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Pollinators Increase Reproductive Success of a Self-Compatible Mangrove, *Sonneratia ovata*, in Southern Thailand

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ABSTRACT.– *Sonneratia ovata* is a true mangrove species that is dwindling in numbers. To save this species, understanding its ecology and reproduction is necessary. We hypothesized that *S. ovata* is self-compatible but is more reproductively successful with biotic pollination. To confirm that, a pollination experiment was conducted and reproductive success was measured through pollination success, fruit set, and seed set. Floral visitors were observed. Pollination success in the hand-self treatment was higher compared to all other treatments, confirming its self-compatibility. However, pollination success was not significantly different for non-hand-pollinated treatments. Fruit set was higher in the animal-pollination treatments than in the spontaneous autogamy treatment, possibly due to herkogamy. Seed set results were similar to fruit set results for non-hand-pollinated treatments, indicating higher reproductive success when animal-pollinated. Only *Macroglossus minimus* was caught near *S. ovata* flowers, suggesting that *S. ovata* may be dependent on a single species of bat for pollination. Therefore, it is important to protect, not only this mangrove species, but also their bat pollinators and the forests where these bats roost.

KEY WORDS: bat pollination; herkogamy, *Macroglossus minimus*; mangrove apple; pollination experiment

INTRODUCTION

Pollination failure may be one of the reasons of rare plant extinction and lack of regeneration in logged forests (Wilcock and Neiland, 2002). This can be exacerbated by habitat fragmentation (Aguilar et al., 2006) and is especially a problem in the tropics where 94 percent are animal pollinated (Ollerton et al., 2011). Although many of these plants can also reproduce without the aid of pollinators, animals bringing pollen from other conspecific flowers reduce the chance of long-term selfing that leads to inbreeding (Ollerton et al., 2011).

In the mangrove ecosystem, many genera are also pollinated by animals; such as *Laguncularia* spp., *Avicennia* spp., and the ambophilous *Rhizophora* spp. (Sánchez-Núñez and Mancera-Pineda, 2012). Additionally, *Sonneratia alba* Sm. and *S. caseolaris* (L.) Engl. were observed to be pollinated by birds, bats, and moths (Pandit and Choudhury, 2001; Stewart and Dudash, 2016; Nor Zalipah and Ahmad Fadhli, 2017) with both producing higher fruit set when animal-pollinated compared to the experimental treatment where all pollinators were excluded (Pandit and Choudhury, 2001; Coupland et al., 2006; Nor Zalipah and Ahmad Fadhli, 2017).

Sonneratia ovata Backer is a widespread true mangrove species (Tomlinson, 1986). However, only a few populations now exist, with few individuals in each population (Zhou et al., 2010). More importantly, Zhou et al. (2010) found that this species is genetically depauperate. Information about the ecology and reproduction of *S. ovata* is needed to understand this species better and prevent it from becoming threatened. Although other *Sonneratia* species' reproductive biology have been studied before, particularly in *S. alba* and *S. caseolaris* (Primack et al., 1981; Pandit and Choudhury, 2001; Coupland et al., 2005; Nor Zalipah and Ahmad Fadhli, 2017), there are no publications about similar studies on *S. ovata*.

The aim of this study is to find out about the reproductive biology of *S. ovata* by (1) conducting a pollination experiment to determine if the species is self-compatible and (2) observing flower visitors that potentially pollinate this mangrove species. We hypothesized that *S. ovata* is self-compatible, yet experiences increased reproductive success when animal pollinators (specifically, bats) promote outcrossing, as was found with *S. alba* and *S. caseolaris* (Nor Zalipah, 2014; Nor Zalipah and Ahmad Fadhli, 2017).

MATERIALS AND METHODS

Study site

This study was conducted in both the dry (April to August 2016) and rainy seasons (October 2016 to March 2017) at three sites in southern Thailand: (1) Khlong Kae and (2) Hua Tang in Satun province on the western side of the peninsula, bordering Malaysia, and (3) Koh Yor in Songkhla province on the eastern side of the peninsula. The populations of *S. ovata* in these study sites, and other areas visited, typically contained fewer than 20 individuals, except for Khlong Kae where a reforestation project was established.

Khlong Kae (6°32'46.5"N 100°03'46.1"E) is a tiny village very close to a river and is surrounded by a mangrove reforestation site that was planted primarily with *S. ovata* and *Rhizophora* trees (Nuevo Diego, pers. obs.). Some of the study trees were planted during reforestation efforts, but three occur naturally. The trees at this site are on soft, muddy ground that dries up when there is no rain. Unlike the hand-planted trees, the naturally occurring individuals have some roots that extend into the main river or its stream offshoots.

Hua Tang (6°37'09.9"N 100°04'48.7"E) is approximately 8 km from Khlong Kae. This area used to be an extensive mangrove forest but has now mostly been developed into an urban area. The trees used in this study are part of the remnants of that forest, and occur along a stream (containing water year-round) next to a local road.

In contrast, Koh Yor is an island that sits within Songkhla Lake. The study site (7°09'20.3" to 7°09'26.7"N 100°32'04.7" to 100°32'07.9"E) is located on the western side of the island, away from the influx of sea water that enters Songkhla Lake from the Gulf of Thailand. Pornpinatepong et al. (2011) categorized this area as a lake-water stagnation zone. The trees used in this study are submerged in low-salinity brackish water (Pornpinatepong et al., 2011) that is approximately 0.05-1.50 m deep throughout the year (Nuevo Diego, pers. obs.).

