Research Article

Pollination in *Lilium sargentiae* (Liliaceae) and the first confirmation of long-tongued hawkmoths as a pollinator niche in Asia

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Received 21 October 2017; Accepted 3 April 2018; Article first published online xx Month 2018

Abstract The geographical distribution of pollinators is an important factor determining the biogeographical pattern of floral evolution and diversification. In Africa, a guild of plant species has converged in a floral syndrome for pollination by long-tongued hawkmoths (predominantly *Agrius convolvuli*). It is hypothesized that such floral convergence could track the geographical distribution of long-tongued hawkmoths, so it may not be confined to Africa. We investigated the pollination biology of *Lilium sargentiae* E. H. Wilson, which is endemic to China and exhibits traits suggestive of long-tongued hawkmoth pollination. *Lilium sargentiae* was visited by *A. convolvuli* as well as small beetles (Nitidulidae) but pollination was mostly or totally effected by the moth. It was consistent with other hawkmoth-pollinated plants in terms of floral tube length, nectar traits, tepal reflectance, and scent composition. We present the first experimental evidence for the hypothesis proposed above and for hawkmoth pollination in the widespread and ornamentally and economically important genus *Lilium* L. in a natural habitat. Our findings imply that long-tongued hawkmoths (especially the extremely widespread *A. convolvuli*) as a distinctive pollinator niche may have underlain the evolution of long-tubed (>8 cm) flowers across different continents.

Key words: *Agrius convolvuli*, floral adaptation, hawkmoth pollination, *Lilium sargentiae*, plant–pollinator interaction, pollination biology.

1 Introduction

Angiosperms have evolved strikingly diverse floral traits adapted to pollinators with different morphologies, visual and olfactory preferences, and energy requirements (van der Niet & Johnson, 2012; Schiestl & Johnson, 2013). Flowers of distantly related plants may evolve to attract a specific functional group of pollinators and exhibit remarkable floral convergence, occupying the same or similar pollinator niches (Johnson, 2010; Schiestl & Johnson, 2013). The biogeographical distribution patterns of pollinator-mediated floral convergence are largely underlain by the distribution of pollinators (Stebbins, 1970; Johnson & Raguso, 2016). This might in turn contribute to the current global biogeographical patterns of floral phenotypic diversity. Notably, a case of floral convergence by a guild of plants can occur across an enormous region if the pollinators for which they converge are widespread. For example, hummingbird-pollinated flowers are concentrated in tropical America, where hummingbirds are resident (Proctor et al., 1996). Some hummingbirds migrate north to temperate North America in summer; unsurprisingly, hummingbird flowers have also evolved there in genera without bird-pollinated species in temperate Eurasia (Cronk & Ojeda, 2008). Different continents in the Old World,

especially Asia and Africa, seem to be more similar to each other in pollinating animals (see Willmer, 2011), suggesting that more cases of floral convergent evolution probably occur between floras of these two continents. However, little evidence is available about this prediction because Asia has even fewer detailed studies on pollination ecology than Africa.

Hawkmoths (Sphingidae) constitute an important component of the pollinating fauna, especially in regions where dusk temperatures are high (Haber & Frankie, 1989; Willmer, 2011). The family Sphingidae is cosmopolitan with species composition varying dramatically with region but many genera occur in both Asia and Africa (see Martins & Johnson, 2013; Pittaway & Kitching, 2018). Hawkmoths generally have relatively long tongues, large bodies, nocturnal activity, and hovering behavior when visiting flowers, but interspecific variations in those traits are considerable (Miller, 1997; Martins & Johnson, 2013; Johnson et al., 2016; Sazatornil et al., 2016). Plants adapted to hawkmoth pollination are characterized by pale floral coloration, overwhelming fragrances, and long floral tubes, providing a distinct and charming group of ornamental flowers (Proctor et al., 1996; Johnson & Raguso, 2016) such as some species in *Jasminum* L., *Clerodendrum* L., *Habenaria* Willd., *Gardenia* J. Ellis, *Lonicera* L., *Nicotiana* L., and *Crinum* L., some of which are indigenous to both Asia and

Africa (Kato et al., 2008; Willmer, 2011; http://foc.eflora.cn/). Moth pollination has been considered to be relatively rare in the Asian flora (Corlett, 2004). However, it is reasonable to doubt this conclusion given that studies of nocturnal pollination by moths have been especially scarce in Asia (see the review by Johnson et al., 2016). Moreover, a number of moth flowers have been revealed there over the past few years, including some cases involving hawkmoths (Zhang et al., 2011; Mizusawa et al., 2012; Yokota & Yahara, 2012; Liu & Huang, 2013; Xiong et al., 2015; Yan et al., 2016).

Agrius convolvuli is a hawkmoth species that can be found in the majority of Asia, Africa, and other regions in the Old World (Johnson & Raguso, 2016; Pittaway & Kitching, 2018). Its tongue, at approximately 100 mm, is longer than those of most hawkmoth species in Asia and Africa (Miller, 1997; Martins & Johnson, 2013). Some hawkmoths in Africa do have longer tongues but their ranges are much narrower and their population density seems to be by no means comparable to that of A. convolvuli in open habitats that cover the majority of that continent (Johnson & Raguso, 2016). A guild of indigenous and introduced plant species from different families in Africa is exclusively visited and pollinated by long-tongued hawkmoths, predominantly A. convolvuli (Johnson & Raguso, 2016). Flowers of these plants are distinguished from other hawkmoth flowers by longer floral tubes (>8 cm), long enough to prevent most other moths from feeding on the nectar. Agrius convolvuli as well as other long-tongued hawkmoths probably also pollinate some plants in Asia and highly specialized pollination systems like the ones in Africa might also have evolved there because this hawkmoth species is also common in Asia (Pittaway & Kitching, 2018). No empirical evidence so far supports this hypothesis, although flowers with long floral tubes (>8 cm) suggestive of long-tongued hawkmoths occur in some lineages in the Asia flora.

