

# Pollination and seed dispersal of *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae): An economic plant species with extremely small populations in China



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## ABSTRACT

Pollination and seed dispersal in angiosperms have long been investigated in order to understand the coevolution of plants and animals. However, the signals from flowers and/or seeds to attract pollinators and/or seed dispersers have received comparatively little attention. In this study, the pollination biology and seed dispersal of the vulnerable agarwood plant *Aquilaria sinensis* (Lour.) Gilg, a traditional medicinal plant in China, was studied in its natural distribution range. The reproductive tactics of *A. sinensis* were studied in detail by employing various tests dealing with fruit set and also seed dispersal. Dynamic headspace extraction followed by GC–MS analysis was also performed in order to reveal the composition of floral scent. The results showed that noctuids and pyralids are the most effective pollinators of pollinator-dependent *A. sinensis*. The main compounds of the floral scent were (*E*, *E*)- $\alpha$ -Farnesene ( $61.9 \pm 3.2\%$ ), *trans*-Ocimene ( $16.6 \pm 1.2\%$ ), and Benzyl salicylate ( $4.6 \pm 1.1\%$ ). The results obtained from seed dispersal experiments indicate that hornets are effective seed dispersers and they may play an important role in long-distance seed dispersal of *A. sinensis*. Based on our findings, we recommend several protection methods for this threatened agarwood plant in China.

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Plant-pollinator relationships may be one of the most ecologically important classes of animal–plant interactions. Without pollinators, many plants cannot reproduce; and without plants as a source of pollen, nectar and other rewards, many animal populations would decline, initiating a series of consequences that would affect other species and the functioning of the ecosystem (Harder and Barrett, 2006; Ollerton et al., 2011; Thomann et al., 2013; Chen et al., 2015a,b). Moreover, pollination is thought to be a key factor in the diversification of some major groups of plants and animals (Smith, 2010; van der Niet and Johnson, 2012). In

addition, human reliance on animal-pollinated crops has demonstrated the value of the ecosystem services provided by pollination (Klein et al., 2007; Gallai et al., 2009; Ren et al., 2014). As far as endangered plant species are concerned, the negative effects of pollinator loss on endemic New Zealand mistletoes has been demonstrated previously (Robertson et al., 1999). Anderson et al. (2011) revealed that the cascading effects of bird functional extinction reduce pollination and plant density of endangered species. Pollen limitation of plant reproduction could deteriorate survival conditions of most threatened plants (Knight et al., 2005). In some small conservation areas, the collapse of the pollination web can negatively influence the reproductive fitness of local “3E plant” species (endangered, endemic, and economically important plant species) (Bennett, 2001; Pauw, 2007; Ma et al., 2013). Therefore, the interaction between threatened plant species and their pollinators should be emphasized in conservation biology.

Seed dispersal by animals is a complex mutualistic interaction involving a great diversity of plant and animal species with significant ecological and evolutionary consequences for the regeneration and survival of species in a plant community (Fenner and

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Thompson, 2005; Rico-Gray and Oliveira, 2007). To date, seed dispersal by all kinds of media has been studied in depth (Tiffney, 2004; Fenner and Thompson, 2005; Thorsen et al., 2009), however, the cause and effect of seed dispersal disruption has received comparatively little attention (Traveset et al., 2012; Rotllan-Puig and Traveset, 2015; Peres et al., 2016). For example, loss of animal seed dispersal increases the risk of extinction in tropical rain forests (Caughlin et al., 2015). The dispersal of seeds by animals can be disturbed significantly by human actions (Markl et al., 2012). It has been shown that the limitation of seed dispersal induces long-term biomass collapse in overhunted Amazonian forests due to the loss of primates as seed dispersers (Peres et al., 2016). Previous studies have suggested that deciphering the interactions between threatened plant species and their seed dispersers can lead to developing scientific conservation and restoration campaigns (Nathan and Muller-Landau, 2000; Sun, 2013). Therefore, as far as threatened plant species are concerned, researchers should explore the relationships between seeds and their dispersers deeply in order to conserve threatened ecological interactions.