Study Species

The genus, *Sonneratia* (mangrove apple), consists of true mangrove trees. *Sonneratia ovata* is a pioneer tree that occupies firm mud along downstream estuaries at high intertidal zones, but still within salt-water influence (Polidoro et al., 2010; Salmo et al., 2010). It can survive in clay soil and can tolerate submersion in low-salinity water (Department of Marine and Coastal Resources, 2012). This species is distributed in Southeast Asia, China (Hainan Island),

FIGURE 1. *Sonneratia ovata* flowers. **(A)** unopened bud (bottom) and unopened bud with exerted style surrounded by red weaver ants (top) **(B)** fully opened flower **(C)** *Sonneratia ovata* fruits **(D)** *Macroglossus minimus*

Palau, north-eastern Australia, and southern Papua New Guinea (Duke and Jackes, 1987; Polidoro et al., 2010). It is fairly common within its range but has experienced extensive losses at the margins of its distribution (Polidoro et al., 2010). Hence, the IUCN Redlist classifies *S. ovata* as Near Threatened and recommends local area protection (Salmo et al., 2010).

Sonneratia ovata has solitary, hermaphroditic brush flowers that form cymes (Tomlinson, 1986). The flowers have no petals (Tomlinson, 1986; Duke and Jackes, 1987). Each flower has a single style surrounded by numerous shorter stamens (Figs. 1A and B) (Tomlinson, 1986; Duke and Jackes, 1987). This species is known to be pollinated by bats and possibly hawk moths (Tomlinson, 1986; Watzke, 2006).

Floral Biology

We observed *S. ovata* flowers at all three sites, noting the time the flowers completely open, anther dehiscence, anther abscission, anther-stigma distance (ASD), stigma receptivity, pollen viability, and nectar production (Supplementary materials 1-5).

Pollination Experiment

A pollination experiment was performed following modified methods used by Bumrungsri et al. (2009) and Nor Zalipah (2014). Five treatments were applied: (1) open pollination, where all pollinators were allowed to visit the flowers; (2) insect pollination, where flowers were placed in baskets with a mesh size of 3x3 cm; (3) hand-self pollination, where flowers were hand-pollinated using pollen from the same flower; (4) hand-cross pollination, where flowers were emasculated before anthesis and then hand-pollinated using pollen from conspecific trees; and (5) spontaneous autogamy, where flowers were bagged before anthesis to prevent any floral visitors. To protect hand-cross pollination, hand-self pollination, and spontaneous autogamy flowers from visitors, flowers were enclosed in nylon mesh bags (mesh size of 1x1 mm) throughout each flower's entire blooming period. These bags were supported by a light frame to prevent the bags from touching the flowers. Hand-cross and hand-self pollination treatments were conducted once between 2000h and 2100h. Zero to six flowers per treatment per tree was randomly chosen throughout each season, using 12 trees in the dry season (99 flowers) and 16 trees in the wet season (201 flowers).

To protect developing fruits from insect and small animal damage, all experimental flowers were enclosed in the same mesh bags described above (without the internal frame to keep the bags light) 3 days after the night of anthesis. Pollination success was calculated as the proportion of flowers surviving for at least 14 days, since most floral abortions were observed to occur within the first 2 weeks following anthesis.

All experimental flowers were checked weekly for abortions and maturity. Fruits are mature after 8-12 weeks, when they are easy to remove from their calyces, have a sour smell, and softer flesh. The seeds from all mature fruits were collected, dried, and counted. All fruits with seed damage (i.e., eaten by insects) were excluded from seed counting.

Floral Visitors

Bat visitation rates were observed for 31 camera trap nights using Moultrie M-1100i (trigger speed: 0.5 s, recovery time: 5.5 s) and D55 IRxt (trigger speed: 1.7 s, recovery time: 8 s) (Alabaster, USA). These were set up before sunset 1.5-3 m away from *S. ovata* flowers and removed early in the morning. To identify bats visiting the flowers, mistnets (2.6 x 9 m) were set as close as possible to *S. ovata* flowers for two nights from 1730h until midnight in Khlong Kae. The nets were checked at least once every 30 mins. The bats were identified using Francis (2008). Insects were observed in person and caught by hand (using forceps for ants and plastic bags for stingless bees).

Data Analysis

Three measures of reproductive success were used in this study: pollination success, fruit set, and seed set. Pollination success is the number of flowers that lasted more than 2 weeks divided by the total number of flowers subjected to treatments. On the other hand, fruit set is the number of mature fruits divided by the number of experimental flowers. Lastly, seed set is the number of mature seeds from all undamaged mature fruits. Thus, pollination success reflects the proportion of flowers receiving sufficient pollen to fertilize ovules, while fruit set shows how each treatment affects the offspring reproduction, and seed count reflects the number of ovules successfully fertilized.

R statistical environment 3.4.2 was used for all analyses with results presented as mean ± SE. Generalized linear mixed modeling (GLMM) using the package "lme4" (Bates et al., 2015) was performed to determine which predictors significantly affected each of our dependent variables (pollination success, fruit set and seed count). Pollination success and fruit set were analyzed with binomial distributions, and seed count was analyzed with a Poisson distribution. The fixed factors for each analysis were treatment, season, location, and any pairwise interactions, while tree individual was included as a random factor.

