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Modern honey bees disrupt the pollination of an ancient gymnosperm, *Gnetum luofuense*

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Bees belong to modern groups of pollinators, which originated concurrently with new angiosperms (flowering plants), and might have contributed to the global gymnosperm-to-angiosperm turnover in the mid-Cretaceous (125–90 million years ago [Ma]; Cardinal and Danforth 2013, Peris et al. 2017). Understanding the role that bees played in this turnover requires an exploration of angiosperm–bee associations as well as an understanding of the potential early interactions between gymnosperms and bees. Because bees are effective pollen collectors that depend on pollen for protein, and the unisexual gymnosperms produce numerous male strobili with plenty of exposed pollen, bees might have collected pollen from coexisting gymnosperm species to meet their nutritional needs (Saunders 2018). However, the gymnosperm–bee interactions from the fossil record

and their ecological and evolutionary consequences, if they ever existed, are still unknown.

Most gymnosperms are wind pollinated and thus rarely interact with pollinating insects, whereas the tropical genus *Gnetum* L. (Gnetaceae, Gnetales), with the oldest potential fossils from the Permian (270 Ma; Wang 2004), is a living gymnosperm clade that attracts a variety of insect visitors (van der Pijl 1953; Kato et al. 1995, Corlett 2001, Gong et al. 2016). However, these visitors are not necessarily pollinators for the dioecious *Gnetum*; because some insects may just “steal” rewards for their own benefit without conducting effective pollination (do Carmo et al. 1990, Hargreaves et al. 2009), which could only be achieved by insects visiting female strobili and simultaneously carrying and transferring conspecific pollen grains from males. Previous studies suggest that nocturnal moths visiting both male and female strobili for sugary fluids can be predominant pollinators of *Gnetum* (Kato et al. 1995, Corlett 2001). Recently, we have found that, unlike fluid-feeding moths, the Asian honey bees (*Apis cerana*) did not visit female strobili but frequently collected pollen from male strobili of *Gnetum luofuense* in a tropical rainforest in China (Fig. 1A). This unique interaction was regularly observed during daytime, when both sterile ovules on male strobili and ovules on female strobili had almost stopped secreting sugary fluids (Fig. 1A, B). Since diurnal pollen consumption may reduce pollen available for transfer by nocturnal pollinators, we hypothesized that pollen theft by honey bees is detrimental to pollination and reproduction of *G. luofuense*.

To test the hypothesis, we conducted field work and collected all data in a natural population of *G. luofuense* on Hainan Island. Our systematic honey bee censuses showed that *A. cerana* was absent before late April (no-bee season) but present during late April to early May (bee-present season). Then, in bee-present season, we quantified honey bee visitation rate, pollen loads on honey bees and pollen remaining in anthers after honey bee visits to assess the intensity of theft. Lastly, we compared pollen loads on pollinating moths, moth visitation rate and seed set between bee-present and no-bee seasons in order to measure the effects of theft on pollen transfer success and plant reproduction (see Appendix S1 for details).

Our two-season observations on the visitors of *G. luofuense* revealed that native *A. cerana* never visited female strobili (strobilus number × observation hours = 453 strobilus hours; Fig. 1B), but frequently visited male strobili at dusk (6,128 visits in 1,210 strobilus hours) and dawn (4,575 visits in 1,053 strobilus hours) for pollen collection, thus acted as pollen thieves (Fig. 1A). The larcenous visitations lasted regularly from 17:10 to 19:30 h and from 05:50 to 08:20 h. The number of honey bee visits per 10 minutes per strobilus was