The native Chinese *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae) is a tropical evergreen tree distributed in Hainan, Guangdong, Guangxi, Yunnan and Taiwan (Wang et al., 2007). This species is the principal source of Chinese agarwood (Chen et al., 2012). The resinous part of the injured *A. sinensis* tree forms agarwood, which is widely used as a sedative, analgesic, and digestive aide in traditional Chinese medicine (Zhang et al., 2012; China Pharmacopoeia Commission, 2015). Owing to the economic value and great demand for agarwood, the natural population of *A. sinensis* has declined severely and is presently listed as a vulnerable species in China (Fu, 1992; IUCN, 2009). Currently, only a few wild individuals of *A. sinensis* can be found in China (Zou et al., 2012). Actually, most of these relict mature individuals grow in conservation areas (Tian et al., 2009; Zou et al., 2012). The species has been listed as one of the 20 PSESP (Plant Species with Extremely Small Populations) for urgent rescuing conservation by the Yunnan provincial government (Sun, 2013). Recently, a number of studies on *A. sinensis* have examined desiccation tolerance and storage characteristics, its threatened status, *ex situ* conservation, the formation mechanism of agarwood, photosynthetic characteristics and the serious pest *Heortia vitessoides* Moore (Su, 1994; Tian et al., 2009; Chen et al., 2011; Zhang et al., 2011; Yuan et al., 2012). However, *A. sinensis* pollination and seed dispersal have received comparatively little attention, even though the pollination biology of *Aquilaria crassna* and seed dispersal of *A. sinensis* by hornets was reported in recent studies (Soehartono and Newton, 2001; Hu, 2012; Manohara, 2013). Due to the importance of pollination and seed dispersal for *A. sinensis* conservation purposes, we studied the reproductive tactics of this vulnerable species.

## 1. Materials and methods

### 1.1. Study sites and plant materials

*Aquilaria sinensis* is a tall tree about 5–20 m. The flowers of *A. aquilaria* are yellowish-green with a diameter about 10 mm, the inflorescence is terminal, a loose panicle of umbels (Fig. 1). The flowers open at night and emit a strong fragrant odor. Obvious floral nectar can be found at the base of the petals. The main flowering time of *A. sinensis* is from April to May in China. Its fruiting time is from mid-June to the end of July (Wang et al., 2007). *A. aquilaria* seeds are dark brown, ovoid, ca.  $10 \times 7.5$  mm. The fruit is a bilocular leathery dehiscent capsule, with each lobe harboring a single seed. Mature fruits split longitudinally while they are still on the tree and the seeds remain hanging through thread-like structures (Fig. 1) for a few days after dehiscence. Each seed bears a

conspicuous white and yellow elaiosome attached to the base of the dark seed (Fig. 1).

In this study, the pollination and seed dispersal experiments were conducted in two locations, Shenzhen Fairy Lake Botanical Garden (FLBG: 22°34'43.1"N; 114°09'54.65"E; 412m), Shenzheng, Guangdong province and Jiangcheng (JC: 22°32'34.43"N; 101°42'6.68"E; 927 m), Yunnan province. The two locations are within the natural distribution range of *A. sinensis* (Wang et al., 2007). There are about 14,000 and 1997 mature individuals of *A. sinensis* planted in JC and FLBG, respectively. The pollination experiments of *A. sinensis* were conducted at JC in 2014 and 2015. The seed dispersal experiments were conducted at FLBG and Kunming Botanical Garden (KBG) in 2013 and 2014. Two voucher specimens of the species from each site have been deposited in KBG.

