E. spelaea*. In the Old World tropics, differential pollen placement is a mechanism that reduces competition among bat-pollinated plant species sharing a common pollinator.

Key words: Chiropterophily, floral morphology, interspecific pollen transfer, pollen placement, bat pollination, Pteropodidae, Thailand, *Ceiba pentandra*, *Durio zibethinus*, *Musa acuminata*, *Parkia speciosa*, *Parkia timoriana*

INTRODUCTION

Early naturalists noted that sympatric plant species likely compete for pollinators, and that such competition could be strong enough to cause species to diverge in habitat affinity, flowering time, pollinator identity or floral morphology (Robertson 1895). Since then, competition for pollinators and the negative effects of interspecific pollen transfer have been well documented in a number of systems (reviewed in Waser 1983; Morales and Traveset 2008). For example, female fitness can be reduced through stigma or style clogging (Waser 1978; Waser and Fugate 1986; Morales and Traveset 2008), while male fitness can be reduced by pollen loss to heterospecific flowers (Inouye et al. 1994; Murcia and Feinsinger 1996; Muchhala and Thomson 2012). A pollinator moving between different plant species can therefore negatively impact the fitness of each plant species simultaneously (e.g. Waser 1983).

Pollination syndromes, or convergently-evolved suites of floral characteristics attractive to specific groups of flower-visiting animals (Faegri and van der Pijl 1966; Fenster et al. 2004, 2015), reflect pollinator-mediated selection and result in visitation fidelity of certain pollinator species while deterring the visits of others (Castellanos et al. 2004). For example, the bat pollination syndrome includes many traits that attract nectarivorous bats (nocturnal anthesis, copious nectar production, pale flowers that are easily visible at night; Faegri and van der Pijl 1966; Fleming et al. 2009), yet deter, for instance, diurnal nectarivores. However, flowering plant species that share the same pollination syndrome can also share the same pollinators, which increases the risk of interspecific pollen transfer and reduced plant reproductive success.

One mechanism that can reduce interspecific pollen transfer is differential pollen placement caused by variation in floral morphology among sympatrically flowering species. By placing pollen on different areas of the pollinator, plant species can limit heterospecific pollen movement even when sharing the same pollinators. Differential pollen placement has been studied in a number of pollination systems, including diurnal bees (Campbell and Motten 1985; Armbruster et al. 1994, 2014; Ollerton et al. 2007; Yang et al. 2007; Flanagan et al. 2009; Waterman et al. 2011; Huang and Shi 2013; Huang et al. 2015) and hummingbirds (Waser 1978; Feinsinger and Tiebout 1991; Murcia and Feinsinger 1996; Kay 2006) and nocturnal nectar bats in the neotropics (Howell 1977; Tschapka et al. 2006; Muchhala 2007; Muchhala and Potts 2007; Muchhala and Thomson 2012). However, these studies rarely demonstrate whether differences in pollen placement on the pollinators actually promote conspecific pollen transfer and reduce interspecific pollen transfer.

Surprisingly, differential pollen placement has never been examined in paleotropical nectar bats (Pteropodidae), despite this family being an attractive study system for two main reasons. First, several pteropodid species have broad diets (Marshall 1985; Bumrungsri et al. 2013) and, compared with their neotropical counterparts, paleotropical bat-pollinated plants are more likely to be pollinated by opportunistic nectar-feeding bats (i.e. bats that predominantly consume non-floral resources) than nectar specialist bats (i.e. bats that primarily or exclusively consume floral resources; Fleming et al. 2009). A mere 13 % of bat-pollinated genera in the New World are visited by opportunistic nectar-feeding bats, compared with 92 % in the Old World (Fleming et al. 2009). We therefore expect high potential for

interspecific pollen transfer. Second, bat-pollinated plant species exhibit a striking diversity of floral designs, which suggests that the reproductive structures of these different flowers contact different areas of the bat's body.

To test whether the various floral designs of bat-pollinated plant taxa limit interspecific pollen movement in the paleotropics, we compared pollen transfer by the dawn bat (*Eonycteris spelaea*) between conspecific flowers and heterospecific flowers. Recent fieldwork has shown that *E. spelaea* is an effective pollinator that promotes pollination between conspecific flowers (Acharya et al. 2015); however, the mechanism limiting interspecific pollen transfer has not been empirically tested. Thus, to determine whether patterns of pollen placement differ by plant species commonly found within a community, we quantified the amount of pollen deposited on four locations of the bat's body. We predicted that *E. spelaea* would transfer more pollen between conspecific flowers than heterospecific flowers, because the variable floral designs would promote pollen placement on different areas of the bat. This study demonstrates a mechanism reducing competition among flowering plant species sharing a common pollinator in an understudied, Old World tropical system.

METHODS

Pollinator study species

Eonycteris spelaea (Pteropodidae) is a colonial, cave-roosting bat species with many adaptations for nectar-feeding, including an elongated muzzle and tongue (Freeman 1995) (Figure 9). We chose to focus on *E. spelaea* because it is the most common pollinator of many chiropterophilous plant species (Gould 1978; Bumrungsri et al.



FIGURE 9. *Eonycteris spelaea*, a common nectar bat and important pollinator in Thailand.

2008, 2009; Srithongchuay et al. 2008) and routinely forages on the flowers of several plant species within a single night (Bumrungsri et al. 2013).

Plant study species

We tested pollen transfer efficiency by *E. spelaea* among four bat-pollinated genera: *Ceiba pentandra* (silk-cotton), *Durio zibethinus* (durian), *Musa acuminata* (banana) and *Parkia* flowers of two species: *P. speciosa* (petai or sator) and *P. timoriana* (tree bean or riang) (Figure 10). These four plant genera account for 50–100 % of the diet of *E. spelaea* across all months of the year (Bumrungsri et al. 2013), and therefore are continually at risk of interspecific pollen transfer. They share many characteristics of the bat pollination syndrome (Faegri and van der Pijl 1966), including nocturnal

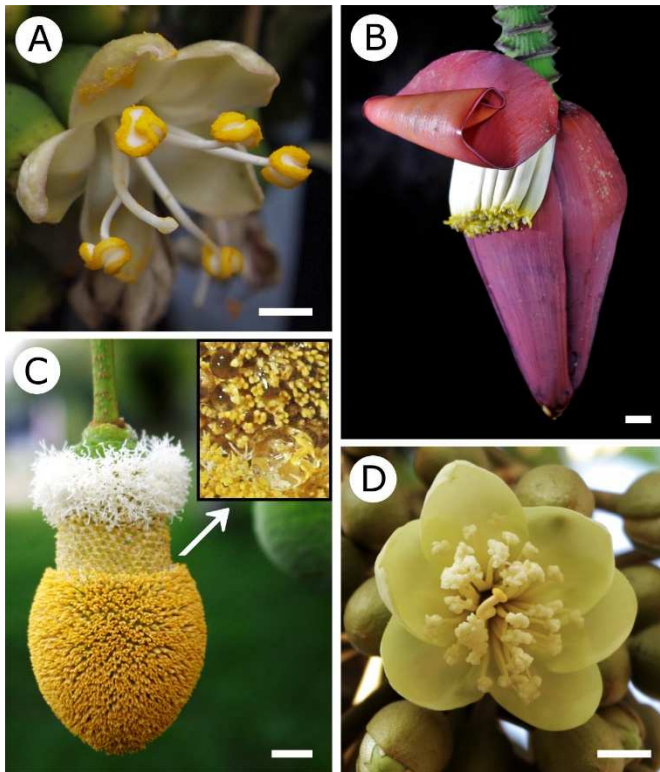


FIGURE 10. Floral designs of plant species pollinated by *Eonycteris spelaea* in Thailand. (A) *Ceiba pentandra*. Five anthers with yellow pollen surround a white stigma. (B) *Musa acuminata*. A red bract curls back to reveal two rows of white flowers. (C) *Parkia speciosa*. Drops of nectar produced by flowers at the neck of the inflorescence (inset) accumulate above pollen-bearing flowers at the base. (D) *Durio zibethinus*. Many stamens surround a pale yellow stigma in the center. Scale bars = 1 cm.

anthesis, abundant pollen that dehisces in early evening, and copious nectar production that steadily declines throughout the night (Elmqvist et al. 1992; Elangovan et al. 2000; Sripaoraya 2005; Vikas et al. 2009). The flowers of these plant species open for a single night, with corollas dropping by morning (Faegri and van der Pijl 1966; Itino et al. 1991; Srithongchuay et al. 2008; Bumrungsri et al. 2009).

Big bang species. *Ceiba pentandra* is found in both the New and Old World tropics, and is primarily bat-pollinated in both regions (Gribel et al. 1999; Singaravelan and Marimuthu 2004). The degree of self-compatibility in this hermaphroditic species varies greatly (Gribel et al. 1999; Lobo et al. 2005), but it has generally been described as self-compatible in the Old World tropics (Toxopeus 1948; Baker and Harris 1959). A single tree can produce hundreds of thousands of flowers (Gribel et al. 1999) presented in clusters along terminal branches

(Singaravelan and Marimuthu 2004) during a short, intense, highly synchronous flowering period (Gribel et al. 1999; Lobo et al. 2005). Flowering occurs during the dry season and varies geographically (Gribel et al. 1999; Lobo et al. 2005); in our study area, all flowering occurred between late November and mid-February (Chapter 2). *Durio zibethinus* is a hermaphroditic, self-incompatible tree species native to Southeast Asia (Bumrungsri et al. 2009). A single tree typically flowers for just 10 d, but can produce over 1000 flowers per night, which are produced in clusters along mature branches (Bumrungsri et al. 2009). Mass flowering is highly synchronous, and all trees in our study area flowered between March and April, consistent with Bumrungsri et al. (2009).

Steady-state species. Wild *Musa acuminata* is an herbaceous plant (Andersson 1998) native to Southeast Asia that requires pollination to set fruit (Itino et al. 1991). Each shoot produces a single inflorescence consisting of bracts covering two rows of flowers (typically 15–40 flowers; Itino et al. 1991). Each night, a bract folds back to expose the flowers, which then fall off by morning (Itino et al. 1991). This species is temporally dioecious; the first 1–30 hands of the inflorescence produce female flowers (which have functional stigmas and vestigial stamens), then 0–4 hands of sterile flowers, followed by 150–300 hands of male flowers (which have functional stamens and reduced, non-functioning stigmas; Pillay and Tenkouano 2012). Because of this temporal separation of reproductive functions, within-inflorescence selfing is very rare (Andersson 1998). Flowering wild *M. acuminata* individuals can be found year-round (Gould 1978; Sripaoraya 2005; Pillay and Tenkouano 2012; Chapter 2). *Parkia* is a pantropical genus (Baker and Harris 1957), and the paleotropical *P.*

speciosa and *P. timoriana* are both self-incompatible (Bumrungsri et al. 2008). Both species produce pendant, spherical inflorescences (i.e. capitula) that are either hermaphroditic or functionally staminate (Bumrungsri et al. 2008). Within each inflorescence, only flowers at the base of the capitulum are fertile; infertile flowers at the neck secrete nectar, while those at the top (where the capitulum connects to the peduncle) provide visual cues for pollinators (Bumrungsri et al. 2008) (Figure 10). Trees of both species can have up to 70 capitula open in a night, and flowering typically lasts 4–5 weeks (Bumrungsri et al. 2008). *Parkia speciosa* has an extended, asynchronous flowering period; while we observed flowering individuals year-round, the majority of flowering occurred from May to November (Bumrungsri et al. [2008] found most flowering to occur from April to October). In contrast, *P. timoriana* has a very short, synchronous flowering season from December to mid-January (Bumrungsri et al. 2008; Chapter 2). We used both *Parkia* species in order to extend the timeframe during which we could conduct experimental trials, and were comfortable pooling the data given their similarity in floral design and the comparable manner in which *E. spelaea* visits them (Bumrungsri et al. 2008).

Pollen transfer experiment

Adult *E. spelaea* were caught in mist nets (polyester, 38-mm mesh; Avinet Inc., Dryden, NY), given as much sugar water as they would consume, placed in cloth bags (following Kunz and Parsons 2009) for 20–24 h, and tested the following evening. This procedure ensured that individuals were similarly food-motivated during testing. Since we did not net at the same site twice, the probability of recapture

was extremely low (less than 0.1% of tagged bats are ever recaptured at a novel site; A. Stewart, unpubl. res.). Each day trials were conducted, we cut and gathered experimental flowers and inflorescences in the late afternoon. Flowers were cut before anthesis (to ensure that only virgin flowers were used) and the stems were kept in water until the start of the experiment. Trials began as soon as flower anthesis was complete and pollen had dehisced (1900–2000 h).

Since our experimental design required separate male (pollen donor) and female (pollen recipient) flowers, we removed anthers as needed from *C. pentandra* and *D. zibethinus* flowers to make them functionally female. Anthers were clipped after anthesis but before pollen dehiscence to minimize the risk of self-pollen falling on the stigmas (verified by examining control stigmas; data not shown). For *M. acuminata*, we could identify the sex of the inflorescence by visual examination of the exposed reproductive structures (female flowers have a large stigma and reduced stamens that lack anthers, male flowers have tiny stigmas and large anthers on long filaments). Since hermaphroditic *Parkia* inflorescences contain thousands of flowers and it was not practical to remove all of the anthers to create functionally female inflorescences, we only used *Parkia* inflorescences as pollen donors.

To quantify how much pollen *E. spelaea* moves between conspecific and heterospecific flower combinations, we released each individual bat into a 3 x 4 x 3 m flight chamber (assembled indoors to standardize lighting and weather conditions) containing two clusters of flowers spaced 1 m apart. Flowers were hung from the ceiling so they were suspended 2–3 m above floor level. One cluster contained only male flowers (pollen donors) and the other contained only female flowers (pollen

recipients). We used clusters of three to five flowers for *C. pentandra* and *D. zibethinus* and a single inflorescence for *M. acuminata* and *Parkia*. These floral arrangements mimic how the flowers are presented in nature. In each trial, the female flowers were initially covered with a plastic bag, a single bat was released into the flight chamber, and all feeding behavior was recorded for 30 min. If the bat fed at the male flowers, a hidden observer pulled a string, which removed the plastic bag from the female flowers. In conspecific treatments, the bat moved from male to female flowers of the same species. In heterospecific treatments, the bat moved from male to female flowers of different species. Observers remained outside the dimly lit flight chamber and viewed bats directly through a small window or with an infrared spotlight and a Sony Nightshot Camcorder. Trials ended as soon as the bat finished feeding at the female flowers, or at the end of the allotted 30 min, and each bat was used only once. Trials were conducted at multiple locations across central and southern Thailand (Phatthalung, Rayong, Satun, Songkla and Trang provinces) so that bats did not have to be transported far from where they were netted in the field.