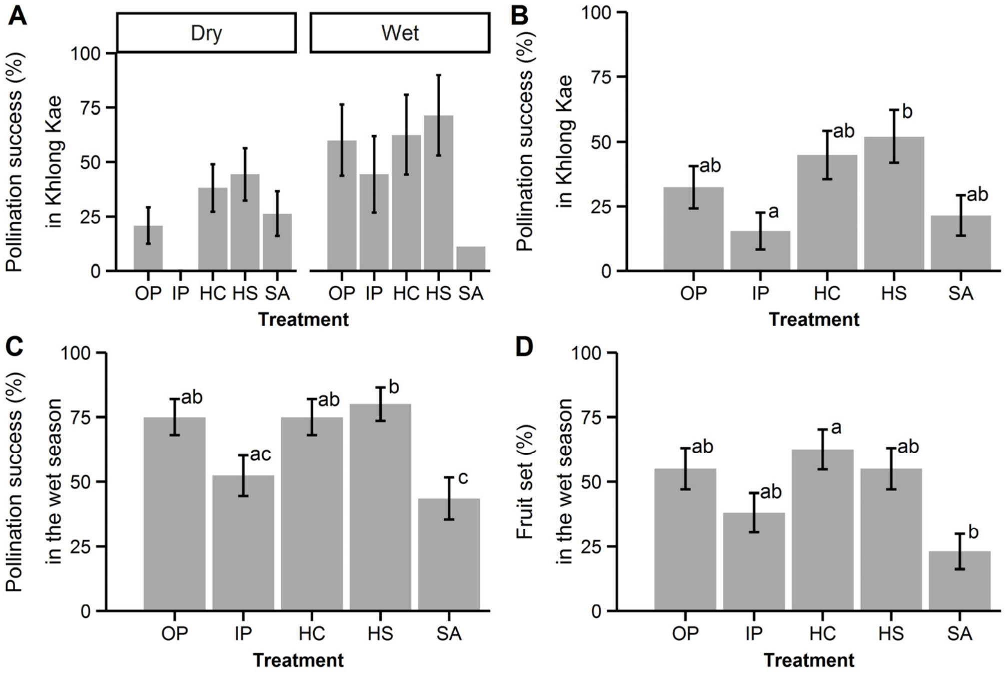

FIGURE 2. (A) Pollination success of *Sonneratia ovata* in Khlong Kae during the dry and wet seasons, where overall wet season pollination success was significantly higher than in the dry season. **(B)** Pollination success of *S. ovata* in Khlong Kae for both the dry and wet season combined. **(C)** Pollination success of *S. ovata* in the wet season. Data were pooled across all three study sites: Khlong Kae and Hua Tang (Satun Province), and Koh Yor (Songkhla Province). Pollination success is the percentage of fruits retained 2 weeks after floral anthesis. **(D)** Fruit set of *Sonneratia ovata* flowers during the wet season, where fruit set is the percentage of mature fruits (8-12 weeks old) collected out of all experimental flowers. Data were pooled across all three study sites: Khlong Kae and Hua Tang (Satun province), and Koh Yor (Songkhla province). Different letters denote significant differences (Tukey's test, $P < 0.05$). The treatments were: OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy. Image rendered in R statistical environment 3.4.2 using ggplot2 (Wickham, 2016)

Nested likelihood ratio tests were used to determine which model best fit the data, and model selection was verified by Akaike Information Criterion (AIC) scores. Tukey's tests using the package "emmeans" (Lenth et al., 2019) were used for posthoc testing.

RESULTS

Pollination Experiment

The model that best explained pollination success in Khlong Kae included both treatment (GLMM, $\chi^2_4 = 13.439$, $P < 0.01$) and season (GLMM, $\chi^2_1 = 7.716$, $P < 0.01$), but not their interaction (GLMM, $\chi^2_4 = 9.106$, $P = 0.585$). Wet season pollination success was significantly higher than that of the dry season (Tukey's test, $z = -2.647$, $P < 0.01$) when averaged over the treatments (Fig. 2A). Moreover, when averaged over the seasons, the hand-self pollination treatment was significantly greater than the insect pollination treatment (Tukey's test, $z = 2.858$, $P < 0.05$) (Fig. 2B).

On the other hand, examining pollination success for all three sites during the wet season revealed that the best model included treatment (GLMM, $\chi^2_4 = 19.736$, $P < 0.001$), but not location (GLMM, $\chi^2_2 = 5.190$, $P = 0.074$) nor their interaction (GLMM, $\chi^2_8 = 9.576$, $P = 0.296$). A post-hoc test (Tukey's) revealed that spontaneous autogamy (43.59 ± 8.04%) had significantly lower pollination success than open pollination (75.00 ± 6.93%; $z = 2.935$, $P < 0.05$), hand-cross (75.00 ± 6.93%; $z = 2.945$, $P < 0.05$), and hand-self (80.00 ± 6.41%; $z = 3.311$, $P < 0.01$) treatments. Additionally, hand-selfed flowers had significantly higher pollination success than insect pollination (52.38 ± 7.80%; $z = 2.774$, $P < 0.05$) (Fig. 2C).