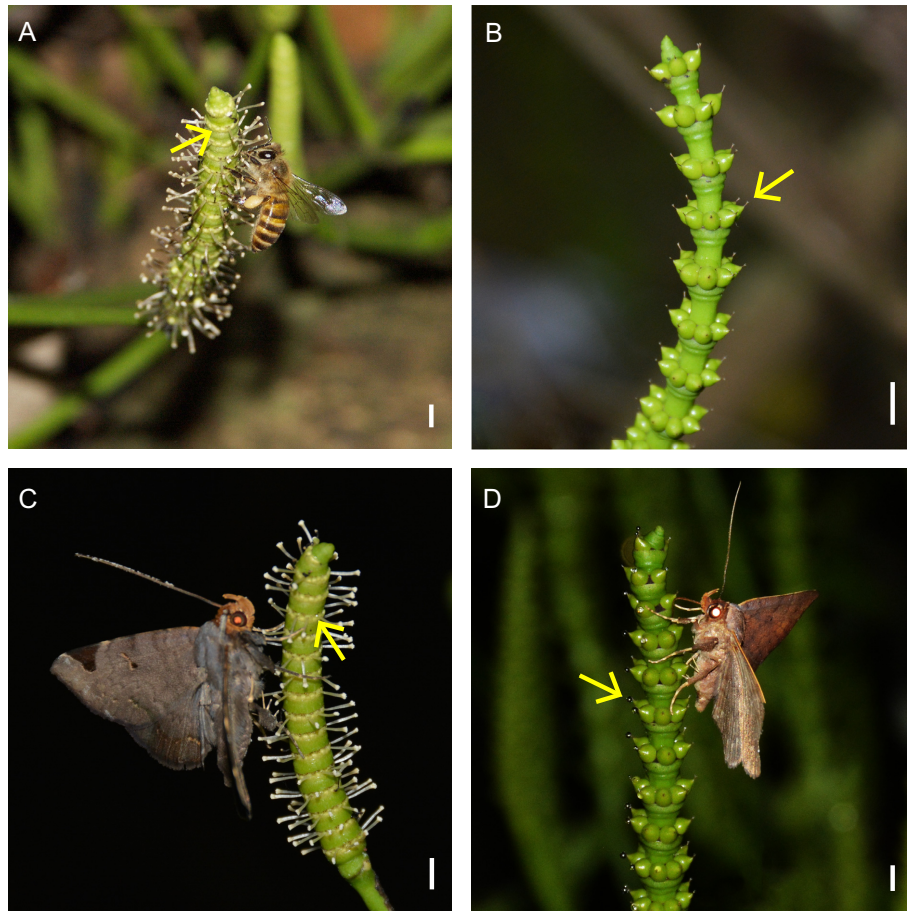


FIG. 1. Insect visitors and strobili of *Gnetum luofuense*. (A) An Asian honey bee *Apis cerana* collecting pollen from a male strobilus during daytime; the arrow shows sterile ovule contains no sugary fluid. (B) No insect visited this female strobilus during daytime; the arrow indicates ovule contains no sugary fluid. (C) A noctuid moth *Mecodina cineracea* visiting a male strobilus during the night; the arrow indicates sterile ovule contains no sugary fluid. (D) A moth species of Noctuidae visiting a female strobilus for sugary fluids emitted by ovule (arrow) at night. Scale bar in panels A–D is 5.0 mm.

greater at dusk (mean \pm SE = 0.98 ± 0.06 , $n = 172$) than at dawn (0.77 ± 0.06 , $n = 163$; $t = -2.56$, $P = 0.011$); however, dusk-visiting honey bees carried fewer pollen grains of *G. luofuense* ($6.1 \times 10^5 \pm 0.9 \times 10^5$, $n = 37$) on their bodies than did dawn-visiting honey bees ($14.5 \times 10^5 \pm 1.8 \times 10^5$, $n = 26$; t test after \log_{10} transformation, $t = 2.41$, $P = 0.020$). This is because pollen accessibility was more restricted at dusk than at dawn by the short-lived anthers (Fig. 2), which started to dehisce at about 17:00 and completely wilted within 21 h.