For each trial in which a bat successfully visited both flower clusters, we collected stigmas from the female flowers. In the case of *M. acuminata*, where a single inflorescence had dozens of flowers, we randomly selected four stigmas. The stigmas were flattened between a microscope slide and cover slip, and then fixed with fuchsin glycerin gelatin (Beattie 1972). The fuchsin dyes pollen grains a bright magenta and facilitates pollen counting and identification to species level, which was accomplished with a compound light microscope at 100–400x magnification. Since

female flowers lack anthers, any pollen found on the stigmas was transferred by *E. spelaea* from the male flowers initially visited in the trials.

Pollen distribution on the pollinator

After each successful trial, we caught the bat with a hand-net and quantified the amount of pollen transferred onto four locations of the bat's body: the top of the head (crown), face, chest and the ventral side of one wing. Pollen was collected using fuchsin glycerin gelatin, as pollen grains readily adhere to the tacky gel. To standardize pollen collection, we dispensed solidified glycerin gelatin from a 1-mL syringe in increments of 0.1 mL per sample (Appendix IX). For each of the four areas on the bat's body, a separate 0.1-mL gel section was gently pressed into the bat's fur five times, and fixed on a slide. Pollen grains were identified to species and counted using a compound light microscope.

Statistical analysis

All analyses were performed with R 3.1.1 (R Development Core Team). To compare the amount of pollen transferred between conspecific flowers versus heterospecific flowers, we performed a generalized linear model (GLM, package glm). The response variable was the lognormal (ln)-transformed mean number of pollen grains per stigma (three or four stigmas were collected per trial, so we calculated the average pollen load per stigma per trial). The predictors were recipient flower species (*C. pentandra*, *D. zibethinus* or *M. acuminata*) and treatment type (conspecific or heterospecific). Model fit was assessed using histograms and q-q plots of the residuals, and variable

importance was determined with Akaike information criterion (AIC) scores (delta AIC < 2) and verified with nested likelihood ratio tests ($P < 0.05$). Contrasts comparing differences in treatment type within each recipient flower species were corrected with the sequential Bonferroni method (Holm 1979). We present back-transformed means and 95% confidence intervals (Cis) throughout the paper.

To determine if patterns of pollen placement on the bat's body vary by flower species, we used a generalized linear mixed model (GLMM, package glmer). We found that modelling the residuals with a Poisson distribution greatly underestimated the dispersion. As an over-dispersed Poisson is not available in the glmer package, we modelled the residuals with a normal distribution utilizing the lme4 package. The response variable was the ln-transformed number of pollen grains collected. The fixed factors were flower species (*C. pentandra*, *D. zibethinus*, *M. acuminata* or *Parkia* spp.), body part where pollen was collected (crown, face, chest or wing; however, only 3 parts were used in the analysis, as total pollen count was used as a covariate) and their interaction. Bat individual was included as a random factor and ln-transformed total pollen count (i.e. all pollen collected from the bat) was included as a covariate. Histograms and q-q plots of the residuals supported an appropriate fit for the normal approximation. Model comparisons were assessed with AIC scores (delta AIC < 2) and verified with nested likelihood ratio tests ($P < 0.05$). Contrasts comparing where different flower species deposited pollen on the bat were corrected with the sequential Bonferroni method (Holm 1979).

RESULTS

We tested 170 *E. spelaea* over 26 nights between February 2014 and January 2015. Only 72 bats successfully visited both donor and recipient flowers, and we therefore present the results from these trials only ($n = 8$ trials for each of the nine floral combinations tested).

Pollen transfer experiment

When comparing pollen transfer between different floral combinations ($n = 8$ trials for each combination), the model that best fitted our data included recipient flower species ($\chi^2_2 = 15.2$, $P = 0.01$) and treatment type ($\chi^2_1 = 120.3$, $P < 0.001$), but not their interaction ($\chi^2_2 = 2.45$, $P = 0.51$). [See Appendix X (A) for all models tested.] *Eonycteris spelaea* transferred significantly more pollen between conspecific flowers than heterospecific flowers for all flower species (pairwise comparisons with sequential Bonferroni correction, $P < 0.001$; Figure 11).

Pollen distribution on the pollinator

The model that best described pollen placement on *E. spelaea* included donor flower species ($\chi^2_9 = 132.0$, $P < 0.001$), location of pollen placement ($\chi^2_8 = 240.9$, $P < 0.001$) and their interaction ($\chi^2_6 = 116.4$, $P < 0.001$) as fixed factors; bat individual as a random factor; and ln-transformed total pollen count as a covariate. [See Appendix X (B) for all models tested.] The significant interaction between donor flower species and location of pollen placement revealed that the flowering plant species deposited pollen on different areas of the bat.

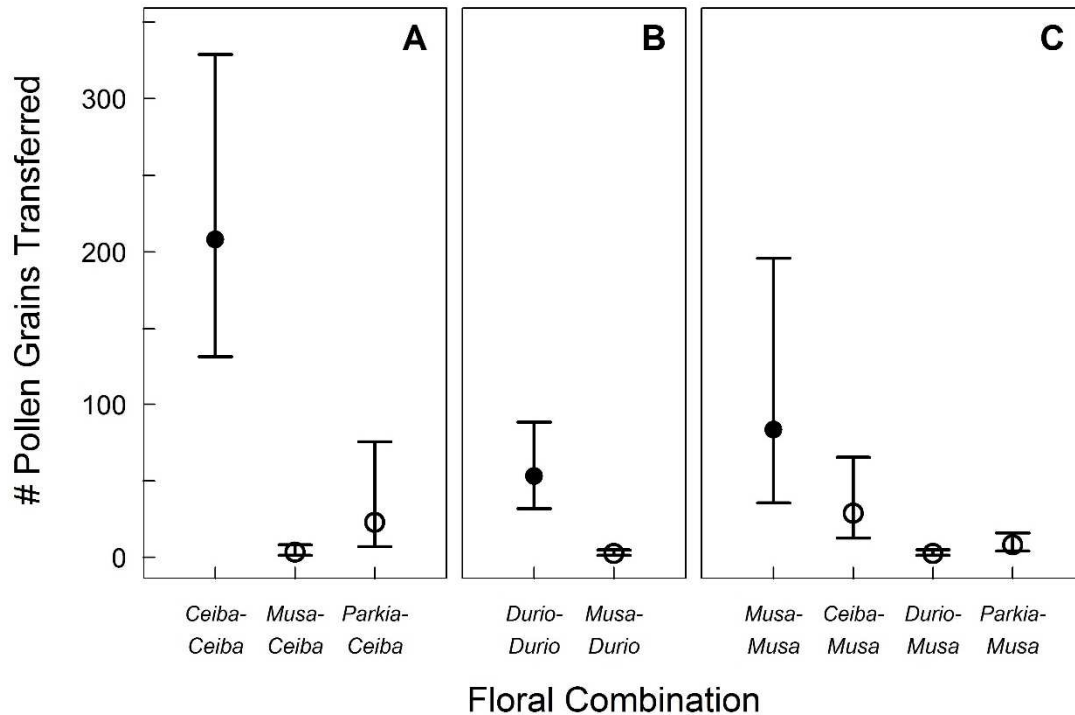


FIGURE 11. The average amount of pollen with 95% confidence intervals (using back-transformed values) transferred by *Eonycteris spelaea* to female (A) *Ceiba pentandra*, (B) *Durio zibethinus* and (C) *Musa acuminata* flowers by male flowers of different plant species. Significantly more pollen was transferred between conspecific flowers (closed circles) than heterospecific flowers (open circles) (GLM pairwise contrasts with sequential Bonferroni correction, $P < 0.001$). Floral combinations ($n = 8$ trials per floral combination) along the x-axis are listed with male flower species preceding female flower species (e.g. ‘*Musa–Ceiba*’ means pollen transferred from male *Musa acuminata* flowers to female *Ceiba pentandra* flowers). *Parkia* inflorescences (*P. speciosa* and *P. timoriana*) were only used as pollen donors (male flowers). *Durio–Ceiba* and *Durio–Parkia* combinations could not be tested because their flowering phenologies did not overlap.

Pairwise contrasts (with sequential Bonferroni correction) demonstrated that pollen deposition patterns were significantly different among all plant study species ($P < 0.001$), except between *C. pentandra* and *D. zibethinus* ($P > 0.9$).

Within each flower species, the ln-transformed number of pollen grains differed significantly among different areas of the bat’s body (multiple comparisons with sequential Bonferroni correction, $P < 0.001$; Figure 12). Our model estimates revealed that *C. pentandra* flowers ($n = 16$ bats) deposited the greatest amount of

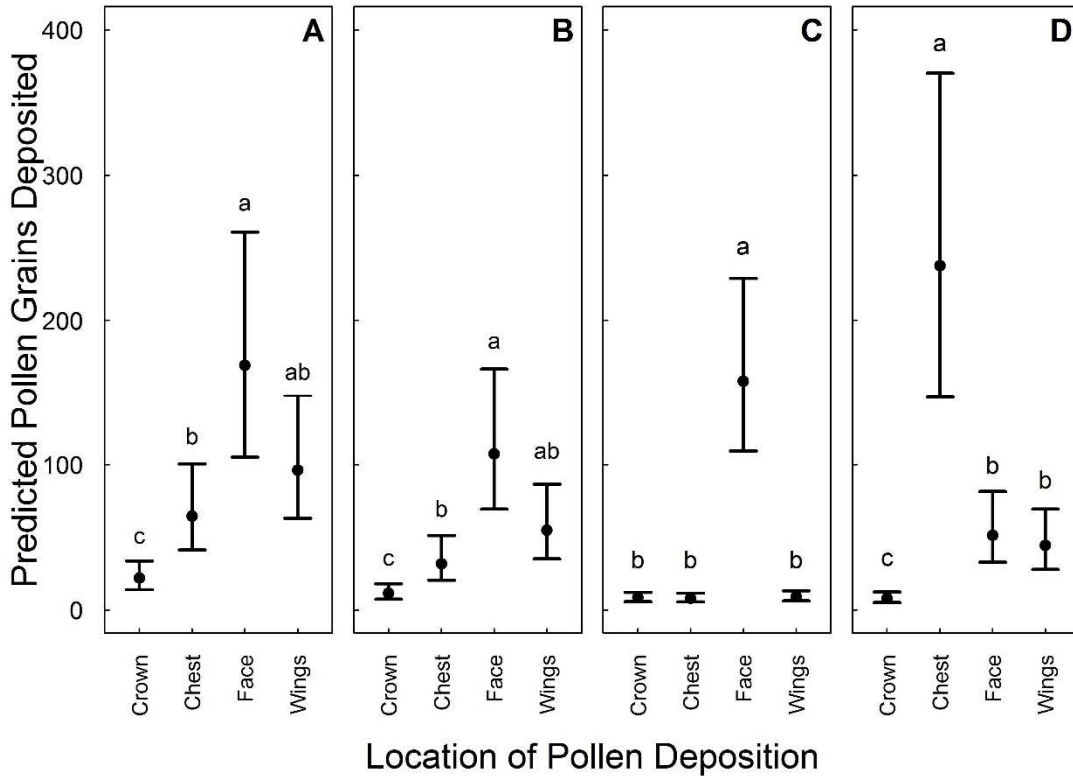


FIGURE 12. Back-transformed model estimates (with 95% confidence intervals) of the number of pollen grains placed on different areas of *Eonycteris spelaea*: the crown of the head, chest, face and ventral side of the wings. The bat-pollinated plant species tested were (A) *Ceiba pentandra* ($n = 16$ bats), (B) *Musa acuminata* ($n = 24$ bats), (C) *Durio zibethinus* ($n = 16$ bats) and (D) *Parkia speciosa* and *P. timoriana* ($n = 16$ bats). Patterns of pollen deposition differed significantly among plant species (GLMM pairwise contrasts, $P < 0.001$), except between *C. pentandra* and *D. zibethinus* ($P > 0.9$). Within each graph, means with different letters are significantly different (GLMM pairwise contrasts with sequential Bonferroni correction, $P < 0.05$).

pollen onto the bat's face, followed by wings, chest and crown. *Durio zibethinus* flowers ($n = 16$ bats) had a very similar pattern of pollen deposition. However, *M. acuminata* pollen ($n = 24$ bats) was placed almost entirely on the bat's face, while *Parkia* flowers ($n = 16$ bats) transferred the large majority of pollen onto the bat's chest.

DISCUSSION

Our study clearly demonstrates that the nectarivorous bat *E. spelaea* transfers significantly more pollen between conspecific flowers than between heterospecific flowers. Furthermore, we found that different bat-pollinated plant species place pollen on different areas of the pollinator's body, which accounts for the low levels of interspecific pollen transfer. Our plant study species represent four of the most common genera visited by *E. spelaea*, and our results provide a mechanism to explain why field observations of flowers visited by *E. spelaea* found only minimal amounts of heterospecific pollen on the stigmas within a community in Southeast Asia (Srithongchuay et al. 2008; Acharya et al. 2015).

The effectiveness of differential pollen placement in limiting interspecific pollen transfer in bees and hummingbirds has received mixed support (Waser 1978; Campbell and Motten 1985; Feinsinger and Tiebout 1991; Armbruster et al. 1994; Murcia and Feinsinger 1996; Yang et al. 2007; Flanagan et al. 2009; Huang and Shi 2013; Huang et al. 2015). However, work in the neotropics suggests that the varied floral designs of bat-pollinated plant taxa successfully partition pollen placement on nectar bats (Howell 1977; Tschapka et al. 2006; Muchhala 2007; Muchhala and Potts 2007). Muchhala (2007) proposed that differential pollen placement is easier to achieve on large pollinators, such as bats, and therefore may be more likely to evolve among bat-pollinated plant species. Our study, using a large (40–70 g) bat pollinator and four bat-pollinated plant species, supports this prediction.