Fruit set (Fig. 1C) was only found in the wet season. The model that best described fruit set included treatment (GLMM, $\chi^2_4 = 14.868$, $P < 0.01$), but not location (GLMM, $\chi^2_2 = 3.876$, $P = 0.144$) nor their interaction (GLMM, $\chi^2_8 = 7.399$, $P = 0.494$), where fruit set from the hand-crossed treatment (62.50 ± 7.75%) was significantly higher than from the spontaneous autogamy treatment (25.64 ± 7.08%) (Tukey's test, $z = 3.197$, $P < 0.05$) (Fig. 2D).

For all mature fruits, the mean total seed count per fruit was 355.39 ± 26.90 (N = 64 fruits from 14 trees). The model that best explained seed count included treatment (GLMM, $\chi^2_4 = 537.660$, $P < 0.001$) and the interaction between treatment and location (GLMM, $\chi^2_8 = 866.220$, $P < 0.001$), but not location alone (GLMM, $\chi^2_2 = 4.500$, $P = 0.105$). A pairwise comparison of treatments within each location revealed that most treatments were significantly different from each other in Khlong Kae (except for hand-cross pollination with both open pollination and hand-self treatment) and Hua Tang (except hand-cross pollination with insect pollination and hand-self treatment with open pollination). In Koh Yor, 5 out of 10 pairwise comparisons among treatments were significantly different; comparisons that were not significantly different consisted of hand-cross treatment with both open pollination and spontaneous autogamy, insect pollination with open pollination and spontaneous autogamy, and open pollination with spontaneous autogamy (Fig.3).

Floral Visitors

We caught only a single bat species during two nights of mistnetting near *S. ovata* flowers during the wet season: the dagger-toothed long-nosed fruit bat (*Macroglossus minimus* Geoffroy) (Fig. 1D). Nine individuals were caught each night; their different forearm lengths and weights indicate that they were all unique individuals, with the exception of one bat that was possibly a recapture. However, camera trapping caught fewer bats than mistnetting did. Out of 31 camera trap nights, the camera traps captured only 6 visitations (twice each for 3 different camera trap nights). In the dry season, 4 visits were captured and occurred once around 2300h, twice around 2400h, and once around 0200h. In the wet season, 2 visits were captured at around 2100h and 2300h. The bats appear likely to be *Macroglossus* individuals, based on morphology (e.g. small body size and long snout) and behavior (e.g. delicate fluttering, compared to the more laborious flight of other pteropodid bat species). However, as the bats caught by the camera traps were moving very fast, it was difficult to confirm their identification.

We observed stingless bees (*Lepidotrigona cf. ventralis* Smith and *L. cf. terminata* Smith), red weaver ants (*Oecophylla smaragdina* Fabricius), yellow crazy ants (*Anoplolepis gracilipes* Smith), small black

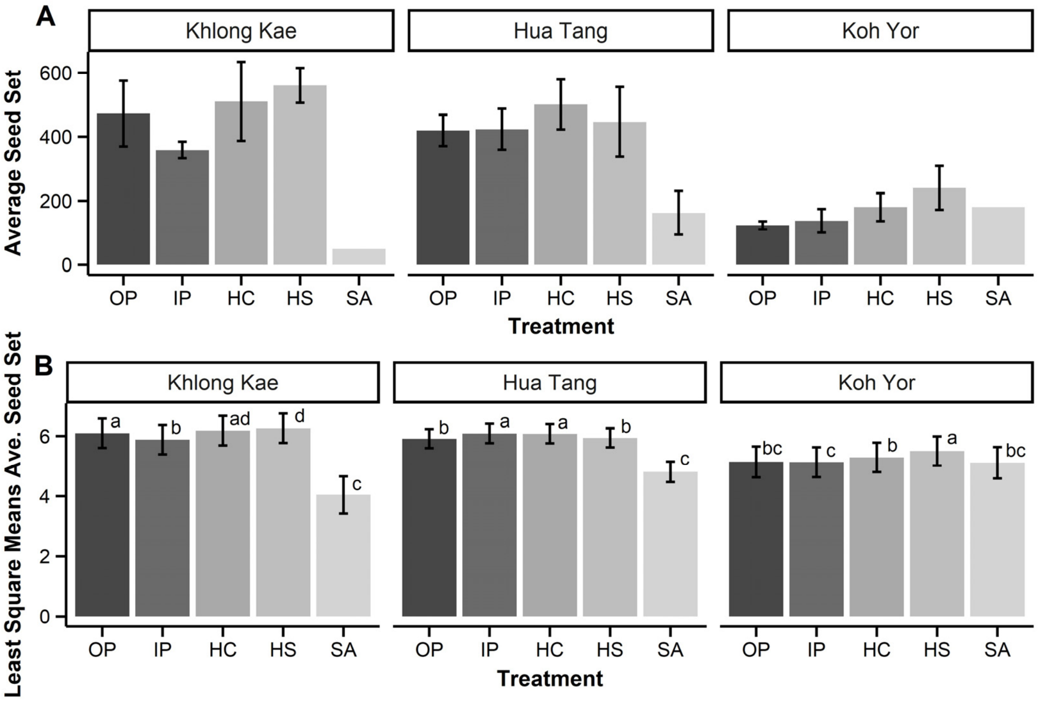

FIGURE 3. (A) Average seed set per *Sonneratia ovata* fruit (mean ± SE) for the five treatments: OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy conducted at 3 locations. **(B)** Least square means of average seed count per fruit (from generalized linear mixed model predictions) for the 5 treatments. Seed set was significantly affected by treatment as well as the interaction between treatment and location (GLMM). Pairwise comparisons were performed among treatments within each location. Different letters denote significant differences (Tukey's test, P<0.05). Image rendered in R statistical environment 3.4.2 using ggplot2 (Wickham, 2016)

