— Short Communication —

Specialized and facultative nectar-feeding bats have different effects on pollination networks in mixed fruit orchards, in Southern Thailand

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Abstract—Recent advances in the study of pollination networks have improved our ability to describe species interactions at the community level. In this study, we compared the abundance and network strength of facultative and obligate nectar-feeding bats to determine their roles in pollinating mixed fruit orchards. We were particularly interested in the effect of distance from forests and caves on the foraging activity of these two bat groups. For this study, we examined 10 pairs of orchards; each pair consisted of one orchard near to (< 1 km) and one orchard far from (> 7 km) the forest edge. We estimated the abundance of each bat group (nectarivorous vs. frugi-nectarivorous) using video observations to determine floral visitation rates. A pollination network was then created for each of the 20 study orchards and network strength was calculated for each bat group at each orchard. We found that nectarivorous bats showed higher abundance and network strength than frugi-nectarivorous bats. Both bat abundance and network strength were negatively correlated with distance to the nearest cave, however, only network strength was affected by distance to the forest. These results corroborate the importance of nectarivorous bats in pollinating crops within southern Thailand's mixed fruit orchards. Higher network strength of bats near forests and caves emphasizes the role of natural habitats as pollinator sources.

Keywords: bat, cave proximity, forest proximity, network strength, pollination network

Introduction

Pollination is a key mutualistic interaction. Although bat pollination is not as common as insect or bird pollination, approximately 250 genera of plants depend on bat pollinators (Sekercioglu 2006; Fleming et al. 2009). A recent study by Stewart et al. (2014) classified paleotropical, phytophagous bats into two feeding guilds, specialized nectarivores (which are obligate nectar feeders) and frugi-nectarivores (which visit flowers opportunistically). Since specialized nectarivores are dependent solely on floral resources, they may be more consistent visitors than frugi-nectarivores, and may provide greater pollination services. In Neotropical studies, nectar specialist and opportunistic bat species differ in their contributions toward plant reproductive success (Frick et al. 2013). However, knowledge about how these two bat groups contribute to plant-pollinator networks within an entire plant community is lacking.

Recent advances in the study of pollination networks have improved our ability to describe species interactions and the underlying structure, function, and stability of communities (Montoya et al. 2006). It has been demonstrated that some properties of pollination networks

are influenced by spatial effects, such as habitat conversion and urbanisation (Geslin et al. 2013). Additionally, decreasing habitat availability at the landscape level can isolate patches of suitable habitat leading to altered pollinator diversity, frequency, and movement patterns (Holyoak et al. 2005; Greenleaf & Kremen 2006; Brosi et al. 2007; Zurbuchen et al. 2010). Proximity to natural habitats is important in enhancing ecosystem services provided by pollinators. However, effects of proximity to natural habitats may vary with the organism. In a previous study, we found that bat visitation and pollination success of durian are significantly negatively correlated with distance to the nearest cave (Sritongchuay et al. 2016). These results correspond to bat roosting behavior, as pteropodid species roost in foliage and limestone karst caves (Kunz & Fenton 2003; Bumrungsri et al. 2009). Previous studies have focused on only one species of plant. However, it is important to understand the effect of distance to natural habitats on the role of flower-visiting bats within the entire bat-pollinated plant community.

In this study, we aim to investigate the role of nectarivorous and frugi-nectarivorous bat species in southern Thailand's mixed fruit orchards (that vary in distance from forests and caves) by addressing these questions: 1) Do nectarivorous and frugi-nectarivorous bats have similar pollination roles? 2) Does the distance to forest patches and caves affect the abundance and/or network strength of either bat group (sum of dependencies across all plant species that

a bat group interacted with), thus influencing the pollination services they provide to the plant community? We hypothesized that nectarivorous bats are more important pollinators, because they feed obligately on nectar and are therefore likely more frequent flower visitors. In addition, we predict that the abundance and network strength of all flower-visiting bats will be negatively correlated with distance to forest patches and caves due to pteropodid bat roosting habitats.