Plant species that exhibited the greatest difference in pollen placement on the bat's body (Figure 12) experienced the least amount of interspecific pollen movement

(Figure 11), consistent with results reported for neotropical nectar bats (Muchhala and Potts 2007). For example, 78% of *M. acuminata* pollen was deposited on the bat's face, while 65% of *Parkia* pollen was deposited on the bat's chest. As a consequence, *E. spelaea* transferred an average of 8.2 pollen grains from *Parkia* to *M. acuminata*, which is ten times fewer than the mean of 84 pollen grains transferred between conspecific *M. acuminata* flowers (Figure 11). Limiting interspecific pollen transfer is likely particularly important between *M. acuminata* and *P. speciosa* since their extended ('steady-state') flowering phenologies overlap almost entirely, and they are both important food resources for *E. spelaea*. Bumrungsri et al. (2013) found that *Parkia* and *Musa* were consistently the most abundant species of pollen in the diet of *E. spelaea* across all months (each constituting between 24 and 34% of the diet). Yet by placing pollen on different parts of the pollinator's body, *M. acuminata* and *Parkia* flowering individuals experience limited heterospecific pollen exchange, as was also observed in nature by Acharya et al. (2015).

Interspecific pollen transfer was greatest between plant species whose reproductive structures contacted similar body parts of the pollinator. Muchhala and Thomson (2012) reported similar findings in the neotropics: the more similar floral competitors were in terms of pollen placement, the more they disrupted pollen transferred by bats between conspecific flowers. We found that *E. spelaea* transferred the greatest amounts of heterospecific pollen from *Parkia* to *C. pentandra*, and from *C. pentandra* to *M. acuminata*. Yet in both instances the amount of heterospecific pollen transferred was still three to nine times less than the amount of pollen transferred in the conspecific treatment (Figure 11). These results suggest our focal

study species experience limited reductions to female plant fitness through stigma clogging (Waser 1978; Waser and Fugate 1986; Morales and Traveset 2008) and to male plant fitness through pollen misplacement (Inouye et al. 1994; Murcia and Feinsinger 1996; Muchhala and Thomson 2012).

Some combinations of our focal plant study species could not be tested due to non-overlapping flowering phenologies, most notably *C. pentandra* and *D. zibethinus*. These two species belong to the same family (Malvaceae) and are much more similar in floral structure than our other plant study species (Figure 10). Furthermore, the similar floral designs produced remarkably similar patterns of pollen deposition (Figure 12), yet heterospecific pollen exchange is unlikely given that they do not flower concurrently. Differential pollen placement and non-overlapping flowering phenologies may be two complementary mechanisms that reduce interspecific pollen transfer, as suggested by Howell (1977) for neotropical pollinating bats and Botes et al. (2008) for paleotropical pollinating birds. Mechanisms reducing heterospecific pollen movement are understudied in the Old World tropics and would benefit from further research, including studies of geographical or ecological isolation, phenological isolation, variation in pollinator assemblages, degree of pollinator fidelity and mechanical isolation due to differences in floral morphology (Ramsey et al. 2003; Kay 2006).

While we only tested four bat-pollinated plant species that are important food resources and commonly co-occur in the field, our results likely apply to other plant species in this system as well. For example, *Oroxylum indicum*, another bat-pollinated plant commonly found in the diet of *E. spelaea* (Bumrungsri et al. 2013; Stewart et al.

2014), has a very different floral design from the four species we tested in this study (Gould 1978). The anthers and stigma of *O. indicum* lie along the roof of the corolla such that contact with *E. spelaea* occurs along the crown of the bat's head (Gould 1978; Srithongchuay et al. 2008). The four plant genera in our study placed very little pollen on this area (Figure 12), suggesting little opportunity for interspecific pollen exchange with *O. indicum*. Supporting this prediction, Srithongchuay et al. (2008) found minimal heterospecific pollen on the stigmas of *O. indicum* pollinated in the wild; *M. acuminata* pollen accounted for less than 6%, *Parkia* pollen less than 4%, and an unknown taxon contributed 2% to the total pollen load found on *O. indicum* stigmas.

Overall, our experimental design reflects bat pollination that occurs in nature, and the observed landing positions and feeding behaviors of *E. spelaea* matched their behavior in the wild (Baker and Harris 1959; Itino et al. 1991; Bumrungsri et al. 2008, 2009; Acharya et al. 2015; A. Stewart, unpubl. res.). Although our results may not account for pollen loss from the bats as they fly (Murcia and Feinsinger 1996; Mitchell et al. 2009) or groom (Flanagan et al. 2009) between foraging bouts, these concerns may be less relevant to bat pollinators. The fur of bats may minimize passive forms of pollen loss, and grooming has not been shown to decrease a bat's pollination efficiency (Muchhala and Thomson 2010). It should be noted that we measured pollen transfer after the bat visited a single cluster of male flowers, whereas in nature the pollen that a bat carries on its body likely accumulates over the course of multiple flower visits throughout the night (Muchhala and Thomson 2010). For this

reason, our measures of pollen transfer may underestimate the actual number of pollen grains transferred in nature.

Our study demonstrates an efficient mechanism that reduces competition among flowering plant species sharing a single pollinator, and illustrates how a floral visitor with a broad diet can still be an effective pollinator for a number of plant species. Although several studies have documented that plants can deposit pollen on different areas of a pollinator's body (Howell 1977; Armbruster et al. 1994; Muchhala 2007; Muchhala and Potts 2007; Muchhala and Thomson 2012; Huang and Shi 2013), they rarely confirm the effects of these differences on conspecific versus heterospecific pollen transfer, and typically examine just two plant species. We illustrate that multiple flowering plant species within a community can effectively share the same pollinator through differential pollen placement, and that these differences in pollen deposition can reduce interspecific pollen transfer among sympatrically flowering plant species.

Chapter 6: Complementary mechanisms limit competition for pollination among paleotropical bat-pollinated plant species across a diverse landscape in southern Thailand

ABSTRACT

Sympatric flowering plant species frequently compete for pollination, which often reduces plant reproductive success. Even distantly related plant species can incur fitness costs when they share pollinators, since interspecific pollen transfer can lead to pollen loss and stigma clogging. Trait differences that reduce reproductive interference are therefore evolutionarily favored. We examined three mechanisms that are thought to reduce competition for pollination, and hypothesized that (1) habitat differences (ecological separation), (2) differences in flowering times (phenological separation), and (3) high pollinator constancy (ethological separation) would minimize reproductive interference among six bat-pollinated plant taxa in southern Thailand. We conducted plant surveys in three broad habitat types, monitored flowering phenologies across the entire year, and examined bat pollen loads to determine the number of plant species visited per night. We found that although spatial and temporal separation play limited roles in reducing competition for pollination among most of the bat-pollinated plant species, ethological separation is very important within this system. This study illustrates that multiple mechanisms act in concert to promote legitimate pollen transfer in a highly diverse tropical community.

Key words: chiropterophily, competition for pollination, ecological separation, ethological separation, phenological separation, Thailand

INTRODUCTION

A major obstacle for sympatric flowering plant species is competition for pollination. Such competition can arise when co-occurring plant species compete for pollinators, and can also arise through interspecific pollen transfer (Stebbins 1951; Levin 1970; Waser 1983). In the latter case, co-occurring plant species share pollinators, which can lead to heterospecific pollen transfer. Costs of heterospecific pollen transfer include hybrid inviability or sterility (Darwin 1876; Rieseberg & Willis 2007), loss of pollen to heterospecific flowers (Levin & Anderson 1970; Morales & Traveset 2008) and loss of stigmatic surface to foreign pollen grains (Waser & Fugate 1986). Given the high fitness costs of competing for pollination, plant species that experience minimal reproductive interference are more likely to coexist (MacArthur & Levins 1967; Waser 1983). Additionally, selection to reduce competition for pollination can lead to reproductive character displacement (Levin 1970; Waser 1983; Armbruster et al. 1994). Thus, we would expect to see varied trait differences among sympatric plant species sharing pollinators.

Several pre-zygotic barriers have been demonstrated to reduce competition for pollination across diverse systems, including spatial, temporal, and ethological separation. Early naturalists such as Darwin (1859) and Robertson (1895) noted that plant species growing in close proximity would experience greater competition for

pollination than more distant species. More recent work has explored the various scales at which spatial partitioning can occur, from geographic (Althoff et al. 2012) to altitudinal (Macek et al. 2009) or ecological separation (Ball & Pidsley 1995; Peterson et al 2013). Spatial separation among plant species appears to be predominantly driven by variation in ecological niches among plant species (including competition for nutrients and differences in physiological requirements; Ball & Pidsley 1995; Linhart & Grant 1996; Macek et al. 2009), yet it can have profound impacts on plant-pollinator interactions. Furthermore, plant species that occupy different patches across a heterogeneous landscape are less likely to receive heterospecific pollen when pollinators are inconstant (Levin & Anderson 1970).

Temporal separation can reduce competition for pollination even when plant species co-occur in close proximity. For example, plant species that flower during different times of the year, such as during different seasons, are phenologically secluded (Robertson 1895; Levin 1970; Mosquin 1971; Pleasants 1980). Phenological separation has frequently been advocated as a mechanism to reduce competition for pollination (Robertson 1895; Mosquin 1971; Stiles 1975; Pleasants 1980). Robertson (1895) observed that plant species which share the same pollinators are more likely to stagger flowering times, compared to plant species pollinated by different pollinators. Alternatively, plant species which overlap in flowering seasons may open their flowers during different times of day, as has been observed with nocturnally-blooming (bat-pollinated) and diurnally-blooming (bird-pollinated) congeners in both the New World (Muchhala 2007; Martén-Rodríguez et al. 2009) and Old World

tropics (Itino et al. 1991). Thus, temporal separation appears to be a common mechanism for minimizing interspecific pollen transfer across diverse systems.

Ethological separation can occur through pollinator preference (Schemske & Bradshaw 1999; Martén-Rodríguez et al. 2009; Fenster et al. 2015) or pollinator constancy (Darwin 1876; Grant 1950; Levin & Anderson 1970; Jones 1978; Aldridge & Campbell 2007). With pollinator preference, a pollinator species forages on a subset of the available floral species, often based on traits associated with attraction or reward. For example, hummingbirds have been shown to strongly prefer nectar-rich, red flowers (Schemske & Bradshaw 1999; Fenster et al. 2006) that are vertically oriented (Fenster et al. 2009) and presented high above the ground (Dudash et al. 2011; Fenster et al. 2015). In contrast, pollinator constancy refers to how faithful a pollinator species is to a specific flowering plant species in its diet. To date, pollinator constancy has mostly been studied in bees (Betts 1920; Brittain & Newton 1933; Grant 1950; Wilson & Stine 1996), but has also been examined in other systems such as hawkmoths and hummingbirds (Aldridge & Campbell 2007), hoverflies (Goulson & Wright 1998), and butterflies (Goulson & Cory 1993; Goulson et al. 1997). However, with long-lived pollinators, it is particularly important to monitor preference and constancy throughout the year, as their diets may change seasonally (Fleming et al. 1993; Sperr et al. 2011). Both pollinator preference and constancy can reduce heterospecific pollen movement, even when sympatric plant species flower simultaneously (Levin & Anderson 1970).

Plant species often experience frequent interactions with non-congeners, particularly when diverse plant taxa share the same pollination syndrome and attract

the same pollinators (Fenster et al. 2004; Rosas-Guerrero et al. 2014). During previous work in southern Thailand, we found that six diverse genera of bat-pollinated plants share a common important pollinator, the dawn bat, *Eonycteris spelaea* (Stewart et al. 2014; Stewart & Dudash 2016). This community of night-blooming plant species is therefore expected to experience strong pressure to minimize competition for pollination. Additionally, because bats are long-lived pollinators that visit flowering species year-round, mechanisms that limit competition among tropical bat-pollinated plants can potentially operate over longer time scales than is observed among temperate insect-pollinated plants. As most pollination work has been conducted in temperate areas with short flowering seasons, we still lack knowledge about tropical plant communities that flower year round and are dependent on long-lived pollinators.

To examine possible mechanisms that facilitate the coexistence of tropical bat-pollinated plant species, we tested whether ecological, phenological, and/or ethological separation reduce competition for pollination. Each of these mechanisms can effectively limit competition, but they impose different consequences on plant populations, and their prevalence has never been examined among Old World bat-pollinated plants. Furthermore, we collected ethological (via bat pollen loads) and phenological data across an entire year, as nectar bats visit flowers year-round and their diets can change seasonally. This study illustrates multiple mechanisms acting in concert to promote reliable intraspecific pollen transfer in a highly diverse tropical community.

METHODS

Study site

This study was conducted in southern Thailand, within and around four protected areas (Khao Banthat Wildlife Sanctuary, Khao Pu Khao Ya National Park, Thale Ban National Park, and Ton Nga Chang Wildlife Sanctuary; Appendix II). The most common habitat within park boundaries is tropical lowland forest, while farmland occupies most of the surrounding area. The major agricultural practices in southern Thailand include rubber plantations, oil palm plantations, and mixed fruit orchards (Aratrakorn et al. 2006). Mangrove forests are scattered along the coast (Thampanya 2006).

Plant study species

Our study focused on six plant taxa that are common to the region and known to be pollinated by bats (Bumrungsri et al. 2008, 2009; Srithongchuay et al. 2008; see also Chapter 3). *Ceiba pentandra* (Malvaceae) is a mast-flowering tree species that occurs sporadically throughout the region, and is sometimes locally cultivated for the cotton-like fibers contained within its seed pods (Nathan et al. 2005). Economically-important *Durio zibethinus* (Malvaceae) is another mast-flowering species; the prized durian fruit is widely cultivated in both monocultures and in mixed orchards (Bumrungsri et al. 2009). *Musa acuminata* (Musaceae) bananas are highly abundant and ubiquitous throughout the region; while seedless cultivated varieties are parthenocarpic, wild banana plants require pollination to set fruit (Andersson 1998). *Oroxylum indicum* (Bignoniaceae) is a small tree species that typically occurs in

relatively open spaces; this steady state species produces a few flowers each night over an extended period (Srithongchuay et al. 2008). Two species of *Parkia* (Fabaceae) are common in the area; *P. speciosa* flowers for much of the year and is often cultivated for its seeds (which are locally consumed), while *P. timoriana* has a much shorter flowering season (Bumrungsri et al. 2008). *Sonneratia* mangroves (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*; Lythraceae) are common in coastal areas and typically produce multiple flushes of flowers per year (Start & Marshall 1976). (See Appendix III detailed descriptions of the plant study species.)