ants, and sunbirds (family Nectariniidae) visiting the flowers daily. However, we never saw any of them touch both the stigma and the anthers. Stingless bees only collected pollen and red weaver ants generally stayed outside the flower, more interested in creating hives and farming aphids (family Aphidoidea). We rarely observed the yellow crazy ants but found some that were collecting nectar. The small black ants collected nectar at night from flowers that were manually emasculated, but not from non-emasculated flowers. The yellow crazy ants and the small black ants were never observed touching either the anthers or the stigmas. Additionally, sunbirds came early in the morning to forage on any remaining nectar. These birds drank nectar from one side of the flower and were never observed to touch the style. Additionally, we observed (but rarely) hawk moths (Family Sphingidae) and bees (possibly carpenter bees of Family Apidae) in the area, but did not see them landing on flowers.

DISCUSSION

Sonneratia ovata is unique in that it is considered a pioneer species, which are typically associated with high reproductive rates (Turner, 2004); yet is at risk of becoming endangered (Duke and Jackes, 1987; Polidoro et al., 2010), and has low genetic diversity (Zhou et al., 2010). Our results confirm that *S. ovata* is self-compatible but also reproduces through outcrossing. With such flexibility, its survival should be assured. However, our data shows that its reproductive success is dependent on both animal pollinators and environments with sufficient water.

Pollination success was higher in the wet season. However, the presence of El Niño during data collection may have intensified the situation, such that the wet season was very wet, while the dry season was very hot and dry (Climatological Center, Meteorological Development Bureau, 2017), resulting in zero fruit set during the dry season. Additionally, the high pollination success in hand-self treatments, compared to all other treatments (Figs.2A-C), suggests that *S. ovata* is highly self-compatible.

However, fruit set was higher in animal-pollinated flowers (open pollination and insect pollination treatments) than when pollinators were excluded (Fig. 2D). This may be due to the fact that *S. ovata* flowers display a xenogamous trait, herkogamy (Tomlinson, 1986; Duke and Jackes, 1987), where the style is longer than the anther filaments. In our study sites, the anthers and stigmas were separated by an average distance of 14.77 ± 0.82 mm (Supplementary material 2). Therefore, it is unlikely that even autogamous pollen can reach the stigma without the help of a pollinator. Supporting this hypothesis, the average seed set was also higher in animal-pollinated flowers than in bagged flowers at two of the three sites (Fig. 3). This implies that *S. ovata* reproduction fares better through biotic pollination.

Higher fruit set in flowers open to biotic pollination is not exclusive to *S. ovata*. Pandit and Choudhury (2001) also observed that *S. caseolaris* flowers that were accessible to animals had significantly higher fruit set than those that were not accessible to animals. Coupland et al. (2006) as well as Nor Zalipah and Ahmad Fadli (2017) also had similar observations with *S. alba* flowers, despite the results not being significantly different. These 2 species also display herkogamous floral morphology. However, in the latter study, the average seed sets of the resulting *S. alba* fruits were not necessarily higher in animal-pollinated flowers, similar to our results for *S. ovata* at the Koh Yor study site. Sutherland (1986) explained that higher seed set in self-compatible species may be due to lower genetic load: when inbreeding increases homozygosity, genetic load is reduced, and higher seed set is produced. If this hypothesis is true, then homozygosity in the Koh Yor population maybe higher than those in the other locations. Hence, higher seed set was produced from spontaneous autogamy flowers compared to both open pollination and insect pollination treatments, as well as from hand-self compared to hand-cross treatments ($P < 0.05$) in Koh Yor. This inbreeding in the population of Koh Yor may be because Koh Yor is an island inside Songkhla Lake and no *S. ovata* trees were observed along the mainland shores of the lake. Thus, cross-fertilization with other conspecific populations may have been more difficult for the Koh Yor population compared to the populations in Satun.

Although *S. ovata* trees were not very far away from other *Sonneratia* tree species that are known to attract different species of bats

(Watzke, 2006; Nor Zalipah, 2014), only the smallest macroglossid species, *Macroglossus minimus*, was caught near *S. ovata* flowers. A possible explanation is that *S. ovata* forms the smallest trees and has the smallest flowers compared to the other three *Sonneratia* species found in Thailand: *S. alba*, *S. caseolaris*, and *S. griffithii* Kurz (Department of Marine and Coastal Resources, 2012; Nor Zalipah, 2014; Nuevo Diego, 2018). Thus, the bigger bat species may have been more attracted to other *Sonneratia* species growing nearby.

Our study illustrates the importance of biotic pollination, even in this self-compatible species, which increases pollination success, fruit set, and seed set. It also demonstrates how this Near Threatened species may be dependent on a single bat species. We therefore recommend that, in addition to local area protection as suggested by Salmo et al. (2010), the bat pollinators of *S. ovata* and the forests where these bats roost should be protected as well.

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Supplementary materials

Supplementary material 1. Floral Biology of *S. ovata*

We observed *S. ovata* flowers (N = 14 trees) from April 2016-March 2017, noting the number of flowers per cyme, as well as the timing of blooming, anthesis, and anther abscission.