MATERIALS AND METHODS

Study sites

Mixed fruit orchards are commonly found around traditional villages in Southeast Asia. Each orchard consists of planted fruit crops and certain native tree species, as well as herb and shrub species. This multi-storied system thus resembles a forest in both structure and diversity. The main fruit trees are durian (*Durio zibethinus* L.), bitter beans (*Parkia speciosa* Hassk.), mangosteen (*Garcinia mangostana* L.), domestic jackfruit (*Artocarpus integer* (Thunb.) Merr.), longon (*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet), rambutan (*Nephelium lappaceum* L.), and mango (*Mangifera indica* L.). Durian, bitter bean (Bumrungsri et al. 2008, 2009), *Oroxylum indicum* (L.) Kurz (Srithongchuay et al. 2008), *Musa acuminate* Colla (Itino et al. 1991), and *Ceiba pentandra* (L.) Gaertn (Lobo et al. 2005; Nathan et al. 2005) are bat-pollinated and nectar-feeding bats commonly forage at the flowers of these species (Bumrungsri et al. 2013; Stewart et al. 2014).

Mixed fruit orchards in southern Thailand are distributed among forest patches. The study took place from September 2012 to June 2013, using 20 mixed fruit orchards situated at varying distances from 10 forest patches in southern Thailand (Nakhon Si Thammarat, Phattalung, Trang, Satun and Songkhla provinces; 6°20'to 8°20'S and 99°40' to 110°00'E). The actual size of the ten patches of tropical rain forest, excluding rubber and oil palm plantations, ranged in area between 3.6 to 650 km2 and occurred at altitudes between 230 to 1,090 m. We determined forest patch size, distance from each orchard to the nearest forest edge, and distance from each orchard to the nearest cave using 1:133 400 scale photographic imagery from Landsat Thematic Mapper data with a geographic information system (ARC GIS 10.2).

For each forest patch, we selected a pair of orchards (one near to and one far from the forest patch) that were managed without pesticide use. We used pollinator foraging distances to determine the cut-off distances for "near" and "far" orchards. Since previous work indicates that the mean foraging distance of local pollinator species ranges between 2-7 km (1,973 km for a stingless bee; 1.7-6.9 km for *Rousettus* bats (Wahala & Huang 2005; Bonaccorso et al. 2014); 4.4 km for *Eonycteris spelaea* bats, (Acharya et al. 2015), we classified orchards as 'near' if they were < 1 km away from the nearest rain forest patch and as 'far' if they were > 7 km away from rain forest. All pairs of orchards were at least 10 km apart. The distance from each study orchard to the nearest cave (potential roosts for nectarivorous bats and some frugi-nectarivorous bat species, such as *Rousettus* bats) ranged from 0.7 to 29 km (mean distance to caves ± SD: 9.42 ± 7.24 km). Bat roosting caves were identified from (Bumrungsri 1997) and the Shepton Mallet Caving Club (http://www.thailandcaves.shepton.org.uk).

Sampling the plant communities

In each study orchard, we marked a 50 × 150 m plot in which we set up 5 parallel 150-m transects at intervals of 10 m. We surveyed the plant communities from January 2012 to June 2013 by recording every individual of all flowering species in the study orchards every month. We counted the number of floral units (either individual flowers or capitula) for each plant. We determined the mean number of flowers in a capitulum from 20 capitula. We estimated the number of individuals of each plant species in each orchard by multiplying plant density (determined from the marked plot) by the total area of the orchard. Additionally, we calculated the total number of flowers by multiplying the number of individual plants by the mean number of open flowers for each plant.

Sampling the flower-visitors

To identify flower visitors and understand how the network of interaction changes with the proximity to forest, flower visitor observations were conducted monthly from April 2012 to June 2013. This was done in calm weather (i.e. sunny and without rain with the temperature ranging from 31°C to 38°C). In each orchard, we observed flower visitors while walking the five 150 m transects described above. Sampling took place 0800 – 1100 h and 1500 to 1830 h, recording both visitor frequency and visitor richness. We only collected data on insects coming into contact with the reproductive parts of the flower. For each plant species, pollinator observations were focally conducted from the four cardinal directions using 15 min observation sessions. Insects were collected with a long-handled net up to a height of 2 m and transferred to a euthanizing bottle containing ethyl acetate. Insects were identified from field guides or by professional taxonomists (see Acknowledgements). Insects that could not be identified to species were morphotyped (Memmott et al. 1993).