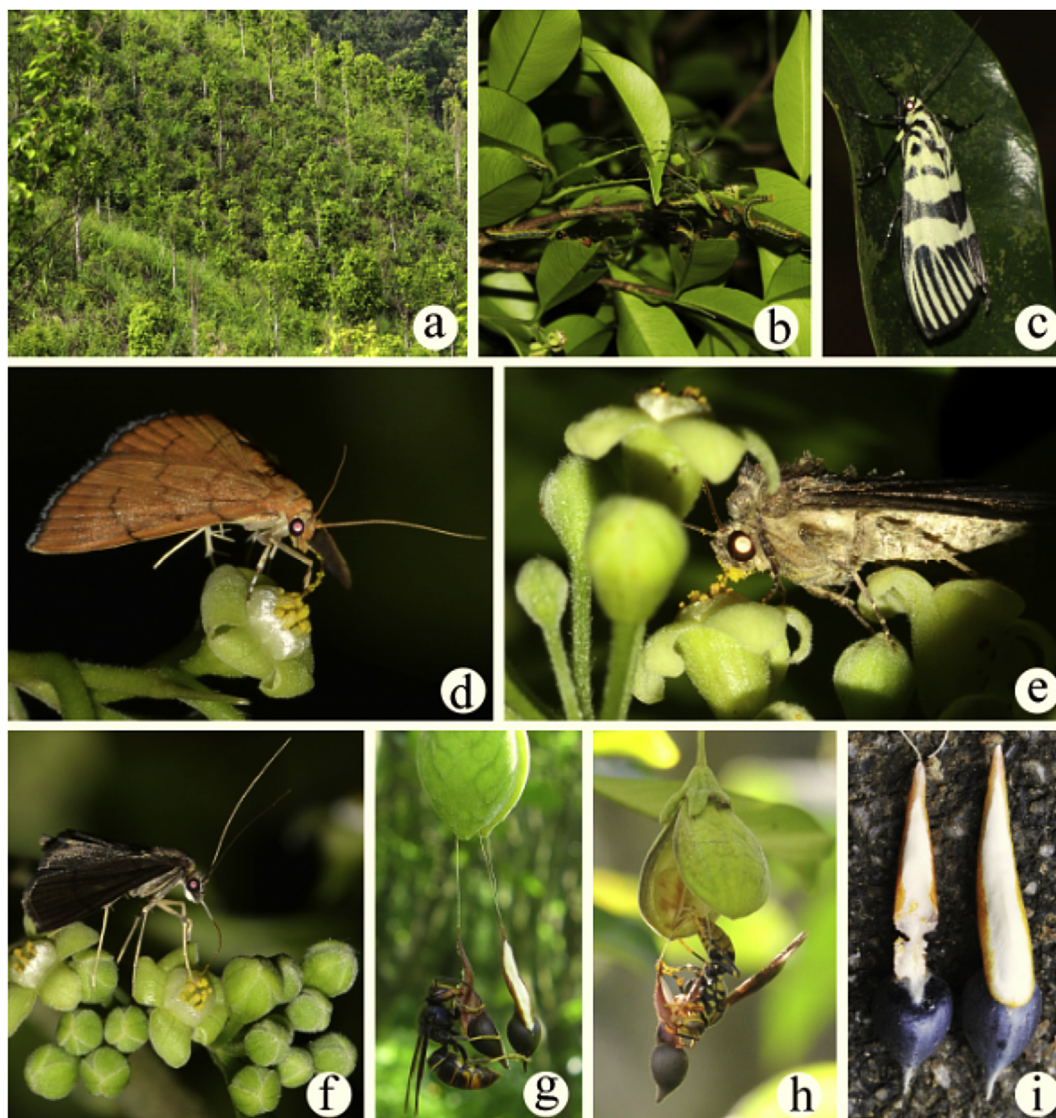
Fairy Lake Botanical Garden is located in Luohu district of Shenzhen, with Wutong Mountain's highest peak standing in the east, and the Shenzhen reservoir in the west, covering an area of 588 ha. The climate at this location is subtropical monsoon. The annual average temperature of FLBG is about 23.7 °C. The mean annual precipitation is about 1608.1 mm. Jiangcheng location is located in southern Yunnan province. It is near Laos and Vietnam. This location is in a subtropical zone with moist climate. The annual average temperature of JC is about 18.7 °C. The mean annual precipitation is about 2283 mm. Kunming Botanical Garden is located in northern Kunming, Yunnan province. This location is very different from the FLBG and JC locations. It is in a mid-subtropical zone with plateau-climate (from 25°07'04.9"N to 25°08'54.8"N, 102°44'15.2"E to 102°44'47.3"E and 1980m). The annual average temperature of KBG is about 14.7 °C. The mean annual precipitation is about 1006.5 mm.