While individual *S. ovata* trees did not flower continuously, at the population level they almost continuously had flowers from April 2016-March 2017. However, we observed that *S. ovata* trees had few or no flowers during continuous days of rain or strong heat, and tended to flower more after the end of a rainy period.

Sonneratia ovata formed cymes composed of 1 to 5 flowers each (1.92 ± 0.11, N = 93 flowers from 14 trees). These white flowers bloom only for a single night, with 1 to 2 flowers per cyme blooming at a time. Only once did we observe all 4 flowers of a cyme bloom in the same night. Full bloom occurs around 1830h during the wet season and around 1930h in the dry season. Stamens start falling around midnight and have generally completely abscised by noon the following day.

Supplementary material 2. Anther-Stigma Distance in *S. ovata*

The white androecium of *S. ovata* is bowl-shaped with the style located in the center. The shortest stamens are closest to the style, while the longest stamens are around the perimeter and point outwards away from the style. To determine anther-stigma distance (ASD), we used a Vernier caliper to measure the distance from the stigma to the tip of the nearest anther in 15 randomly chosen flowers from 5 trees in the dry season, as well as 39 randomly chosen flowers from 10 trees in the wet season.

The mean length of the style is 35.05 ± 0.64 mm, while those of the stamens are 20.28 ± 0.55mm (short stamens) to 31.07 ± 0.50 mm (long stamens). The average ASD is 14.77 ± 0.82 mm (N = 54 flowers from 15 trees). The model that best explained ASD included season (GLMM, χ_1^2 = 7.1951, P = 0.00731), but not location (GLMM, χ_2^2 = 2.6061, P = 0.2717). The interaction between season and location could not be tested, as we only had data for both seasons at a single location. Stigmas are significantly closer to the nearest stamens in the dry season (ASD 8.48 ± 0.95, N =15 flowers from 5 trees) compared to wet season (ASD 17.19 ± 0.78, N = 39 flowers from 10 trees) (Tukey's test, t = -2.575, $P < 0.05$). This is because the center stamens (which are closest to the stigma) were longer (dry: 23.108 ± 0.78 mm; wet: 19.19 ± 0.62 mm) and the styles were shorter (dry: 31.59 ± 0.74; wet: 36.38 ± 0.74) in the dry season.

Supplementary material 3. Stigma Receptivity in *S. ovata*

We observed the stigma receptivity of 22 flowers from 12 trees. Prior to full bloom, we emasculated and bagged the flowers to exclude all pollinators. We tested stigma receptivity using the hydrogen peroxide test (with 3% H_2O_2) following Kearns and Inouye (1993). With the aid of a magnifying glass, we checked each stigma for the presence of bubbles, which indicates receptivity. We tested stigma receptivity at approximately 1700h, 1900h, 2100h, and 2300h on the first night of blooming; at 0500h the following morning; and at 1900h and 0700h each day thereafter until the stigma was unreceptive or the flower abscised.

We observed that the stigmas of *S. ovata* were already receptive upon exsertion from the flower (Fig. 1A in main text), approximately 2 hours before anthers begin to unfold (around 1630h). We did not test receptivity before exsertion. After exsertion, the stigmas started browning at varying rates starting near the edges, before the entire surface turned brown. Brown areas no longer responded to the hydrogen peroxide test, indicating that these areas were no longer receptive. Unpollinated flowers remained receptive or partially receptive until the flowers fell on the second or third day.

Supplementary material 4. Pollen Availability and Viability in *S. ovata*

We checked for pollen availability by brushing a glass capillary tube against the anthers and checking it for the presence of any pollen grains. Data were collected from 56 randomly-chosen flowers (15 flowers from 3 trees in Khlong Kae during the dry season, as well as 41 flowers from 2 trees in Khlong Kae, 4 trees in Hua Tang, and 5 trees in Koh Yor during the wet season).

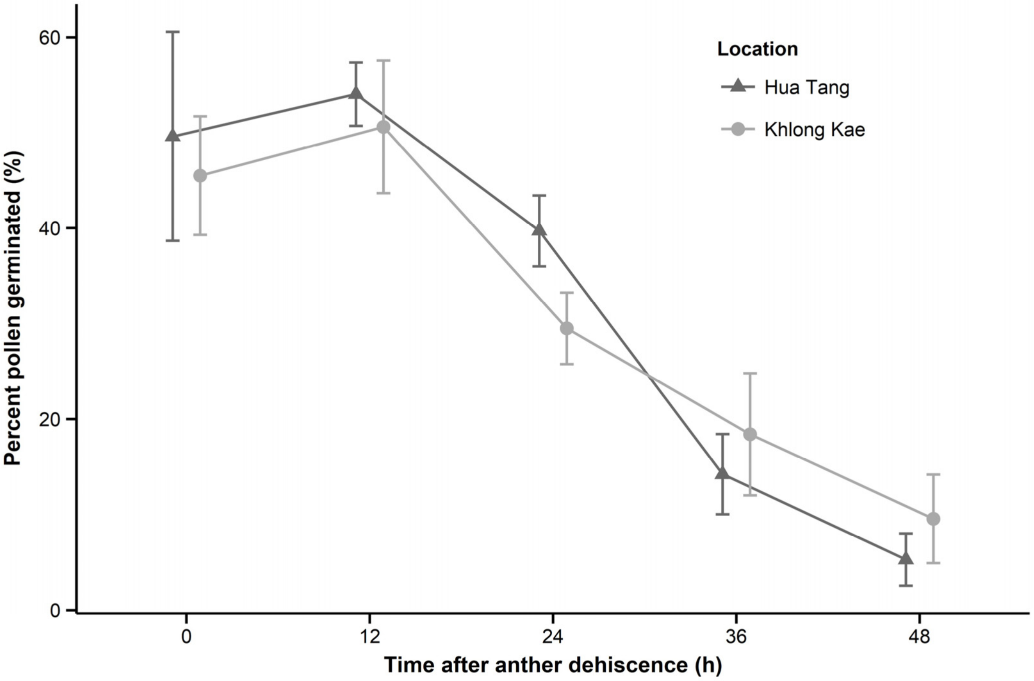
Anthers dehisced around 1930h to 2030h, approximately an hour after the flowers were completely open, when it was already dark. Pollen remained available on attached stamens until midmorning the following day.