Plant habitat surveys

To assess whether differential habitat use (ecological separation) precludes competition for pollination among bat-pollinated plant species, we conducted plant surveys from March – July 2013 in three broad habitat types occupied by our study species: agriculture, forest, and mangrove. Given that our study species are long-lived tree and herbaceous plant species, this 5-month assessment is an accurate depiction of their year-round physical locations and distributions. Within each habitat type, we walked 50-m transects ($n = 18$ transects per habitat type) and recorded all individuals of our plant study species within 10 m on either side of each transect. Transects in forested and agricultural areas were conducted in all four protected areas, and data were pooled by habitat type as there were no significant differences between protected areas ($\chi^2_{15} = 9.3$, $P = 0.86$). Mangrove transects were only conducted in Thale Ban National Park (as the other three parks are landlocked), and were limited to areas that bordered roads or had boardwalks. We compared differences among habitat

types using a negative binomial generalized linear model (GLM). The response variable was number of observed plants, and the predictors were plant species, habitat type, and their interaction. Likelihood ratio tests were used to compare models. Within each plant species, differences in abundance across the three habitat types were compared with 95% confidence intervals of the model predictions, where non-overlapping confidence intervals indicate significant differences ($P < 0.05$). All analyses were performed in R 3.1.1 (R Development Core Team).

Flowering phenology

To determine whether differences in flowering times (phenological separation) reduce competition for pollination among our plant study species, we recorded flowering phenologies monthly (for *Sonneratia* species) or biweekly (for all other plant species) between March 2013 and May 2014. We rotated surveys among the four protected areas for all plant species except *Sonneratia* species, which were only observed in Thale Ban National Park (the other three parks are situated inland and lack mangroves). For each plant species, we selected ten random individuals and noted whether or not they were in flower (*S. caseolaris* was the exception; as there were only three *S. caseolaris* trees at our mangrove study site, we observed the same three individuals every month). Temporal segregation of flowering phenologies was examined with the TimeOverlap program (Castro-Arellano et al. 2010). This program uses a randomization algorithm that preserves temporal autocorrelation to generate a null distribution of assemblage-wide temporal overlap, against which the observed overlap is compared. Overlap was calculated using Pianka's index, where values near

zero indicate less overlap and values near one indicate more overlap (Castro-Arellano et al. 2010).

Pollinator pollen loads

To assess whether pollinator constancy (ethological separation) limits competition for pollination among our plant study species, we examined pollen loads collected from the bodies of flower-visiting bats. Pollen loads allowed us to identify the flowering plant species a bat foraged at during the night without having to track individual bats. We collected pollen loads from 1,219 bats (caught with mist-nets during their nightly foraging) between March 2013 and May 2014 ($n = 122$ nights).

To obtain a random sample of each bat's pollen load, we dabbed 0.4 mL of solidified fuchsin glycerin gelatin (Beattie 1972) uniformly across the bat's head, chest, and ventral side of the wings (for detailed description, see Stewart & Dudash 2016). Samples were fixed on microscope slides and viewed at 100-400x magnifications. Pollen grains were counted and identified to species (or to genus, in the case of *Parkia* and *Sonneratia*) by comparison to a reference collection. As we were only interested in competition among our focal bat-pollinated plant species, we excluded all other plant species pollen from this analysis (other pollen species were uncommon, see Appendix V for details). We counted how many plant pollen species each bat carried, omitting pollen species occurring in trace amounts (fewer than 5 pollen grains). Bats found with pollen carried between one to four plant pollen species. As polynomial analyses require testing the observed distribution against a null distribution, and the null distribution of plant pollen species carried was

unknown, we opted to perform binomial tests. Thus, we classified each bat individual into one of two categories: “high” pollinator constancy (1-2 pollen species) or “low” pollinator constancy (3-4 pollen species) and used binomial tests to determine if each bat species is more or less constant than expected from chance by comparing the observed probabilities to the random probability of $P = 0.5$. We chose a null probability of 0.5 (i.e., half the bats exhibit low constancy and half exhibit high constancy) as the most neutral scenario to compare the observed data against.

RESULTS

Ecological separation

When assessing the role of habitat type in reducing competition among plant species, the model that best described plant abundance included plant species ($X^2_{18} = 299.2$, $P < 0.001$), habitat type ($X^2_{14} = 239.2$, $P < 0.001$), and their interaction ($X^2_{12} = 189.3$, $P < 0.001$) (see Appendix XI for all models tested). *Ceiba pentandra*, *D. zibethinus*, *M. acuminata*, and *O. indicum* were significantly more abundant in agricultural areas than in forests or mangroves (Figure 13). Both species of *Parkia* were equally abundant in agricultural and forested areas, and significantly more so than in mangroves (Figure 13). In contrast, *Sonneratia* trees were significantly more abundant in mangroves than in the other two habitat types (Figure 13).

Phenological separation in forest and agricultural areas

Overall, the bat-pollinated plant assemblage within forested and agricultural areas did not significantly stagger flowering phenologies throughout the year

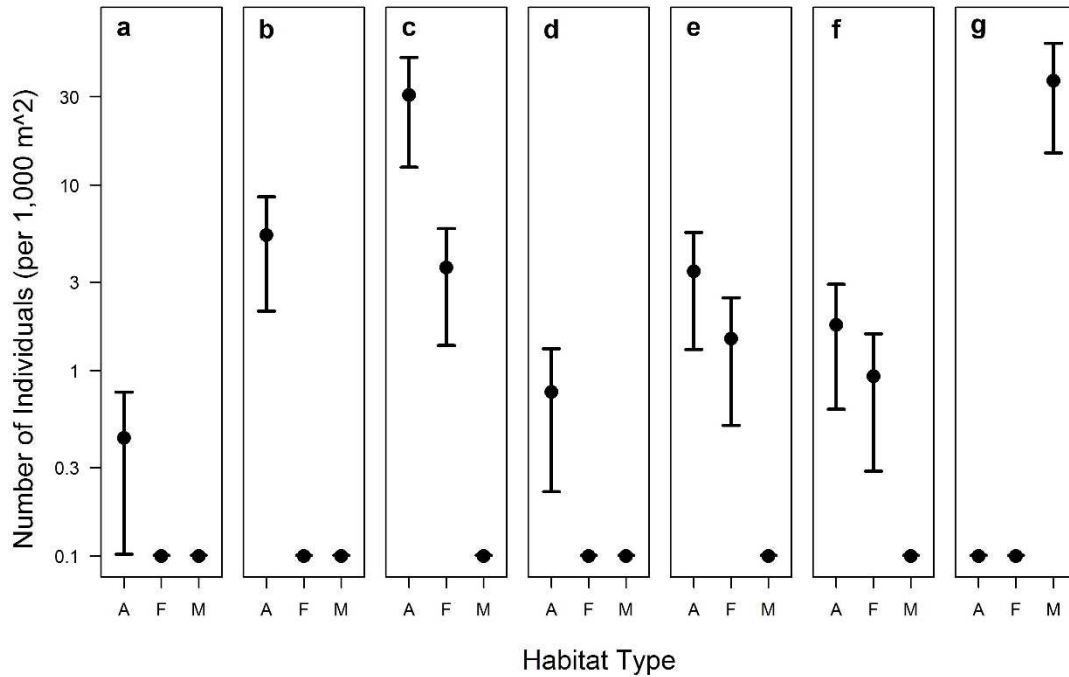


FIGURE 13. Model predictions of the abundance of bat-pollinated plant species (mean \pm 95% confidence interval) in three broad habitat types within southern Thailand (x-axis: A, agriculture; F, forest; M mangrove). The plant study species were: (a) *Ceiba pentandra*, (b) *Durio zibethinus*, (c) *Musa acuminata*, (d) *Oroxylum indicum*, (e) *Parkia speciosa*, (f) *Parkia timoriana*, and (g) *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*). Habitat types with non-overlapping confidence intervals are significantly different (negative binomial generalized linear model, $P < 0.05$).

(Pianka's index = 0.49, $P = 0.47$; Figure 14). Three of the plant study species (*C. pentandra*, *D. zibethinus*, and *P. timoriana*) exhibited big-bang phenologies, with short, highly synchronous flowering periods (Figure 14a). *Durio zibethinus* never overlapped with *C. pentandra* (Pianka's index = 0) and only slightly overlapped with *P. timoriana* (Pianka's index = 0.08). However, there was considerable overlap between *C. pentandra* and *P. timoriana* (Pianka's index = 0.69). The three other sympatric plant species (*M. acuminata*, *O. indicum*, and *P. speciosa*) flowered year-round (Figure 14b) and thus overlapped continually (Pianka's index: *M. acuminata* and *O. indicum* = 0.86; *M. acuminata* and *P. speciosa* = 0.88; *O. indicum* and *P. speciosa* = 0.96).

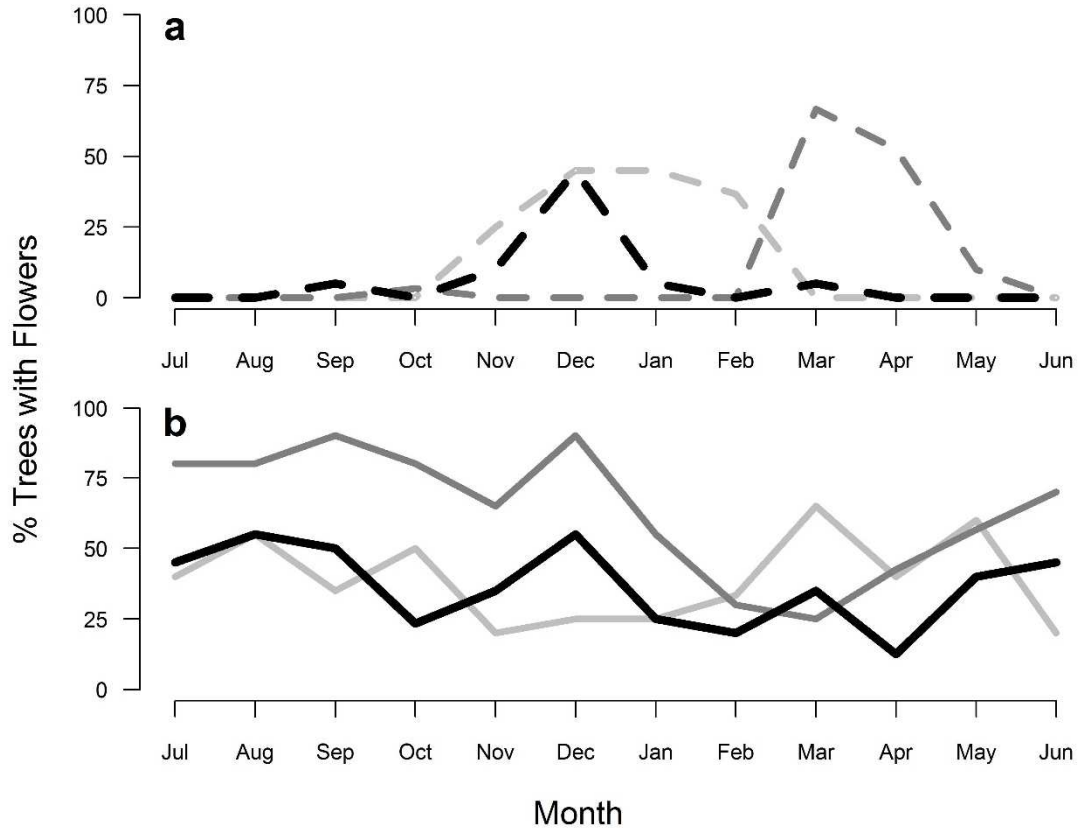


FIGURE 14. Flowering phenologies of bat-pollinated plant species in southern Thailand. (a) Plant species with big-bang flowering phenologies: *Ceiba pentandra* (dashed, light gray), *Durio zibethinus* (dashed, medium gray), and *Parkia timoriana* (dashed, black). (b) Plant species with steady-state flowering phenologies: *Musa acuminata* (solid, light gray), *Oroxylum indicum* (solid, medium gray), and *Parkia speciosa* (solid, black). Phenological overlap of the entire community was not significantly less than expected by random chance (Pianka's index of overlap = 0.49, $P = 0.47$).

Phenological separation in mangroves

Flowering phenologies of the four *Sonneratia* species also did not exhibit greater segregation than expected by random chance (Pianka's index = 0.68, $P = 0.44$; Figure 15). *Sonneratia griffithii* had two distinct mast-flowering events (September and April), while *S. caseolaris* flowered continuously throughout the year (Figure 15). The other two species (*S. alba* and *S. ovata*) had less clear-cut phenologies; flowering trees of each were generally observed year-round, but the number of individual trees in flower fluctuated greatly (Figure 15).