For nocturnal pollinators such as bats and moths, we used video cameras set to record for 15 min every hour from 1900 h to 0500 h. Because it is difficult to identify bats to species from camera traps, we also mist-netted at each site to identify the local species, allowing us to confirm our video identification. The mist nets were placed close to the flowering trees to avoid capturing the bats that visit to other fruit trees in the same orchard. Bats were identified to species following Francis (2008), mainly from external morphology and size. We categorized fruit bats into two groups, nectarivorous (*Eonypteris spelaea* (Dobson), *Macroglossus minimus* (Geoffroy), *M. sobrinus* (Andersen)) and frugi-nectarivorous (*Cynopterus brachyotis* (Muller), *C. horsfieldi* (Gray), *C. sphinx* (Vahl) and *Megaerops ecaudatus* (Temminck)), following criteria in Stewart et al. 2014.

Constructing the flower-visitation networks

The overall pollination network structures across all seasons were visualized using the bipartite package implemented in R (ver. 2.13.0, R. Development Core Team 2011 http://www.R-project.org). For each network, interactions were summarized as a bipartite matrix, with each cell containing the frequency of the pairwise interaction between a plant and animal species. To assess the abundance of each bat group (nectarivorous vs. frugi-nectarivorous), we summed all bat sightings captured by camera traps at each plant species. We then calculated a network strength value for each bat group by summing the dependencies across all plant species with which a bat group interacted. Dependency is calculated as the proportion of interactions performed by each animal species (Bascompte et al. 2006).

Statistical analyses

We used generalized linear mixed models (GLMM) to examine the effect of distance to forest edge (near vs. far), distance to the nearest cave, and bat group (nectarivorous vs. frugi-nectarivorous) on both bat abundance and network strength of each bat group in the pollination networks. We modelled the residuals with a normal distribution. Distance to forest, distance to nearest cave, and bat group were included as explanatory variables. Study orchard pairing was treated as a random factor. To determine the best predictive model, we selected the GLMM with the lowest AIC score.

RESULTS

Overall, we recorded 61 species of plant. The five bat-pollinated plant species were visited by 87 species of insect, 2 species of bird and 7 species of bat. Hymenoptera were common visitors to both orchard types; within this order, 32 species belonged to the family Apidae. Bats contributed to 0.2% to 0.4% of all visits at orchards near the forest and 0.3% to 0.8% at orchards far from the forest. We netted 553 individuals of six fruit bat species (193 *E. spelaea*, 81 *Macroglossus sobrinus*, 52 *Rousettus amplexicaudatus*, 30 *R. leschenaulti*, 29 *Cynopterus horsfieldi*, 126 *C. sphinx* and 46 *C. brachyotis*) during 480 hours of mist-netting. One pair of bipartite matrix, interaction between a plant and animal groups are shown in Fig. 1.

The model that best described bat abundance included negative effect of distance to cave ($P < 0.001$), bat group ($P < 0.001$), and the distance to cave x bat group interaction ($P = 0.024$) (Tab. 1). The overall abundance of nectarivorous bats (mean $\pm$ SD = 238 $\pm$ 81.41 visits) was greater than that of frugi-nectarivorous bats (155.2 $\pm$ 33.58 visits). Additionally, abundance was negatively correlated with distance from the nearest cave for both nectarivorous and frugi-nectarivorous bats. The negative correlation was much more pronounced in nectarivorous than frugi-nectarivorous bats, demonstrating the significant distance to cave x bat group interaction (Fig. 2).

The model that best described network strength included distance to the forest edge ($P = 0.01$), distance to the nearest cave ($P = 0.23$), and bat group ($P = 0.001$) (Tab. 1). The network strength of nectarivorous bats (0.83 $\pm$ 0.54) was greater than that of frugi-nectarivorous bats (0.41 $\pm$ 0.22). Since there is no bat group x forest interaction or bat group × cave interaction, we analyzed all bats together. Network strength of all bats was negatively correlated with both the distance to forest and distance to cave (Tab. 1).