### 1.2. Floral biology

To describe and determine the anthesis and longevity, respectively, of *A. sinensis*, 30 flower buds from five individuals (six buds per individual) at the JC location were tagged with thin inconspicuous green threads and monitored in May 2015. To determine if flowers of *A. sinensis* supply nectar to pollinators, 30 flowers at JC location were examined for nectar. Fully developed inflorescences were individually bagged with nylon nets to exclude floral visitors before the measurement of nectar took place. The floral nectar volume was determined with capillary tubes and the nectar sugar concentration was measured using a handheld refractometer (Sugar/Brix Refractometer, 0–50%; Bellingham and Stanley, Telford, UK). The reproductive success (fruit set) of *A. sinensis* was examined at the JC location in July in 2014 and 2015. Flower buds (578) from ten individual plants (five inflorescences per individual) were randomly selected, labeled, and enclosed in nylon nets to exclude flower visitors and allowed only spontaneous autogamous self-pollination in 2014. Untreated, open-pollinated flowers (563 flowers from 10 individuals) served as controls. The fruit set was recorded at the JC population about 60 d after the main flowering peak. The identical procedure was followed in 2015, using 612 flowers for spontaneous autogamous self-pollination and 602 flowers for open pollination, respectively.

### 1.3. Flower visitor observations

The floral visitors of *A. sinensis* were observed at the JC location, which is situated within the natural range of this plant species. In 2014, the flowering period of the population started April 23rd and ended May 17th. The floral visitors were observed continuously during the flower opening time from 08:30 pm to 02:30 am for 5 days from May 1st to 5th. Flower visitor observations were conducted in parallel by three observers (3 × 2 individuals observed



**Fig. 1.** Plantation, pest, pollination and seed dispersal of *A. sinensis*. Plantation of *A. sinensis* at JC location (a); larvae and imago of *Heortia vitessoides*, an obligate pest of *A. sinensis* (b) and (c); three moth pollinators of *A. sinensis* (d–f); hornets attack diaspores of *A. sinensis* (g) and (h); discarded diaspores with partly eaten (left) and intact (right) elaiosomes of *A. sinensis* (i). Photos taken by G. Chen (a, b, c, e), Chang-Qiu Liu (d and f) and Meng Guo (g–i).

per day). In 2015, the flowering period lasted from April 19th to May 26th, and observations were carried out from May 3rd to 7th by two observers using the same observation protocol as in 2014. During these observations the behavior of the flower visitors, such as movement on the flower and contact with floral organs, was recorded and it was noted whether flower visitors were carrying pollen on their proboscises or bodies. Some flower visitors were caught and preserved for later identification by insect taxonomists.

#### 1.4. Collection and analysis of floral volatiles

Scent samples of three *A. sinensis* inflorescences were collected between 9:30 pm and 00:30 am at the JC locality, which coincided with the time of floral scent emission. Floral scent was collected using dynamic headspace adsorption by enclosing a single newly opened inflorescence with a Tedlar bag (Dupont, USA). The scent was drawn from the bag into a tube containing the adsorbent Porapak Q (150 mg, mesh 60/80, Waters Associates, Inc.) using a pump with an inlet flow rate of 350 mL min<sup>-1</sup>. Trapped VOCs were eluted with 300 µL dichloromethane (99.5%) and concentrated to

one-fifth of the original volume by a gentle stream of nitrogen (200 mL min<sup>-1</sup>). Next, 3 µg *n*-nonane was added as an internal standard to each sample for quantification. Volatiles were also collected from natural branches without flowers as control to identify any background compounds in floral scent samples.