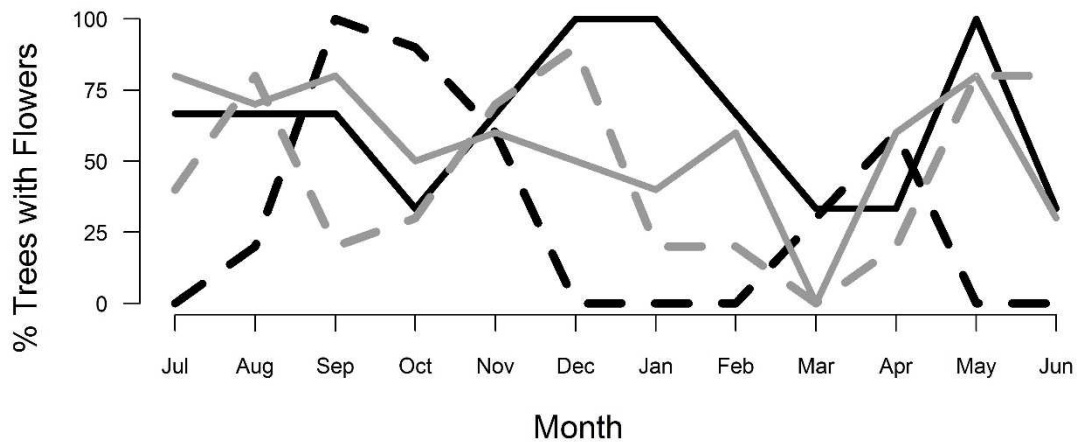


FIGURE 15. Flowering phenologies of four *Sonneratia* mangrove species in southern Thailand: *S. alba* (dashed, gray), *S. caseolaris* (solid, black), *S. griffithii* (dashed, black), *S. ovata* (solid, gray). Phenological overlap of the mangrove community was not significantly less than expected by random chance (Pianka's index of overlap = 0.68, $P = 0.44$).

Ethological separation

All bat species exhibited significantly higher constancy (with individuals carrying only 1 or 2 pollen species per night) than expected by chance (Figure 16). There were no significant differences between sexes, so we pooled males and females of each bat species (t-test, *E. spelaea*: $t = 1.1$, $df = 573$, $P = 0.28$; *M. minimus*: $t = 0.71$, $df = 110$, $P = 0.48$; *M. sobrinus*: $t = 0.14$, $df = 67$, $P = 0.89$; *R. leschenaulti*: $t = 0.35$, $df = 28$, $P = 0.73$; *Cynopterus* species: $t = 1.5$, $df = 34$, $P = 0.15$). Of the 746 *E. spelaea* bats sampled, 612 carried pollen, with over 88% (537 bats) exhibiting high constancy (binomial test, $P < 0.0001$). All but two *M. minimus* individuals carried pollen, and 99% (112/113 bats) exhibited high constancy ($P < 0.0001$). Of the 73 *M. sobrinus* bats, 69 carried pollen, with 88% (61 bats) carrying just one or two plant pollen species ($P < 0.0001$). *Rousettus leschenaulti* individuals were less likely to carry pollen in general (only 41 out of 81 bats carried pollen), but nearly all of the

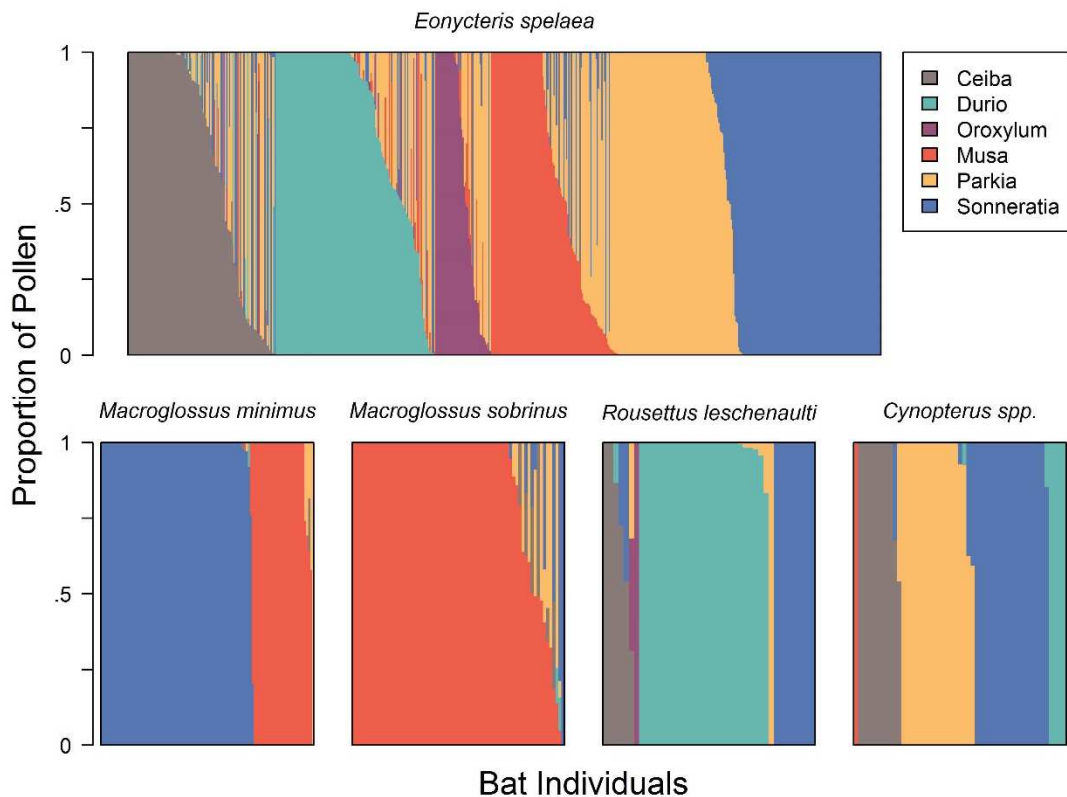


FIGURE 16. Pollinator constancy of five nectar-feeding bat taxa in southern Thailand, illustrated by the proportions of different pollen species in the diets of individual bats. Vertical lines represent the diets of individual bats, with different colors corresponding to different pollen species (i.e., a solid colored line denotes a bat that carried only one species of pollen, a line composed of two colors denotes a bat that carried two species of pollen, etc.). Top row: *Eonycteris spelaea* ($n = 612$ bats). Bottom row, left to right: *Macroglossus minimus* ($n = 113$ bats), *Macroglossus sobrinus* ($n = 69$ bats), *Rousettus leschenaulti* ($n = 41$ bats), *Cynopterus* species ($n = 49$ bats; *C. brachyotis*, *C. horsfieldii*, *C. sphinx*). For all bat species, individuals carrying one or two pollen species were encountered significantly more often than individuals carrying three or four pollen species (binomial test, $P < 0.0001$). Pollen species: *Ceiba pentandra* (grey), *Durio zibethinus* (green), *Oroxylum indicum* (purple), *Musa acuminata* (red), *Parkia* species (yellow; *P. speciosa* and *P. timoriana*), *Sonneratia* species (blue; *S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*).

bats carrying pollen exhibited high constancy (98%, or 40/41 bats; $P < 0.0001$). Less than a quarter of *Cynopterus* individuals carried pollen (49 out of 204 bats), but 100% of pollen-carrying individuals carried two or fewer pollen species ($P < 0.0001$).

DISCUSSION

In diverse tropical communities, where multiple plant species potentially compete for pollination, mechanisms that reduce the risk of incorrect pollen transfer can confer high fitness benefits (reviewed in Levin 1970; Waser 1983; Mitchell et al. 2009).

Within our study system of paleotropical bat-pollinated plants, we found some evidence for ecological separation and limited evidence for phenological separation. Ethological separation appears to be much more prominent in limiting interspecific pollen transfer, as bat pollen loads reveal high pollinator constancy. Although not all three competition-reducing mechanisms (ecological, phenological and ethological separation) are universally used by all plant species, together they greatly minimize reproductive interference among sympatrically occurring plant species in a mixed forest-agricultural landscape.

Ecological separation

Plant species composition differed markedly among the three broad habitat types (agriculture, forest, and mangrove). Some bat-pollinated plant species (*M. acuminata* and *Parkia* species) were found ubiquitously throughout southern Thailand, as they are commonly cultivated on farms yet also occur in the wild (Andersson 1998; Bumrungsri et al. 2008). Other species were observed primarily in agricultural areas, and rarely in forests. For example, the distribution of *D. zibethinus* was heavily influenced by human activities, as durian trees are extensively cultivated in fruit orchards (Bumrungsri et al. 2009). Two other species, *C. pentandra* and *O. indicum*, prefer open areas with lots of sunlight (Kyereh 1999; Srithongchuy 2008);

unsurprisingly, individuals of these species were never observed in the forest interior. In general, however, we frequently found these bat-pollinated plant species co-occurring in close proximity (sometimes less than a meter apart) throughout non-coastal areas.

The only bat-pollinated plant species observed in mangroves were the four *Sonneratia* species, which are well adapted to high salt concentrations and water-logged soils (e.g., via pneumatophores and accumulation of inorganic ions to maintain osmotic potential; Parida & Jha 2010). Given this spatial separation (coastal versus inland), *Sonneratia* individuals appear to experience minimal competition for pollination with other bat-pollinated plant species. Furthermore, other studies indicate that *Sonneratia* species may partition ecological niches on an even finer scale based on salinity and inundation frequency (Ball & Pidsley 1995). Duke et al. (1998) reported that *S. alba* and *S. ovata* typically grow downstream while *S. caseolaris* and *S. griffithii* are often found upstream, which corroborates our personal observation (A. Stewart) that *S. alba* grew nearest to the ocean and *S. griffithii* farthest away.

Spatial separation can certainly limit interspecific pollen transfer if the distance between patches of different plant species is greater than pollinator movement, but would not ensure complete separation if the pollinator can travel great distances, such as nectarivorous bats can (Start & Marshall 1976). Thus, ecological separation does not appear to prevent interspecific pollen transfer except, perhaps, between coastal-growing *Sonneratia* and other bat-pollinated plant species growing further inland. Supporting this prediction, studies examining flowers of *O. indicum*, *D. zibethinus*, and *Parkia* trees reported that the only heterospecific pollen found on

floral stigmas belonged to inland plant species, and never to *Sonneratia* species (Srithongchuay et al. 2008; Acharya et al. 2015).

Phenological separation

Multiple bat-pollinated plant species often flowered concurrently, so phenological separation appears unlikely to prevent interspecific pollen transfer. Indeed, three of the inland plant species flowered year-round (Figure 14b), and thus overlapped continuously. Such steady state plant species are important food resources for sustaining pollinator populations (Gentry 1974; Peters et al. 2013), particularly long-lived pollinators such as bats (Baker 1963). Additionally, by producing a few flowers over an extended period, steady state plants encourage trap-line foraging behavior in pollinators (Gentry 1974; Machado & Vogel 2004), which promotes outcrossing and likely leads to greater reproductive success (Ohashi & Thomson 2009).

Even the plant species with big bang phenologies did not overlap less than expected by random chance (Figure 14a). While a few studies have reported phenological partitioning of co-occurring plant species (Heithaus et al. 1975; Stiles 1975; Pleasants 1980), a growing body of evidence indicates that flowering phenologies are often limited by phylogenetic (Kochmer & Handel 1986; Davies et al. 2013; Du et al. 2015) and/or climatic constraints (Ashton et al. 1988; Totland 1993; Inouye et al. 2003). Two of the big bang species (*C. pentandra* and *D. zibethinus*) belong to the same family (Malvaceae) and phylogenetic constraints may prevent their flowering phenologies from diverging greatly. It is noteworthy,

however, that phenological overlap between *C. pentandra* and *D. zibethinus* is actually very minimal (Figure 14a); this temporal divergence may be important in reducing competition between the two plant species, given that they often grow in close proximity and share the same pollinators (Chapter 3).

Flowering phenologies of the three big bang species may also be constrained by climatic (seasonal) factors, as all three species flower during the area's drier months (December through May; Thai Meteorological Department, www.tmd.go.th/en). Correspondingly, Janzen (1967) proposed that flowering during the dry season is advantageous within the tropics because it allows plants to maximize vegetative growth during the rainy season. Thus, other selective pressures may outweigh the benefits of phenological partitioning (Brody 1997), causing plant species to rely on other mechanisms for reducing competition for pollination.

Ethological separation

The nectarivorous bats in our study area exhibited high floral constancy, suggesting that pollinator behavior limits interspecific pollen transfer, as has been demonstrated in other studies (Jones 1978; Waser 1986; Aldridge & Campbell 2007; Yang et al. 2007). For most bat species that we observed, this constancy stems from specializing on specific plant species (*M. minimus* bats on *Sonneratia* flowers, *M. sobrinus* bats on *M. acuminata* flowers, *R. leschenaulti* on *D. zibethinus* flowers; Figure 16; see also Chapter 2). *Eonycteris spelaea* was the only bat species to consistently carry pollen loads for all of our plant study taxa (Figure 16), and thus appeared to be a generalist forager at the population level. Yet individual pollen loads

indicate that *E. spelaea* forages on just one or two pollen species per night. Moreover, this result was consistent throughout the night (Appendix XII) and across the entire year, even as *E. spelaea* changed diets seasonally between big-bang and steady-state floral resources (Figure 16).

Ethological separation has several advantages over other competition-reducing mechanisms. Perhaps most importantly, it does not require plant species to adapt to new habitats, or to alter flowering times. Thus, our plant study species are able to occupy the same habitat types, resulting in high local diversity as is commonly observed in tropical communities. Our study species are also able to have overlapping phenologies, which is particularly important for the continuously-flowering species that provide critical food resources for long-lived nectar bats. As temperate regions do not support plant species that flower year-round (Gentry 1974), and are overall less diverse than tropical areas (Pianka 1966; Mittelbach et al. 2007), pollinator constancy may be more important for tropical systems than temperate systems, although this hypothesis has not been examined.

Several explanations have been proposed for why foraging individuals might specialize on a subset of available resources (reviewed in Bolnick et al. 2003), such as improving foraging efficiency (Lewis 1986), having different physiological requirements (e.g., according to sex or reproductive state; Rose 1994), or differing in competitive dominance and thus access to preferred resources (Holbrook and Schmitt 1992). Furthermore, *E. spelaea* appears to specialize on big bang resources (*C. pentandra*, *D. zibethinus*) when possible, and forage on steady state resources (*M. acuminata*, *O. indicum*, *Parkia* spp.) during other times of the year (Figure 16).

Alternatively, bats may be grooming pollen from their fur throughout the night, which could explain why the number of plant pollen species carried remained constant over time (Pearson's correlation coefficient, $r = 0.03$, $P = 0.35$, $n = 847$; Appendix XII) rather than increasing, as might be expected. Given that individuals carry pollen from few plant species at any given time (Figure 16), ethological separation is likely important in minimizing reproductive interference between heterospecifics of paleotropical bat-pollinated plants.