DISCUSSION

Through the use of pollination networks, we demonstrate that nectar and frugi-nectarivorous bats differ in their impact on bat-pollinated plant species within southern Thailand's mixed fruit orchards. Additionally, these two groups are differentially affected by distance to caves and forests. Both bat groups are strongly integrated into pollination networks, playing important roles in the networks where they occur. However, nectar bats were more important than frugi-nectarivorous bats (higher network strength), and plants received more visits from nectar bats than frugi-nectarivorous bats. This pattern may result from foraging strategies; nectar bats only forage on floral resources, while frugi-nectarivorous bats predominantly forage on fruit resources (Stewart et al. 2014). Moreover, nectar-specialist *E. spelaea* has strong fidelity to its foraging area and visits the same area each night (Acharya et al, 2015), whereas frugi-nectarivorous bats visit up to six feeding areas each night (Bumrungsri, 2002). Additionally, species-specific morphological traits may constrain the opportunity for interactions between bats and flowers. Nectar-specialist *Eonycteris* and *Macroglossus* species have elongated rostrums and tongues. In contrast, the other (frugi-nectarivorous) bat species have relatively robust rostrums and short tongues (Bumrungsri et al. 2008, 2013; Francis 2008; Hodgkison et al. 2004; Marshall 1983, 1985). Consequently, tubular flowers (e.g. *Musa, Oroxylum*) are more likely to be visited by nectar-specialist bats (Srithongchuay et al. 2008; Fleming et al. 2009; Stewart et al. 2014), while frugi-nectarivorous bats may be more likely to visit flowers with "shaving brush" morphologies (e.g. *Parkia*) because tubular shaped corollas limit frugi-nectarivorous bat access to flowers.