Under natural conditions, *G. luofuense* produced $7,428 \pm 80$ ($n = 105$) pollen grains per anther. After the first round of honey bee visits at dusk, the pollen remaining in each anther significantly decreased to 5594 ± 158 grains ($n = 60$), an average of 22.3% pollen loss after accounting for pollen dispersal by abiotic agents, which was insignificant during this period (see netted treatment; Fig. 2). As expected, nocturnal pollination also significantly affected pollen remaining in each anther (Fig. 2);

however, an average of only 2.1% of pollen production was removed by pollinators at night. In contrast, the second round of honey bee visits at dawn reduced pollen remaining in each anther to $2,638 \pm 147$ grains (Fig. 2), an average loss of another 25.4% of pollen production.

All the 13 moths collected on male strobili and 11 of the 12 moths collected on female strobili carried pollen grains of *G. luofuense* on their bodies, confirming that moths were effective pollinators (Fig. 1C, D). We found that moths carried significantly fewer pollen grains in bee-present season ($9.5 \times 10^3 \pm 5.6 \times 10^3$, $n = 12$) than in no-bee season ($31.2 \times 10^3 \pm 13.8 \times 10^3$, $n = 13$; ANOVA: $F_{1,21} = 6.59$, $P = 0.018$). There was no significant difference in moth visitation rate between bee-present (0.0145 ± 0.0044 visit per strobilus in 10 minutes, $n = 220$) and no-bee seasons (0.0147 ± 0.0040 , $n = 140$; Tweedie GLMM, $\chi^2 = 0.02$, $P = 0.877$). Furthermore, the presence of honey bees negatively impacted seed set (bee-present season

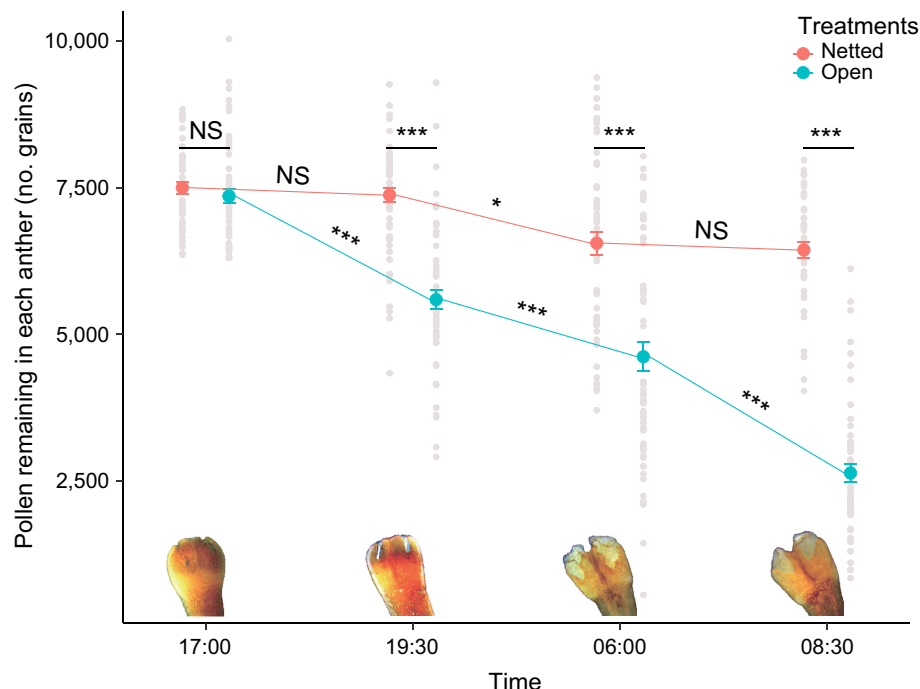


FIG. 2. Comparisons of pollen remaining in each anther between open-visited and netted treatments on *Gnetum luofuense* at four collecting times: 17:00 (before dusk honey bee visits), 19:30 (after dusk honey bee visits but before pollinator visits), 06:30 (after nocturnal pollinator visits but before dawn honey bee visits), and 08:30 (after dawn honey bee visits) on 27–28 April 2017. Error bars depict mean \pm SE and gray points depict pollen remaining in each anther. The status of anthers under natural conditions are presented above the x-axis (corresponding to the four collecting times). See Appendix S1 for details on statistics. *** $P < 0.001$; * $0.01 < P < 0.05$; NS, $P > 0.05$.