The extracts were analyzed using an Agilent Technologies HP 6890 gas chromatograph, equipped with a HP-5MS column, and linked to a HP 5973 mass spectrometer. Helium was used as a carrier gas at a flow rate of 1 mL min<sup>-1</sup>. Split inlet and FID were held at 250 °C. The column temperature was programmed to rise from 40 °C (5-min hold) to 250 °C (20-min hold) at 3 °C/min. Compounds were tentatively identified by comparing their MS spectra and relative retention times with that provided in the Wiley 7n.1 mass spectral library and their relative proportions (%) were determined by calculation of the peak area. For some compounds, identification was confirmed by comparing their GC retention time and mass spectrum with those of standard compounds purchased from Sigma–Aldrich, USA. The mean amounts of floral scents from *A. sinensis* were counted in SPSS 13.0 software (SPSS Inc., Armonk, NY, USA).



### 1.5. Seed dispersal of *A. sinensis* by hornets

A mature capsule of *A. sinensis* often includes two diaspores. When capsules dehisce, the diaspores are exposed to air, awaiting potential dispersers (Fig. 1). Without dispersers, the diaspores will fall down on the ground via gravity. To calculate the average mass of a fresh mature diaspore, 30 diaspores from different individuals were weighed with an analytical balance.

A previous study observed that different species of hornets visit *A. sinensis* diaspores (Hu, 2012), with 99% of these hornets belonging to the genus *Vespa*. Hornets were found to visit more than 72.1% of *A. sinensis* seeds (Hu, 2012). However, seed dispersal distance and seed destiny were not examined. In our study, 84 and 57 capsules from various individuals of *A. sinensis* were observed in July 2013 and 2014 at the FLBG location, respectively. The foraging behavior of hornets on *A. sinensis* diaspores was recorded. During weather conducive to hornet activity, 6–17 fresh mature capsules were observed between 10:30 am and 05:00 pm. The total observation period continued nine days in 2013 and five days in 2014. Visitors of 93 randomly selected diaspores were recorded in order to identify effective seed dispersers; 57 in 2013 and 36 in 2014. The dispersal distance of 40 diaspores was evaluated by meter ruler and Zeiss 8 × 30 binoculars. The flight paths of the foragers were tracked and the rough dispersal distance was measured.

### 1.6. Seed or the elaiosome of *A. sinensis* attract hornets

To compare the relative importance of different parts of the diaspores to the hornet *Vespa velutina*, behavioral experiments were conducted at KBG. All bioassays were conducted between 11 am and 4 pm, a time of peak patrolling activities for *V. velutina* foragers. The removal rates of whole diaspores, seeds or elaiosomes by hornets were recorded at the KBG within a flight cage ( $8.6 \times 6.4 \times 2.4 \text{ m}^3$ ). A table was placed in the center of the flight cage and was used as a foraging area for the hornets. Three dishes (diameter 10 cm) each containing 10 diaspores, seeds or elaiosomes were placed on top of the table. To avoid location effects, the position of the dishes was changed every 5 min. Each test lasted 30 min and the bioassays were performed 11 times. Four nests of *V. velutina* were used in this study in 2014. To identify whether hornets destroyed the seeds of *A. sinensis*, 20 recovered seeds from the flight cage were examined microscopically for evidence of seed coat scarification. To investigate whether the seeds were kept in nests of *V. velutina*, 80 diaspores were fed to hornets from four nests in a flight cage in September in 2014. After the experiment was finished, the nests were opened to determine if any seeds had appeared in the nests.