Nectar bats showed higher network strength in orchards closer to the caves. Our study indicates that there is a higher abundance of bats visiting flowers near caves, and this emphasizes the role of caves as sources of pollinators for surrounding trees. We found that the most abundant bat species was the nectar bat *E. spelaea*, which roosts in caves (Bumrungsri et al. 2009), although most other pteropodid bat species roost in foliage (with the exception of cave-roosting Rousettus bats; Campbell et al. 2006, Kunz & Fenton 2003). Previous studies have shown that *E. spelaea* is the main pollinator of *Parkia* (Bumrungsri et al. 2008; Acharya et al. 2015), durian (Bumrungsri et al. 2009), and *Oroxylum indicum* (Srithongchuay et al. 2008). Furthermore, we previously found that bat visitation to durian flowers is significantly negatively correlated with distance to the nearest cave. Additionally, the number of durian fruits set per inflorescence was not significantly affected by distance to forest, but it was influenced by distance to the nearest cave (Sritongchuay et al 2016). In our current study, by examining the entire plant community, we
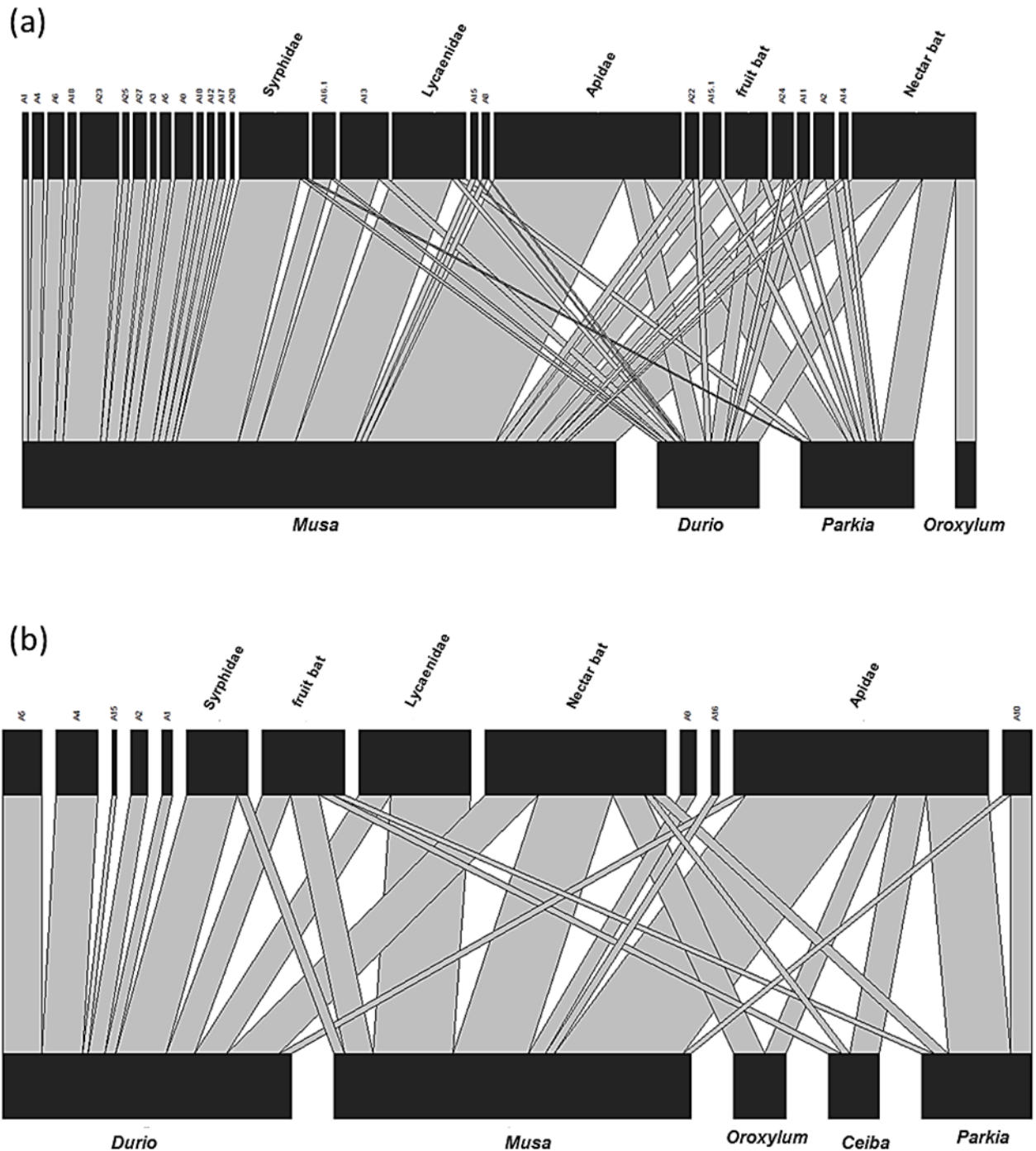
FIGURE 1. Quantitative pollination networks at mixed fruit orchards in southern Thailand (A) near the forest edge and (B) far from the forest edge. For each web, lower bars represent plant abundance and upper bars represent animal visitor abundance. Linkage width indicates frequency of each plant-animal interaction.

have also demonstrated that forests (not just caves), are important sources for bat pollinators. Similarly, previous authors have also found that the pollination success of chiropterophilous plants in the neotropics was affected by forest fragmentation (Stoner et al. 2002; Quesada et al. 2003, 2004).

Our findings emphasize how plant-bat interactions within mixed fruit orchards may undergo severe

transformations due to isolation from pollinator sources. Moreover, our study provides solid evidence that increasing the distance to pollinator sources limits the abundance and network strength of pollinators. In quantifying the impact of forest and cave proximity on pollination networks, our results can provide potential conservation recommendations concerning both plants and animals. Conservation practices aiming to preserve plant-pollinator interactions should promote the maintenance of both groups of bats and

TABLE 1.   Results of generalized linear mixed models for (A) bat abundance and (B) network strength of bat. Fixed effects include distance to the forest edge, distance to cave, and bat group.