To test pollen viability, we randomly collected 20 flowers from 9 trees in the afternoon before anthesis and allowed them to bloom under room conditions in the laboratory, following a modified version of Kearns and Inouye (1993). We placed a Petri dish under each flower to collect falling pollen. Between 2000h and 2100h, we collected pollen and mixed it into a drop of 15% sucrose solution on a microscope slide, then covered each drop with a cover slip before placing the slides upside down inside a humid, dark chamber. After 12 hours, we added a drop of basic fuchsin to the sample (which dyed pollen grains to increase visibility) and observed the slides under a microscope at 100x magnification. We calculated the percentage of germinated pollen grains at 10 non-overlapping fields of view per slide, each field of view containing 50-100 pollen grains. We repeated this method every 12 hours for five days, in 1 to 3 replicates per flower, and averaged the results for each tree.

A small proportion of pollen grains remained viable for up to 5 days in the laboratory, although percent germination started to decline rapidly from the first 12 hours (Supplementary Fig. S1). The highest percent germination observed for a single flower was 83.95%, from pollen collected at anther dehiscence. On average, percent germination remained close to 50% during the first 12 hours and decreased steadily thereafter, with only about10% germination for pollen collected 48 hours after anther dehiscence.

Supplementary material 5. Nectar Production in *S. ovata*

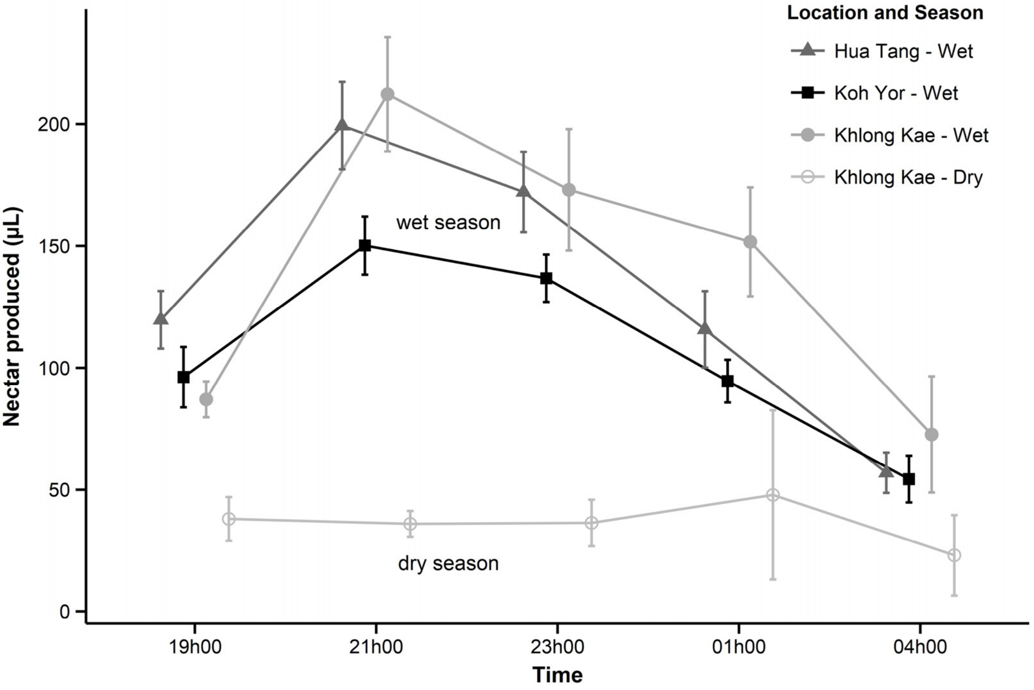
We collected nectar from the same flowers used to examine pollen availability (Supplementary material 4). We followed a modified version of the methods used by Bumrungsri et al. (2009), Kearns and Inouye (1993), and Nor Zalipah (2014). To prevent visitors from gaining access to the flowers, we enclosed them in nylon mesh bags before anthesis and kept them bagged throughout nectar collection. Using 75-µL microcapillary tubes, we collected nectar at 1700h, 1900h, 2100h, 2300h, 0400h, and 0700h during anthesis. We



SUPPLYMENTARY FIGURE S1. The percentage of *Sonneratia ovata* pollen that germinated (mean ± SE) in 15 percent sucrose solution when collected from 0 to 48 hours after anther dehiscence (N = 9 trees at 2 study sites in Satun, Thailand). Image rendered in R statistical environment 3.4.2 using ggplot2 (Wickham, 2016)