## 2. Results

### 2.1. Floral biology of *A. sinensis*

Flowers of *A. sinensis* typically opened between 08:30 pm and 02:30 am for around one day. The average nectar volume was  $1.2 \pm 0.1 \mu\text{L}/\text{flower}$  ( $n = 30$ ), and the nectar concentration was  $10.1 \pm 0.2\%$  ( $n = 30$ ). The natural fruit set was 11.4% and 8.6% at the JC location in 2014 and 2015, respectively. However, the field experiments showed that spontaneous self-pollination did not occur since bagged flowers at JC location did not set fruits.

All 728 flower visitors observed in 2014 and 2015 at the JC population were moths from three families: Noctuidae (314), Pyralidae (263), and Geometridae (151). Most of them (611 individuals, 83.9%) belonged to *Condica illecta*, *Blasticorhinus* sp., *Hydrillodes* spp., and *Hypsopygia* spp. The visiting frequency was 2.43 times per hour at the JC location. Moths carried pollen grains

**Table 1**

Average relative amounts (%) of floral scents from inflorescences of *A. sinensis* ( $n = 3$ ).

No.	Compound	CAS No.	Rel. amount $\pm$ S.E. (%)
1.	<i>trans</i> -Ocimene	13877-91-3	$16.6 \pm 1.23$
2.	Methyl benzoate	93-58-3	$2.6 \pm 0.31$
3.	Linalool	78-70-6	$0.9 \pm 0.24$
4.	4,8-Dimethyl-1,3,7-nonatriene	51911-82-1	$1.4 \pm 0.42$
5.	Methyl-2-methoxybenzoate	606-45-1	$0.8 \pm 0.34$
6.	<i>cis</i> -hex-3-Enyl hexanoate	31501-11-8	$1.1 \pm 0.43$
7.	$\beta$ -Caryophyllene	87-44-5	$1.2 \pm 0.22$
8.	( <i>E</i> , <i>Z</i> )- $\alpha$ -Farnesene	502-61-4	$0.6 \pm 0.16$
9.	( <i>E</i> , <i>E</i> )- $\alpha$ -Farnesene	502-61-4	$61.9 \pm 3.22$
10.	<i>Z</i> -3-Hexenyl benzoate	25152-85-6	$3.6 \pm 0.98$
11.	Benzyl benzoate	120-51-4	$2.5 \pm 0.46$
12.	Benzyl salicylate	118-58-1	$4.6 \pm 1.11$

on their proboscises during the collection of nectar (Fig. 1) and the emission rate of floral scents from *A. sinensis* was about  $1721.4 \pm 283.6 \text{ ng/inflorescence h}^{-1}$  ( $n = 3$ ) according to the results obtained by dynamic headspace extraction combined with GC–MS using internal standard method for calibration.

The floral scent samples of *A. sinensis* contained four major compounds (Table 1): (*E*, *E*)- $\alpha$ -Farnesene ( $61.9 \pm 3.2\%$ ), *trans*-Ocimene ( $16.6 \pm 1.2\%$ ), Benzyl salicylate ( $4.6 \pm 1.1\%$ ), and *z*-3-Hexenyl benzoate ( $3.6 \pm 0.9\%$ ).