$n = 32.3\% \pm 3.8\%$, $n = 24$ vs. no-bee season $n = 42.7\% \pm 2.5\%$, $n = 25$; t test, $t = -2.30$, $P = 0.027$).

Our data suggest that diurnal honey bees disrupt the pollination of an insect-pollinated gymnosperm by reducing the pool of pollen available for nocturnal pollinators, resulting in decreased pollinator pollen loads and reduced seed set. These detrimental effects usually occur when effective pollinators are scarce (Lau and Galloway 2004) and/or pollen thieves are abundant (Hargreaves et al. 2010), both conditions that are met in our population. Despite the lack of quantitative data, honey bees were also described collecting pollen from *G. luofuense* in Hong Kong (Corlett 2001), and the visitation rate of moth pollinators to *Gnetum gnemon* in Malaysia (Kato et al. 1995) is as low as in our study. Together with the widely sympatric distribution of *A. cerana* and *Gnetum* species in tropical Asia (Hepburn and Radloff 2011, Ickert-Bond and Renner 2016), pollen theft by honey bees may be ubiquitous and its ecological consequences need to be investigated in more *Gnetum* populations.

In our population, dusk-visiting honey bees clearly act as pollen thieves, whereas honey bees at dawn are just pollen scavengers, because they collect pollen from anthers of *G. luofuense* that wilt before the pollination of the upcoming night. But given the remaining 35.5% of

pollen production in post-dawn anthers (Fig. 2), why do the anthers wilt so quickly? The reason may be that anther maintenance is costly of limited resources and contributes little to reproductive fitness (Ashman and Schoen 1994), it could be more profitable for *G. luofuense* to produce new anthers than to maintain existing anthers.

In addition to pollinator pollen loads, one may argue that the reduced seed set in bee-present season could also be explained by temporal variation in resource allocation (Dai et al. 2018). Unfortunately, we lack evidence to account for the potential effect of resource allocation on seed set, that is, *G. luofuense* may allocate fewer resources to female reproduction in bee-present season than in no-bee season. Further work should explore this confounding effect by comparing seed set of supplemental pollinated strobili between honey bee-present and no-honey bee seasons.

The nocturnal pollen presentation in *G. luofuense*, which moth pollinators may select for, could also be an evolutionary response to diurnal pollen thieves (escape in time; see Hargreaves et al. 2009). Similar anti-theft adaptation has also been found in bat-pollinated *Crescentia alata*, whose late-dehiscid anthers at night suffered less pollen theft by bees than early-dehiscid anthers (del Río and Bullock 1990). Other potential adaptations include diurnal wilting of anthers and

restricted pollen accessibility at dusk, both of which are able to mitigate the impact of daytime pollen theft. Although the evolutionary history of the interaction between *Gnetum* and native honey bees is unknown, the above adaptive traits suggest that pollen thief-mediated selection may play an unappreciated role in the evolution of *Gnetum* pollination systems.

Bees are among the most important pollinators but are also the most commonly documented pollen thieves of angiosperms (Hargreaves et al. 2009). For living gymnosperms, the collection of pollen by bees, a prerequisite for pollen theft, has been previously observed in all four lineages, namely wind-pollinated conifers and *Ginkgo* (Saunders 2018), specialist insect-pollinated cycads (Schneider et al. 2002), and generalist insect-pollinated gnetophytes (Corlett 2001). Furthermore, bees diversified in a period that included many pollen-providing gymnosperms (Ollerton 2017). We therefore propose that pollen theft by bees and its detrimental effects on pollination may be more common in extant and even extinct gymnosperms than previously thought.