| Explanatory fixed variable	Estimate	SE	t-value	P-value
A) Bat abundance (AIC = 84.21)				
Intercept	322.422	19.543	16.498	<0.001***
Distance to forest edge (Near)	-32.127	15.798	-2.034	0.0523
Distance to cave	-7.185	1.595	-4.505	<0.001***
Bat group	-134.079	26.229	-5.112	<0.001***
Distance to cave * Bat group	5.371	2.240	2.398	0.024*
B) Network strength of bat groups (AIC = 62.24)				
Intercept	1.251	0.146	8.555	<0.001***
Distance to forest edge (Near)	-0.294	0.105	-2.788	0.010*
Distance to cave	-0.029	0.012	-2.416	0.023*
Bat group	-0.676	0.174	-3.884	0.001**
Distance to cave * Bat group	-0.027	0.015	-1.861	0.074
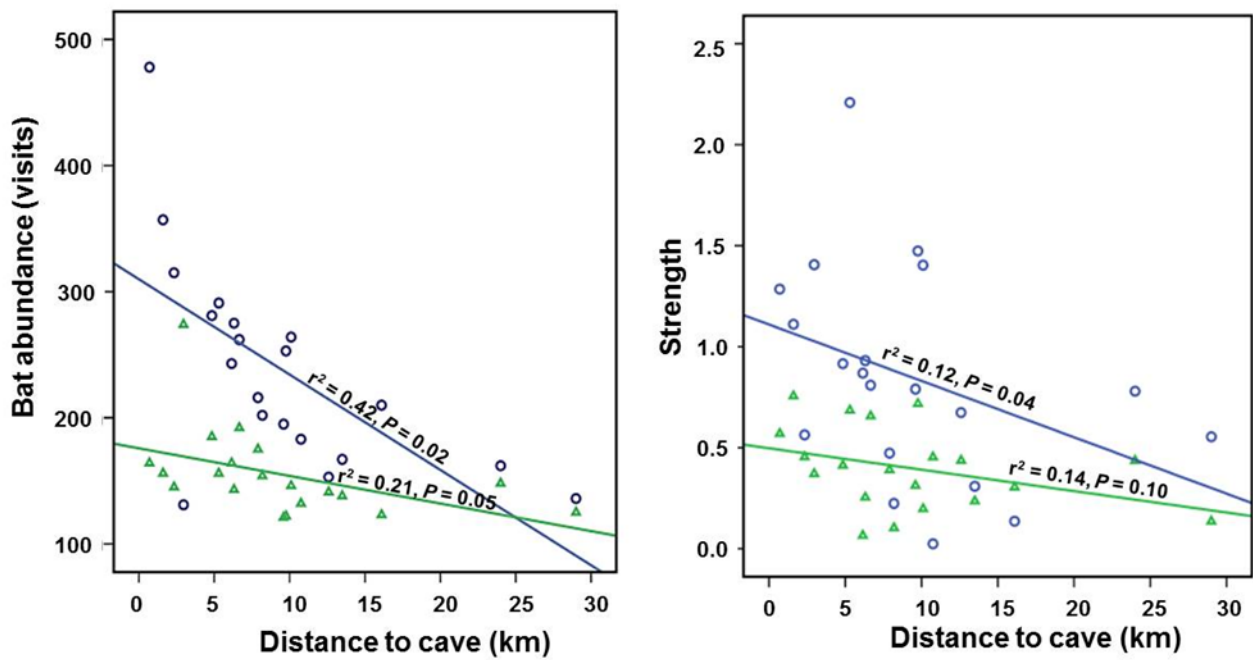
FIGURE 2.   The (A) abundance and (B) network strength of nectarivorous bats (blue circles) and frugi-nectarivorous bats (green triangles) plotted against distance from the nearest cave. Each point represents a single fruit orchard in southern Thailand.

specialist plant species (e.g., *Oroxylum indicum*). Moreover, to maintain pollinators in orchards, we recommend including plant species that flower year-round, such as *Musa*. Disseminating information about the ecological and economic significance of pollination services to farmers can help raise awareness about natural habitats and nectarivorous bats, which can promote local protection of forest and caves. This knowledge will further advance our understanding of how sustainable conservation policies and practices can be adopted.

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