### 2.2. Seed dispersal of *A. sinensis*

Each capsule of *A. sinensis* often includes two diaspores. The seeds remain hanging for 4–7 days after capsule dehiscing, and drop to the ground in the absence of suitable seed dispersers. We found that the average mass of a mature fresh diaspore (seed and elaiosome) is about  $128.7 \pm 4.84$ ,  $76.2 \pm 2.82$  and  $52.5 \pm 2.02 \text{ mg}$ , respectively ( $n = 30$ ). Foraging hornets can easily carry away diaspores in the field. Actually, hornets removed 33.7% of diaspores from 141 capsules (276 diaspores) within three days after dehiscence. Individuals of *V. velutina* (68.8%) are the most effective seed dispersers of *A. sinensis*. *Vespa* hornet foragers were observed attacking the diaspores of *A. sinensis* as if they were trying to ‘kill’ them by biting, much like their behavior when attacking prey (Fig. 1; Movie S1). Diaspore carrying experiments indicated that diaspores and elaiosomes elicited significantly more carrying behavior than controls from *V. velutina* [Diaspore:  $6.73 \pm 0.52 \text{ time}/30 \text{ min}$ ; Elaiosome:  $7.09 \pm 0.51 \text{ time}/30 \text{ min}$ ; Seed:  $0.36 \pm 0.18 \text{ time}/30 \text{ min}$  ( $n = 11$ )] (Movie S2). Elaiosomes elicited the same amount of carrying behavior as intact diaspores. Hornets did not damage the seed coat of *A. sinensis*, often only eating the elaiosomes. No seeds were found in the nests of hornets in this study. In the flight cage, foraging hornets discarded seeds of *A. sinensis* during the flight back to their nests. This behavior implies that hornets also treated seeds with similar behavior in natural habitats. The average dispersal distance of *A. sinensis* diaspores taken by hornets was about 80 m according to the flight tracks of hornets.

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.pld.2016.09.006>.

## 3. Discussion

### 3.1. Pollination biology of *A. sinensis*

Plant-pollinator relationships are important ecological interactions in angiosperms, because successful fruit set after pollination is important for most flowering plants (Harder and Barrett, 2006). Signals produced by flowers to attract all kinds of potential

pollinators are an important theme in the evolution of plant–pollinator relationships (Dobson, 2006; Raguso, 2008; Chen et al., 2015a). Flowers or inflorescences can use a variety of cues, including visual, olfactory, gustatory, and even thermal information to signal their presence and to guide pollinators to their flowers (Raguso, 2008; Schiestl, 2010). However, floral recognition by pollinators is mainly mediated by floral color and scent cues (Chittka and Raine, 2006; Chen et al., 2015a). For moth-pollinated taxa, white flowers and fragrant floral scents are typical convergent flower characteristics (Knudsen and Tollsten, 1993; Dobson, 2006). In the present study, our results indicated that the white flowers of *A. sinensis* emit the predominant and indicative floral scent (*E*, *E*- $\alpha$ -Farnesene (61.9%), which is a common floral scent compound in angiosperms (Knudsen et al., 2006). Previous studies also revealed that floral scent compounds elicit strong antennal responses of different moth species (Casado et al., 2006; Guédot et al., 2008). Therefore, we inferred that floral scents and color may attract moths to locate *A. sinensis* flowers. Floral nectar reward may encourage moths to visit flowers of *A. sinensis* persistently.

The effectiveness of animals as pollinators depends on the reproductive system of the plant and the availability of the associated pollinators (Proctor et al., 1996; Kato et al., 2003; Okamoto et al., 2008). Given that the reproductive success of endangered plant species are related to effective pollinators (Robertson et al., 1999; Knight et al., 2005; Anderson et al., 2011), the interactions between threatened plants and their pollinators should be studied deeply in conservation biology. Previous studies revealed that most members of the Thymelaeaceae family are entomophilous (Whitehead et al., 1987; Soehartono and Newton, 2001; Tasen et al., 2009). More than 100 insect species visited flowers of *A. crassna*, and *Endotricha* species were the most abundant and frequent visitors of *A. crassna* (Soehartono and Newton, 2001). The study also indicated that pollen transfer from anthers to stigma by vectors was required for fruit setting of *A. crassna* flowers. In the present study, the results of the pollinator exclusion experiment clearly indicate that *A. sinensis* is a pollinator-dependent species, and moth pollinators mediate the fruit set of *A. sinensis*. Nocturnal moths seem to be the most important floral pollinators, especially noctuids, pyralids and geometrids. To the best of our knowledge, this investigation has provided the first observation of pollination for *A. sinensis*. However, further studies need to be conducted on populations in the field, particularly for pollination and reproductive tactics, as the behavior of pollinators in plantations may differ from that in natural habitats.