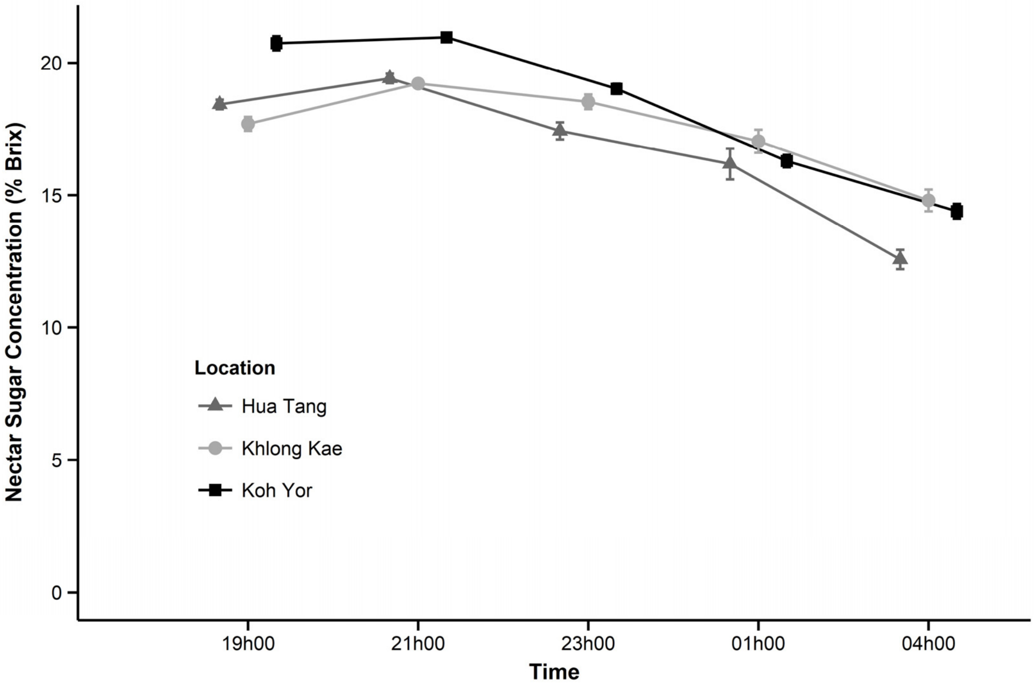
used an Atago N-1α hand refractometer (Tokyo, Japan) to measure sugar content. We estimated nectar volume by using a Vernier caliper to measure the length of nectar within the microcapillary tube, and then converted the length to µL, where 1 mm equals 1 µL of nectar.

Flowers generally began secreting nectar before they had completed blooming (N = 49 out of 52 flowers from 15 trees observed). The model that best explained the total amount of nectar produced included both season (GLMM, χ_1^2 = 16.514, $P < 0.001$) and location (GLMM, χ_2^2 = 10.022, $P < 0.01$). In Khlong Kae (the only site where nectar was collected in both seasons), the total amount of nectar produced during the dry season (316.95 ± 45.37 µL) was significantly lower (Tukey's test, z = -5.128, $P < 0.0001$) than in the wet season (616.38 ± 23.57 µL). Post-hoc analysis of differences across locations revealed that Hua Tang flowers produced significantly more nectar (695.88 ± 40.21 µL) than did Koh Yor flowers (537.72 ± 21.11 µL) (Tukey's test, t = 2.780, $P < 0.05$). Additionally, wet season nectar production peaked at around 2100h while there was no substantial peak during the dry season (Supplementary Fig. S2).



SUPPLYMENTARY FIGURE S2. Average amount of nectar per *Sonneratia ovata* flower (mean ± SE) collected at different times during anthesis; flowers from 3 locations were used during the wet season while only 1 location was examined during the dry season. Image rendered in R statistical environment 3.4.2 using ggplot2 (Wickham, 2016)

A comparison of nectar sugar concentration in Khlong Kae revealed no significant differences between seasons (GLMM, $\chi_1^2 = 0.0013$, $P = 0.9716$), we therefore pooled data across seasons. Nectar sugar concentration peaked around 2100h (approximately 20% Brix) and slowly decreased throughout the night (Supplementary Fig. S3). Further examination of the effect of location on nectar sugar concentration revealed this factor was significant (GLMM, $\chi_2^2 = 15.526$, $P < 0.01$), with the average nectar sugar concentration from Koh Yor flowers (18.36 ± 0.13) being significantly higher than from the flowers of both Khlong Kae (17.42 ± 0.28) (Tukey's test, $z = -3.241$, $P < 0.01$) and Hua Tang (16.81 ± 0.20) (Tukey's test, $z = -4.802$, $P < 0.01$).



SUPPLYMENTARY FIGURE S3. Average sugar concentration (mean ± SE) of *Sonneratia ovata* nectar collected at different times during anthesis. Season did not significantly affect sugar concentration, while location did. Koh Yor flowers had higher concentrations than both Khlong Kae flowers (Tukey's test, z = -3.241, P < 0.01) and Hua Tang flowers (Tukey's test, z = -4.802, P < 0.01). Image rendered in R statistical environment 3.4.2 using ggplot2 (Wickham, 2016)

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