Moreover, bat-pollinated plant species exhibit a striking diversity of floral designs, which has been found to deposit pollen on different areas of the bat's body (Howell 1977; Tschapka et al. 2006; Muchhala & Potts 2007; Stewart & Dudash 2016). We recently demonstrated that strong mechanical separation through differential pollen placement on a pteropodid nectar bat significantly reduces interspecific pollen transfer within our study system. Thus mechanical differences can provide an additional mechanism to ensure correct or legitimate pollen transfer (Darwin 1877) when ethological separation is not absolute.

Incomplete reproductive separation among congeners?

While we found evidence that various mechanisms reduce competition for pollination among diverse and distantly related plant taxa, these mechanisms seem to be less effective for congeneric species. The two *Parkia* species (*P. speciosa*, *P. timoriana*) and four *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*) were often observed in close proximity to, and flowering concurrently with, congeneric species. Additionally, congeneric flowers are very similar (to human

senses) both morphologically and in terms of reward, so it seems unlikely that bats would discriminate between congeners, or that congeneric flowers would place pollen on different areas of the pollinator's body. Supporting this assertion, hybrid individuals have been observed in nature. For example, crosses between *P. speciosa* and *P. timoriana* result in *P. intermedia* hybrids (Hopkins 1994), and several *Sonneratia* hybrids have been documented, including *S. alba* x *S. caseolaris* and *S. alba* x *S. ovata* (Muller & Hou-Liu 1966; Duke 1984; Zhou et al. 2005). Another study examining *Sonneratia* individuals found that hybrids had low germination rates (23 – 64%), high percentages of sterile pollen grains (55 – 95%), and that hybridization never progressed beyond F1 plants (Zhou et al. 2005). Together, the strong fitness costs associated with incorrect pollen placement (Grant 1994) may drive reinforcement (Levin 1970), and may lead to the evolution of multiple competition-reducing mechanisms such as ecological, phenological, ethological, and mechanical separation.

Conclusions

Flowering plant species in highly diverse tropical communities experience high competition for pollination, particularly when they share common pollinators (Start & Marshall 1976; Stewart et al. 2014). Within our paleotropical bat-pollinated study system, multiple mechanisms appear to function in parallel, although ethological (this study) and mechanical differences (Stewart & Dudash 2016) are likely much more prominent in limiting competition than ecological or phenological differences (this study). It is unclear whether the observed trait differences among co-occurring plant

species were driven by selection to reduce competition for pollination, or whether they arose through other processes (e.g., they may have been shaped by other biotic or abiotic selective pressures, or perhaps arose entirely in allopatry). Regardless of their origin, trait differences among plant species that share pollinators likely confer substantial fitness benefits by reducing pollen loss to heterospecific flowering individuals (Murcia & Feinsinger 1996; Muchhala & Thomson 2012), and by minimizing stigma clogging (Waser 1978; Morales & Traveset 2008). At the very least, we would expect to see some combination of ecological, phenological, ethological, and mechanical differences maintained, although they may vary depending on environmental context. While most studies have focused on the role of pre-zygotic isolating mechanisms in facilitating divergence of sister species (i.e., speciation; Stiles 1975; Morrison et al. 1994; Muchhala & Potts 2007; Yang et al. 2007; Macek et al. 2009; Huang & Shi 2013; Peterson et al 2013), they are also important in minimizing reproductive interference among diverse plant species that share pollinators across a diverse landscape.

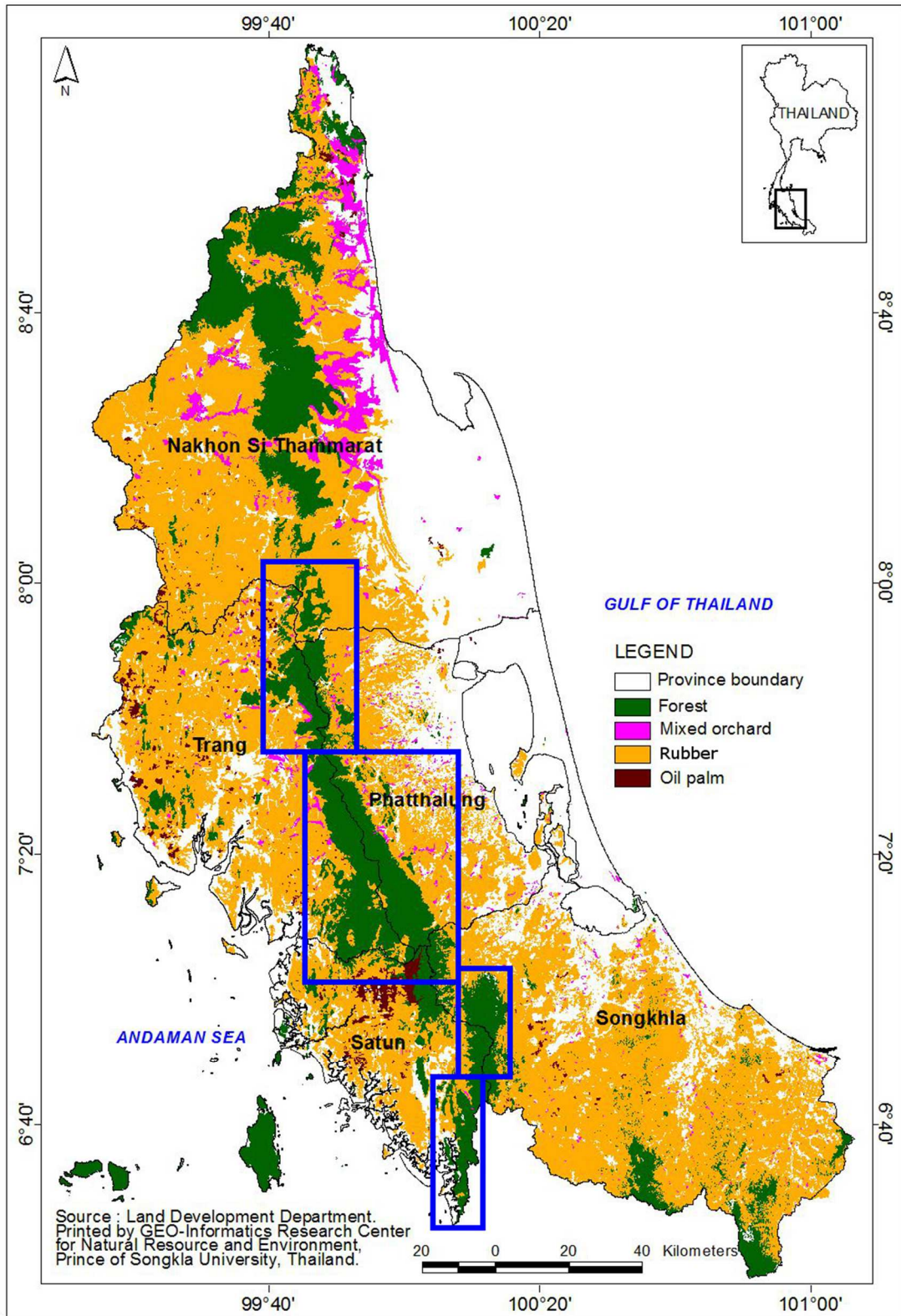
Appendix I

Number of bats (per species, sex, and reproductive class) caught foraging at bat-associated plant species in southern Thailand. Bat species, left to right: *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*, *Megaerops ecaudatus*, *Penthetor lucasi*, *Rousettus amplexicaudatus*, *R. leschenaulti*. Asterisks denote nectarivorous species, all others are frugi-nectarivorous. Non-repro. = non-reproductive.

	<i>*E. spe</i>	<i>*M. min</i>	<i>*M. sob</i>	<i>C. bra</i>	<i>C. hor</i>	<i>C. sph</i>	<i>Me. eca</i>	<i>P. luc</i>	<i>R. amp</i>	<i>R. les</i>	Total
Male											
Juvenile	31	7	8	0	27	23	2	1	1	0	100
Adult	19	13	11	11	25	39	4	0	0	0	122
Female											
Juvenile	15	4	5	2	7	18	2	0	0	0	53
Lactating	20	3	8	3	7	17	2	0	0	1	61
Pregnant	8	0	0	0	1	7	0	0	0	0	16
Non-repro.	12	8	7	0	2	7	0	0	0	0	36
Total	105	35	39	16	69	111	10	1	1	1	388

Appendix II

Map of our study area in southern Thailand, showing the major habitat types: forested (green), mixed fruit orchards (pink), rubber plantations (orange), and oil palm plantations (brown). Data collection occurred within and around the four protected areas highlighted in the blue rectangles (N to S): Khao Pu Khao Ya National Park, Khao Banthat Wildlife Sanctuary, Ton Nga Chang Wildlife Sanctuary, and Thale Ban National Park. (Map courtesy of the GEO-Informatics Research Center for Natural Resource and Environment, Prince of Songkla University.)



Appendix III

Descriptions of plant study species.

Ceiba pentandra (Malvaceae; common name “kapok”) is a self-compatible, pantropical tree species with a short, highly-synchronous flowering period (Gribel et al. 1999). In our study area, all flowering occurred between late November and mid-February (Chapter 2), with individual trees producing hundreds of thousands of hermaphroditic flowers per season (Gribel et al. 1999). Each flower contains over 200 ovules (Gribel et al. 1999). Studies in Brazil (Gribel et al. 1999), Costa Rica (Lobo et al. 2005), Ghana (Baker & Harris 1959), India (Singaravelan & Marimuthu 2004), and Samoa (Elmqvist et al. 1992) have all found that nectarivorous bats are the main visitors, including the pteropodid species *C. sphinx* and *R. leschenaulti* in India (Singaravelan & Marimuthu 2004).

Durio zibethinus (Malvaceae; durian) is a self-incompatible tree species native to southeast Asia (Bumrungsri et al. 2009). Hermaphroditic flowers are produced in clusters along mature branches and the mass flowering is highly synchronous (Bumrungsri et al. 2009). All trees in our study area flowered between March and April, consistent with Bumrungsri et al. (2009). A single tree typically flowers for just 10 days, but can produce over 1,000 flowers per night (Bumrungsri et al. 2009). Each flower has five locules, with 5-7 ovules per locule (Kozai et al. 2014). A prior study of *D. zibethinus* found that bat-pollinated flowers set significantly more fruit than insect-pollinated flowers, indicating that bats are the primary pollinator

(Bumrungsri et al. 2009). However, they did not compare the relative importance of different bat species, which is the goal of this study.

Musa acuminata (Musaceae; banana) is a herbaceous plant species native to southeast Asia (Itino *et al.* 1991), with wild individuals requiring pollination to set fruit (Andersson 1998). Flowering individuals can be found year-round (Gould 1978; Sripaoraya 2005; Pillay & Tenkouano 2012; see also Chapter 2). Each shoot produces a single inflorescence consisting of bracts covering two rows of flowers (around 15 to 40 flowers; Itino *et al.* 1991). Each night, a bract folds back to expose the flowers, which are female during the first 1-30 hands, sterile during the next 0-4 hands, and male during the last 150-300 hands (Pillay & Tenkouano 2012). As this species is temporally dioecious, within-inflorescence selfing is very rare (Andersson 1998). The ovary of each flower within an inflorescence contains three locules, with 100-500 ovules per locule (Fortescue & Turner 2005). *Eonycteris spelaea* (Nur 1976; Gould 1978), *M. minimus* (Nur 1976; Gould 1978), *M. sobrinus* (Itino et al. 1991), and *Cynopterus* species (Gould 1978) have all been observed visiting the flowers of *M. acuminata* in Southeast Asia.

Oroxylum indicum (Bignoniaceae; Indian trumpet flower) is a self-incompatible tree species with hermaphroditic flowers that is distributed throughout much of Asia (Srithongchuay et al. 2008). Flowering individuals are found year-round, with some trees flowering continuously (Sritongchuay et al. 2010; see also Chapter 6). Only one or two flowers open per inflorescence per night (Srithongchuay et al. 2008), but trees can have dozens of inflorescences flower simultaneously (Gould 1978). Flowers have over 300 ovules (Srithongchuay et al. 2008). Prior work

has found that *E. spelaea* is the main visitor in Southeast Asia (Gould 1978), where they reliably transfers large amounts of pollen (Srithongchuay et al. 2008). In India, *C. sphinx* has been reported as the major visitor (Vikas et al. 2009).

Parkia (Fabaceae) is a pantropical genus (Baker & Harris 1957), and the paleotropical *P. speciosa* (petai, or sator) and *P. timoriana* (tree bean, or riang) are both self-incompatible (Bumrungsri et al. 2008). We observed flowering *P. speciosa* trees year-round, but the majority of flowering occurred from May to November. In contrast, *P. timoriana* has a very short, synchronous flowering season from December to mid-January (Bumrungsri et al. 2008; see also Chapter 2). Individuals of both species can have up to 70 capitula (pendant, spherical inflorescences) open per night, which are either hermaphroditic or functionally staminate (Bumrungsri et al. 2008). Each inflorescence contains 2,500-4,000 flowers; the majority are fertile, but about 25-30% are nectar-secreting or staminoidal (Bumrungsri et al. 2008). Each fertile flower contains 16-19 ovules (Bumrungsri et al. 2008). *Parkia* species are predominantly bat-pollinated in the New World (Hopkins 1984; Piechowski et al. 2010) and Old World tropics (Baker & Harris 1957; Bumrungsri et al. 2008; Hopkins 1994; Lassen et al. 2012; Vanlalnghaka 2014), although a few nectar-less neotropical species are insect-pollinated (Hopkins 1984; Hopkins et al. 2000). In Asia, *E. spelaea* (Gould 1978; Bumrungsri et al. 2008) and *Cynopterus* species (Bumrungsri et al. 2008; Vanlalnghaka 2014) are common visitors.

Sonneratia (Lythraceae) is a genus of mangrove trees distributed throughout the Old World tropics (Tomlinson 1994). Four species are commonly found in our study area (*S. alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*), all of which have

hermaphroditic flowers (Bureau of Mangrove Resources Conservation 2009). *Sonneratia caseolaris* flowers year-round while the other three species have multiple bang phenologies (Start & Marshall 1976; A. Stewart, pers. obs.). Each *S. alba* flower contains around 220 ovules (Primack et al. 1981), and other *Sonneratia* species likely have similar numbers of ovules (Watson & Dallwitz 1992). *Sonneratia* species are assumed to be bat-pollinated based on observations of bats visiting the flowers, particularly by *M. minimus* (Start & Marshall 1976), but hawkmoths were observed visiting *S. alba* in Australia (Primack et al. 1981).