### 3.2. Seed dispersal of *A. sinensis*

Seed dispersal by animals is a complex mutualistic interaction involving a great diversity of plant and animal species (Fenner and Thompson, 2005; Rico-Gray and Oliveira, 2007). In this study, we showed that hornets are effective seed dispersers of *A. sinensis*. The average dispersal distance of *A. sinensis* diaspores taken by hornets was about 80 m. We infer that seed dispersal by hornets may have evolved as a strategy to occupy a wider distribution range at different spatial scales and reduce sib–sib competition in *A. sinensis*. Long-distance seed dispersal of *A. sinensis* might already have important consequences for the population dynamics of *A. sinensis* in its natural range. Actually, some studies have revealed that hornets also disperse diaspores of both *A. sinensis* and the closely related species *Aquilaria malaccensis* (Hu, 2012; Manohara, 2013). However, the dispersal distance and the destiny of dispersed seeds were not tested. Several species of hornets were recorded in Hu's study: *Vespa nigrithorax*, *Vespa bicolor*, *Vespa carbro* and *Ropalidia hongkongensis*. According to Manohara (2013), *Vespa affinis* is the only seed disperser of *A. malaccensis*. That study found

that hornets feed on the caruncle of *A. malaccensis*. The flight of the seed-carrying hornets was followed up to 500 m away from the parent tree (Manohara, 2013). In our study, the results indicated that *A. sinensis* elaiosomes, but not the seeds, promote seed carrying behavior by hornets. It is possible that some rewards from elaiosomes promote hornet-carrying behavior. The hard coat of seeds did not attract foraging hornets. Further studies are needed to decipher the location, recognition, and acceptance behavior of hornets to diaspores of *A. sinensis* in order to clarify the interactions between plants and insects. A similar study has been conducted in *Stemona tuberosa* Lour., whose seeds are dispersed by ants as well as hornets (Chen et al., 2016). In conclusion, our findings shed new light on a central issue in seed dispersal by hornets.

### 3.3. Protection tactics of *A. sinensis*

*Aquilaria sinensis* is a vulnerable plant species in China (IUCN, 2009). Because of its economic value and enormous pressure due to the loss of its natural habitat, the species has been listed as a plant species with extremely small populations since 2012 in China (Sun, 2013). Compared to widely distributed species, the pollination and seed dispersal of threatened plant species is closely related to their pollinators and seed dispersers (Sun, 2013; Caughlin et al., 2015; Peres et al., 2016), as loss of animal pollinators and seed dispersers may increase the risk of extinction of endangered species (Markl et al., 2012; Sun, 2013). Knowledge of the interactions between threatened plant species and their pollinators and seed dispersers supports effective conservation tactics and management methods (Nathan and Muller-Landau, 2000; Sun, 2013; Chen et al., 2015c). Based on the comparison of available data related to *A. sinensis*, such as seed germination and seed sensitivity to desiccation (Zhang et al., 2011), whole-tree agarwood-induction technology (Zhang et al., 2012), photosynthetic characteristics (He et al., 2011; Yuan et al., 2012), existence of the monophagous *H. vitesoides* pest (Chen et al., 2011; Qiao et al., 2012), and genetic diversity and differentiation (Zou et al., 2012), we recommend several methods to rescue the threatened agarwood plant in China. (1) Wild *A. sinensis* germplasm and seedlings from seed germination should be preserved; local residents should be encouraged to plant *A. sinensis* in home gardens. (2) Inoculation of agarwood trees with appropriate fungi to stimulate agarwood production should be conducted to meet economic needs. (3) Researchers should reintroduce cultivated *A. sinensis* seedlings to natural habitats based on genetic analysis. (4) Hornet and moth species involved in *A. sinensis* pollination and seed dispersal should be protected. (5) Because *A. sinensis* is a well-known economic tree, and artificial plantation is very common in China, previous studies suggest that cultivation of this species is likely to be a very effective conservation strategy. (6) Conservation science is not merely a technical matter; it also poses social and political questions; therefore, integrating multi-disciplinary methods and developing multi-stakeholder coalitions is imperative.

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