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LITERATURE CITED

- Ashman, T.-L., and D. J. Schoen. 1994. How long should flowers live? *Nature* 371:788–791.
- Cardinal, S., and B. N. Danforth. 2013. Bees diversified in the age of eudicots. *Proceedings of the Royal Society B* 280:20122686.
- Corlett, R. T. 2001. Pollination in a degraded tropical landscape: a Hong Kong case study. *Journal of Tropical Ecology* 17:155–161.
- Dai, C., W.-J. Luo, Y.-B. Gong, F. Liu, and Z.-X. Wang. 2018. Resource reallocation patterns within *Sagittaria trifolia* inflorescences following differential pollination. *American Journal of Botany* 105:803–811.
- del Río, C. M., and S. H. Bullock. 1990. Parasitismo floral por abejas sociales (Meliponinae; Apidae) en el árbol quiropterófilo *Crescentia alata* (Bignoniaceae). *Botanical Sciences* 50:69–76.
- do Carmo, R. M., E. V. Franceschinelli, and F. A. da Silveira. 1990. Introduced honeybee (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* 36:371–376.
- Gong, Y.-B., M. Yang, J. C. Vamosi, H.-M. Yang, W.-X. Mu, J.-K. Li, and T. Wan. 2016. Wind or insect pollination? Ambiphily in a subtropical gymnosperm *Gnetum parvifolium* (Gnetales). *Plant Species Biology* 31:272–279.
- Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews* 84:259–276.
- Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2010. Native pollen thieves reduce the reproductive success of a hermaphroditic plant, *Aloe maculata*. *Ecology* 91:1693–1703.
- Hepburn, H. R., and S. E. Radloff. 2011. Biogeography. Pages 51–67 in H. R. Hepburn, and S. E. Radloff, editors. *Honeybees of Asia*. Springer, Berlin, Germany.
- Ickert-Bond, S. M., and S. S. Renner. 2016. The Gnetales: Recent insights on their morphology, reproductive biology, chromosome numbers, biogeography, and divergence times. *Journal of Systematics and Evolution* 54:1–16.
- Kato, M., T. Inoue, and T. Nagamitsu. 1995. Pollination Biology of *Gnetum* (Gnetaceae) in a Lowland Mixed Dipterocarp Forest in Sarawak. *American Journal of Botany* 82:862–868.
- Lau, J. A., and L. F. Galloway. 2004. Effects of low-efficiency pollinators on plant fitness and floral trait evolution in *Campanula americana* (Campanulaceae). *Oecologia* 141:577–583.
- Ollerton, J. 2017. Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics* 48:353–376.
- Peris, D., R. Pérez-de la Fuente, E. Peñalver, X. Delclòs, E. Barrón, and C. C. Labandeira. 2017. False blister beetles and the expansion of gymnosperm-insect pollination modes before angiosperm dominance. *Current Biology* 27:897–904.
- Saunders, M. E. 2018. Insect pollinators collect pollen from wind-pollinated plants: implications for pollination ecology and sustainable agriculture. *Insect Conservation and Diversity* 11:13–31.
- Schneider, D., M. Wink, F. Sporer, and P. Lounibos. 2002. Cycads: their evolution, toxins, herbivores and insect pollinators. *Naturwissenschaften* 89:281–294.
- van der Pijl, L. 1953. On the flower biology of some plants from Java with general remarks on fly-traps. *Annales Bogorienses* 1:77–99.
- Wang, Z. Q. 2004. A new Permian gnetalean cone as fossil evidence for supporting current molecular phylogeny. *Annals of Botany* 94:281–288.
- Yang, M., T. Wan, C. Dai, X.-C. Zou, F. Liu, and Y. Gong. 2021. Modern honeybees disrupt the pollination of an ancient gymnosperm, *Gnetum luofuense*. Figshare, data set. <https://doi.org/10.6084/m9.figshare.14673369.v1>

SUPPORTING INFORMATION

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OPEN RESEARCH

Data and R scripts (Yang et al. 2021) are available on Figshare: <https://doi.org/10.6084/m9.figshare.14673369.v1>.