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Appendix IV

Sample sizes and measurements of flower-visiting bat species netted from March 2013 – August 2014 in southern Thailand. FA = forearm length.

	Bat Species	Sex/Age	<i>N</i>	Mass ± SE (g)	FA ± SE (mm)
nectarivorous	<i>Eonycteris spelaea</i> (Cave nectar bat, Dawn bat)	male	139	59.1 ± 0.73	67.7 ± 0.30
		female	154	52.9 ± 0.46	66.3 ± 0.21
		juvenile	459	37.2 ± 0.40	60.6 ± 0.21
	<i>Macroglossus minimus</i> (Long-tongued nectar bat)	male	30	17.7 ± 0.66	42.2 ± 0.37
		female	26	18.1 ± 0.96	42.3 ± 0.53
		juvenile	42	14.7 ± 0.69	41.1 ± 0.47
	<i>Macroglossus sobrinus</i> (Greater nectar bat)	male	25	22.2 ± 0.47	45.6 ± 0.30
		female	23	22.9 ± 0.52	45.7 ± 0.31
		juvenile	25	18.4 ± 0.72	43.6 ± 0.31
(primarily) frugivorous	<i>Cynopterus brachyotis</i> (Lesser short- nosed fruit bat)	male	28	30.5 ± 0.87	59.5 ± 0.41
		female	26	34.7 ± 1.2	61.4 ± 0.66
		juvenile	5	31.9 ± 1.8	60.0 ± 0.82
	<i>Cynopterus horsfieldii</i> (Horsfield's fruit bat)	male	33	47.5 ± 1.2	69.6 ± 0.45
		female	22	49.2 ± 1.8	70.3 ± 0.49
		juvenile	49	42.2 ± 0.71	68.1 ± 0.37
	<i>Cynopterus sphinx</i> (Greater short- nosed fruit bat)	male	4	42.6 ± 3.7	66.8 ± 1.64
		female	10	42.4 ± 1.4	66.2 ± 0.95
		juvenile	35	34.7 ± 1.2	64.2 ± 0.69
	<i>Rousettus leschenaulti</i> (Leschenault's Rousette)	male	13	89.2 ± 5.4	83.7 ± 0.95
		female	20	73.4 ± 3.1	79.7 ± 0.72
		juvenile	43	58.2 ± 2.3	73.4 ± 0.98

Appendix V

Most pollen carried by nectar-feeding bats in southern Thailand belonged to our plant study species (*Ceiba pentandra*, *Durio zibethinus*, *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, *P. timoriana*, *Sonneratia alba*, *S. caseolaris*, *S. griffithii*, and *S. ovata*); other pollen morphotypes were uncommon. Unknown morphotypes are noted below. Sampling was conducted on a subset of 297 bats caught between March-August 2013. *N* = number of bats carrying each pollen taxa; Mean = average number of pollen grains carried; SE = standard error of the number of pollen grains carried

Pollen taxa	<i>N</i>	Mean	SE
<i>Ceiba pentandra</i>	19	16.6	7.0
<i>Durio zibethinus</i>	58	47.3	11.0
<i>Musa acuminata</i>	86	10.8	1.9
<i>Oroxylum indicum</i>	25	11.7	5.1
<i>Parkia</i> spp.	95	58.8	10.6
<i>Sonneratia</i> spp.	168	555.8	144.5
Unknown A	2	0.1	0.1
Unknown B	5	0.3	0.1
Unknown C	5	7.9	6.4
Unknown D	1	0.1	0.1
Unknown E	2	0.6	0.5
Unknown F	1	0.0	0.0
Unknown G	2	0.1	0.1
Unknown H	3	0.2	0.1
Unknown I	2	7.2	7.2
Unknown J	1	0.0	0.0
Unknown K	2	0.5	0.5
Unknown L	1	0.0	0.0
Unknown M	4	2.8	2.5

Appendix VI

Verifying pollinator pollen load as an accurate metric of pollen transfer effectiveness.

Adult bats were caught in mist-nets, liberally fed sugar water, and held for 20-24 hours to be tested the following evening. During testing, individual bats (24 *Eonycteris spelaea*, 7 *Rousettus leschenaulti*, and 4 *Macroglossus sobrinus*) were placed in a flight cage containing two clusters of flowers: one cluster consisted of emasculated (“female”) flowers, the other consisted of unaltered (“male”) conspecific flowers. Three flower taxa were used: *Ceiba pentandra* ($n = 8$ trials), *Durio zibethinus* ($n = 15$ trials), and *Musa acuminata* ($n = 12$ trials). Initially, only the male flowers were exposed (female flowers were covered with a plastic bag). After the bat visited the male flowers, a hidden observer pulled a string, which removed the plastic bag and exposed the female flowers. A trial was complete once the bat finished foraging at the female flowers.

To determine pollinator pollen load acquired during the experimental trial, each bat’s fur was uniformly swabbed for pollen using solidified fuchsin glycerin gelatin (0.1 mL gelatin for each of four sample areas: the crown of the head, face, chest, and wings). Pollen samples were fixed on microscope slides, viewed at 100-400x magnifications, and pollen grains were counted.

To determine the number of pollen grains transferred from male to female flowers, stigmas of the female flowers were fixed on microscope slides using fuchsin glycerin gelatin, and all pollen grains found on the stigma were counted.

The correlation between total pollinator pollen load and pollen grains transferred (Figure A) was assessed using a linear model and Pearson's correlation coefficient.

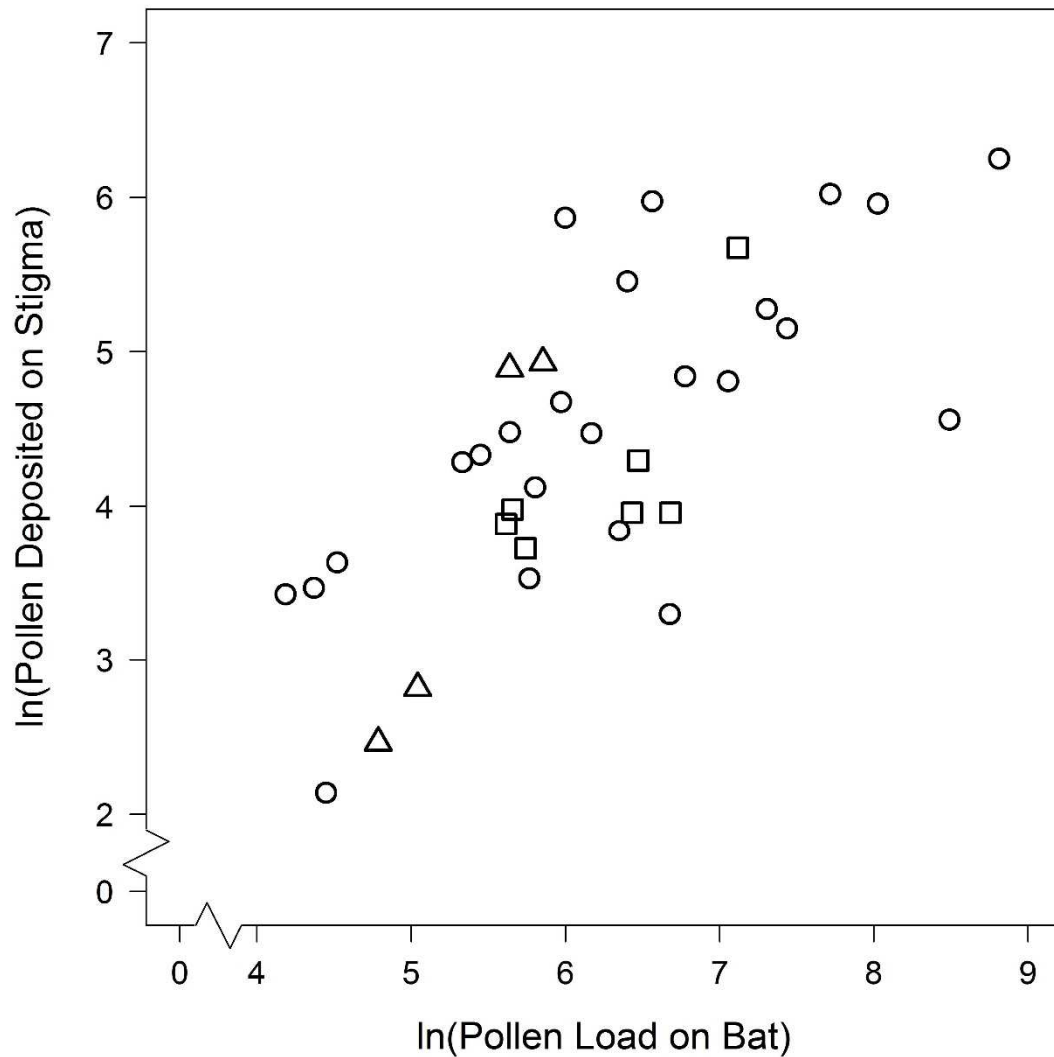


FIGURE A. The pollen load carried by a bat (after visiting male flowers) is positively correlated with the amount of pollen that the bat deposits on the stigmas of female flowers (linear model, $F_{1,34} = 38.9$, $P < 0.0001$, $r^2 = 0.53$; Pearson correlation coefficient = 0.73). Circles: *Eonycteris spelaea* ($n = 24$); rectangles: *Rousettus leschenaulti* ($n = 7$), triangles: *Macroglossus sobrinus* ($n = 4$).

Appendix VII

Variation in visitation rates (Figure B) and pollen loads (Figure C) of seven pteropodid bat species (in southern Thailand) across four bat-pollinated plant species and three years.

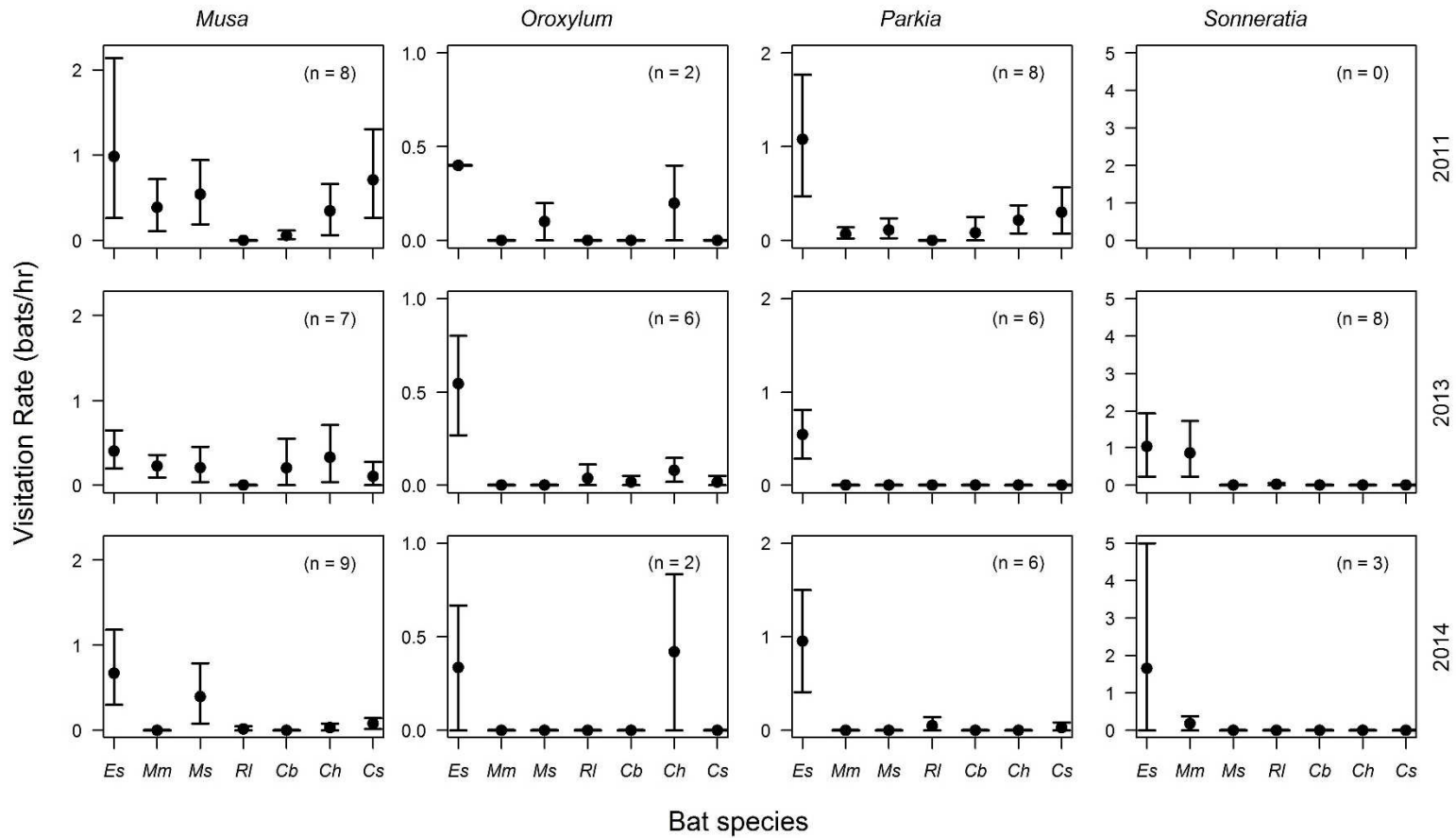


Figure B. Visitation rates (bats netted per hour; bootstrapped means and 95% confidence intervals) of pteropodid bats in southern Thailand across four bat-pollinated plant species and three years. Bat species with non-overlapping 95% confidence intervals are significantly different. Sample sizes in parentheses in the top right corner of each plot indicate the number of nights mist-netted. Plant species in columns (left to right): *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*). Data was not collected at *Sonneratia* trees in 2011. Bat species along x-axes (left to right): *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Rousettus leschenaulti*, *Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*.

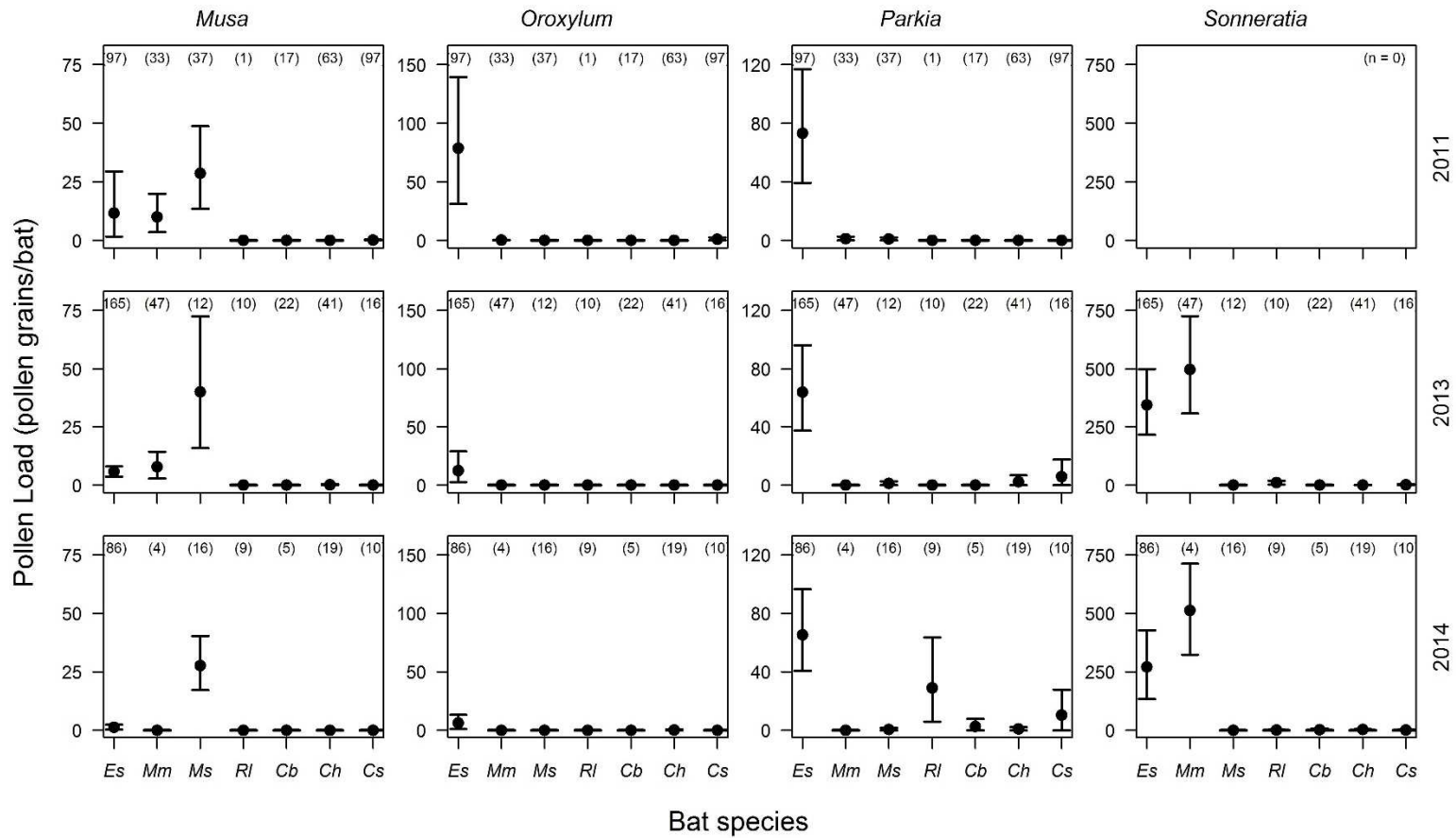
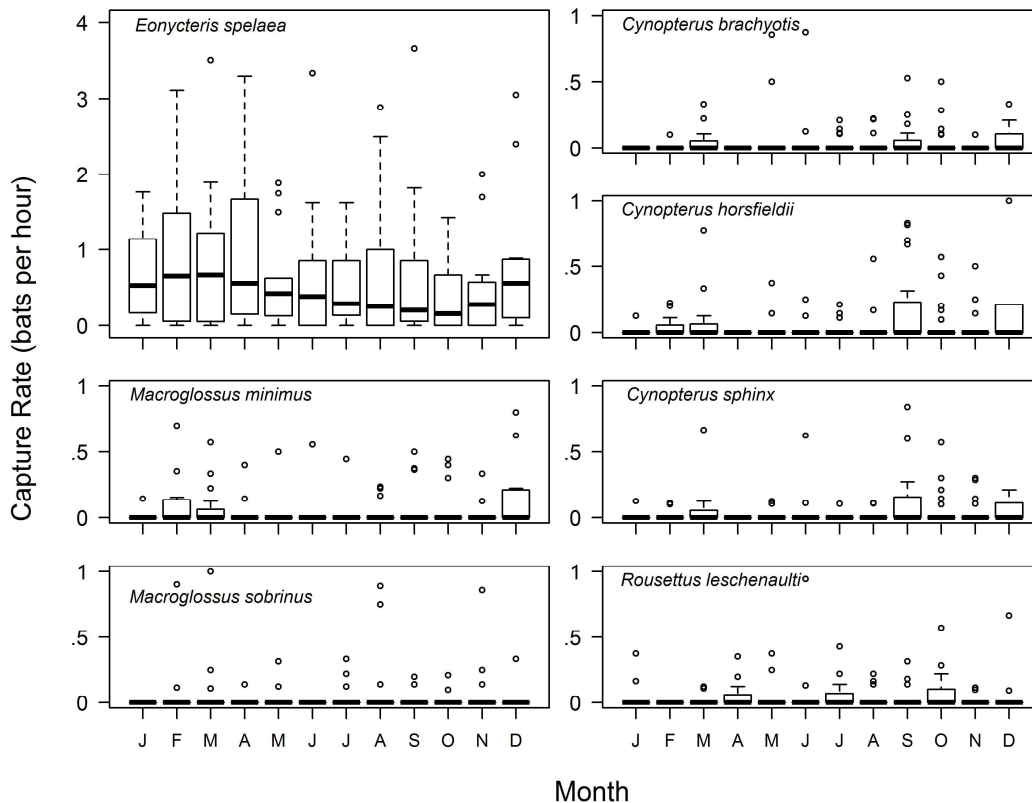


Figure C. Pollen loads (number of pollen grains collected per bat; bootstrapped means and 95% confidence intervals) of pteropodid bats in Thailand across four bat-pollinated plant species and three years. Bat species with non-overlapping 95% confidence intervals are significantly different. Sample sizes in parentheses along the top of each plot indicate the number of bats that pollen was collected from. Plant species in columns (left to right): *Musa acuminata*, *Oroxylum indicum*, *Parkia speciosa*, *Sonneratia* species (*S. alba*, *S. caseolaris*, *S. griffithii*, *S. ovata*). Data was not collected at *Sonneratia* trees in 2011. Bat species along x-axes (left to right): *Eonycteris spelaea*, *Macroglossus minimus*, *M. sobrinus*, *Rousettus leschenaulti*, *Cynoptyerus brachyotis*, *C. horsfieldii*, *C. sphinx*.

Appendix VIII

Capture rate (bats per hour) of pteropodid bat species caught in southern Thailand throughout the year. Individuals of each species were caught nearly every month, indicating that pteropodid bats are non-migratory residents. Capture rates did not differ significantly across months (ANOVA, *Eonycteris spelaea*: $F_{11,181} = 0.78$, $P = 0.66$; *Macroglossus minimus*: $F_{11,181} = 0.67$, $P = 0.76$; *Macroglossus sobrinus*: $F_{11,181} = 1.0$, $P = 0.45$; *Rousettus leschenaulti*: $F_{11,181} = 0.83$, $P = 0.61$; *Cynopterus brachyotis*: $F_{11,181} = 1.2$, $P = 0.31$; *Cynopterus horsfieldii*: $F_{11,181} = 1.6$, $P = 0.09$; *Cynopterus sphinx*: $F_{11,181} = 1.6$, $P = 0.09$). Note the y-axis for *E. spelaea* is different than the other y-axes.



Appendix IX

Description and photos of the syringe method for dispensing glycerin gelatin and collecting pollen grains.



FIGURE D. A syringe provides a convenient and efficient way for standardizing pollen collection.



FIGURE E. An empty 1-mL syringe with the tip cut off (i) and a syringe filled with fuchsin glycerin gelatin (ii). The syringe plunger is easily manipulated to control how much gel is used during pollen collection (iii).

Preparing the syringe:

- 1) Cut the tip off of a syringe so that the entire length of the syringe barrel is the same diameter (Figure E-i). (Any size syringe will work.)
- 2) Prepare fuchsin glycerin gelatin following Beattie (1972), or use the solution of your choice. At this point, the solution should be in a liquid state.
- 3) Use the syringe to draw the fuchsin glycerin gelatin up into the barrel of the syringe, and let the solution solidify in the syringe (Figure E-ii).

Appendix X

AIC statistics for models describing (A) the number of pollen grains transferred between male (pollen donor) and female (pollen recipient) flowers, and (B) the number of pollen grains collected from different body parts of the bat. Predictors for the number of pollen grains transferred include “recipient” (species of the female flower), “treatment” (conspecific or heterospecific trial), and/or their interaction. Predictors for the number of pollen grains collected include “donor” (species of the male flower), “part” (body part where the pollen was collected from), and/or their interaction; “individual” (bat individual) was included as a random factor, and “total” (total number of pollen grains collected from each bat) was included as a covariate. df = degrees of freedom, AIC = Akaike’s information criterion, Δ AIC = difference between the AIC scores of the top model and given model. Models are listed by ascending AIC scores.

Response Variable	Model	df	AIC	ΔAIC
(A) ln(pollen grains transferred +1)	recipient + treatment	5	251.87	0
	recipient * treatment	7	254.40	2.53
	treatment	3	256.39	4.52
	recipient	4	299.49	47.62
(B) ln(pollen grains collected +1)	donor * part + individual + ln(total+1)	15	621.12	0
	donor + part + individual + ln(total+1)	9	720.01	98.90
	part + individual + ln(total+1)	6	725.38	104.26
	donor * part + individual	14	743.80	122.68
	donor + individual + ln(total+1)	7	834.35	213.23
	donor * part + ln(total+1)	4	835.05	213.93

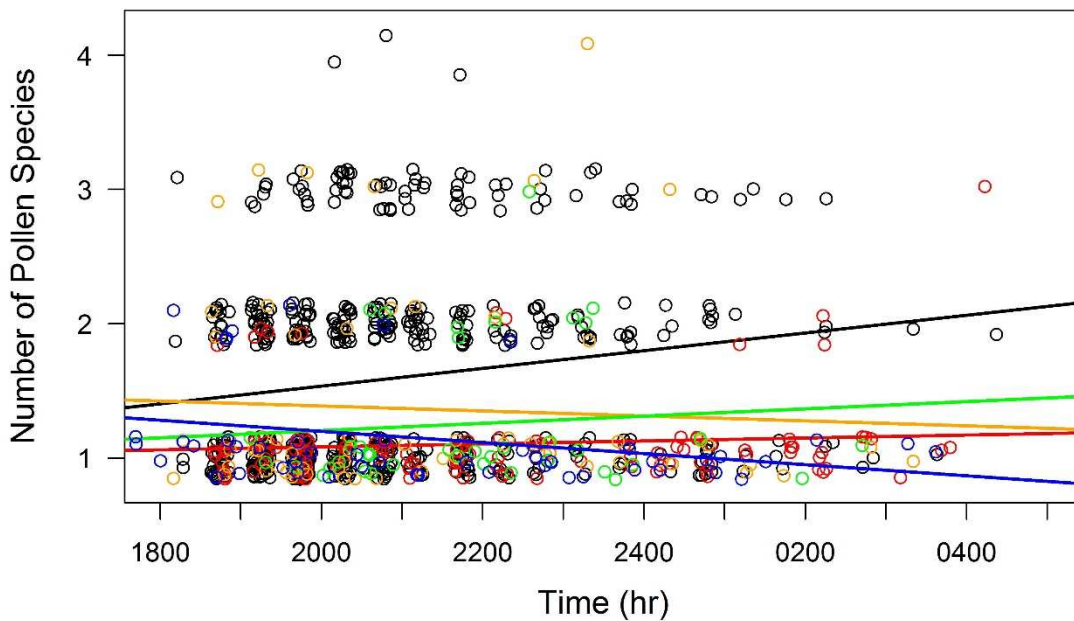
Appendix XI

AIC statistics for models describing plant abundance across different habitat types in southern Thailand. The predictors include “habitat” (agriculture, forest, or mangrove), “species” (plant species), and/or their interaction. df = degrees of freedom, AIC = Akaike's information criterion, ΔAIC = difference between the AIC scores of the top model and given model. Models are listed by ascending AIC scores.

Response Variable	Model	df	AIC	ΔAIC
Number of observed plants	habitat * species	22	863.63	0
	habitat + species	10	1028.90	165.27
	species	8	1074.83	211.20
	habitat	4	1126.84	263.21

Appendix XII

Changes in the number of pollen species carried by bats throughout the night. Across all bat species, there was not a significant correlation between time of night and number of pollen species carried (Pearson's correlation coefficient, $r = 0.03$, $P = 0.35$, $n = 847$). Within species, the correlation was not significant for *Macroglossus minimus* (red; $r = 0.09$, $P = 0.35$, $n = 113$), *M. sobrinus* (orange, $r = -0.06$, $P = 0.64$, $n = 69$), or *Rousettus leschenaulti* (green, $r = 0.10$, $P = 0.55$, $n = 41$), while there was a slight positive correlation for *Eonycteris spelaea* (black, $r = 0.16$, $P < 0.001$, $n = 612$), and a slight negative correlation for *Cynopterus* species (blue, $r = -0.31$, $P = 0.03$, $n = 49$) [*Cynopterus* species include *C. brachyotis*, *C. horsfieldii*, *C. sphinx*].



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