Here we use Lilium sargentiae E. H. Wilson, which is endemic to China, to preliminarily examine the above hypothesis. The genus Lilium L. is distributed in temperate regions and rarely in tropical alpine regions in the Northern Hemisphere. Long trumpet-shaped fragrant flowers with pale perianths in Lilium have multiple evolutionary origins, at least two of which probably occurred in eastern Asia and one of which gave rise to L. sargentiae (see Gao et al., 2013). Such a floral syndrome represents adaptation to long-tongued hawkmoths (Johnson & Raguso, 2016). Given that A. convolvuli is widespread and often rather abundant, it could have played a major part in the convergent evolution of those Asian Lilium species, including L. sargentiae, although we cannot rule out the possibility that other long-tongued hawkmoths play a role in the pollination of L. sargentiae. Our study addresses the following four specific questions. (i) What are the flower visitors to L. sargentiae? (ii) What is the floral syndrome of L. sargentiae like? (We attempted to quantify the floral traits rather than to present a qualitative description.) (iii) Which visitors are the real pollinators? (iv) How does the floral morphology match the pollinators?

2 Material and Methods

2.1 Study species and site
Lilium sargentiae is a summer-flowering perennial deciduous geophyte growing on slopes without dense trees in Sichuan Province, China. Each stem bears one or several terminal flowers that are strongly fragrant, especially after dark. The tepals are connivent to form a trumpet-shaped perianth (the functional floral tube). Our study site was the slopes near Dadu River, Jiajun Town, Luding County, Sichuan Province, China (29°40′05″N, 102°15′51″E; approximately 1213 m a.s.l.). Our voucher specimens were deposited in the herbarium of Guangxi Institute of Botany (Guilin, China) under the following accession numbers: L. sargentiae (LCQ 201701) and L. sargentiae (LCQ 201702).

2.2 Floral visitor observations
Both diurnal and nocturnal floral visitors were observed in 2015 and 2016. Diurnal observations began at dawn and were scattered over the entire day. Nocturnal observations were carried out during the 3 h after sunset. Each observation session lasted 30 min and several neighboring stems with 15–27 flowers in total were observed during each session. Twenty and 19 observation sessions were undertaken by day and by night in 2015 and 2016, respectively. We recorded the visitor species and the total numbers of their visits to individual flowers during each observation session. Visiting beetles were rather inactive and usually stayed in flowers throughout an observation session, so the above observation method did not apply to them. Instead we recorded the number of beetles within each flower. All of the flowers whose beetles were counted came from different individual plants. The beetles on one flower of each of 20 plants were counted 10 min before sunset. To assess whether and how the moth-exclusion treatment (see the following paragraph) affected beetle visitation, we also counted the beetles in these caged flowers for the moth-exclusion treatment. All the beetles were counted after the flowers had been open for 2 days.

2.3 Trait measurements
To understand how the floral morphology matches the pollinator morphology, in 2015, the floral tube length of L. sargentiae of 26 flowers and the tongue length of 12 moth visitors were measured to the nearest 1 mm using a plastic ruler. All measured flowers came from different individual plants. Flower visitors with measurable tongues were caught and anesthetized in a plastic bag with a few drops of ethyl acetate. Their tongues were measured immediately and then they were left on stones to revive and to fly away. Nectar in 20 flowers from different plants was extracted into glass capillary tubes (inner diameter, 0.3 mm) and the length of the column of nectar in the tubes was measured to calculate volume. Nectar solute concentration was determined using a Bellingham & Stanley (Tunbridge Wells, UK) hand-held sucrose refractometer (0–50%, g solute per 100 g solution). The nectar measurements were carried out 10 min before sunset.

To reveal the details of the floral color, the reflectance spectra of the outer surface in eight flower and that of the inner surface in 12 flowers were measured using a spectror-adiometer (USB Ocean Optics 2000+), equipped with a UV-VIS light source (DH-2000; both by Ocean Optics, Dunedin, FL, USA). Given that the inner and outer surfaces of tepals differ in color, we measured both of them. We measured the inner or outer surface of each flower three times and used the

average as one replicate. The three measurements were carried out on three different tepals of each flower. For an inner surface, the measurements were carried out on the perianth opening. For an outer surface, the measurements were undertaken on the center of the brown part. During measurement, the probe was held at 45° to the surface of the target, to avoid glare. Polytetrafluoroethylene material WS-1 (Ocean Optics) was used here as the white reference. The raw floral reflectance spectra (300–700 nm) were smoothed and visualized using the R package pavo (Maia et al., 2013).

The floral scent of six flowers from different plants was collected and analyzed in 2016. Scent was collected from 20:00 to 22:00 h when visitors were active. Each flower used came from a different plant. Each of the flowers was put into a thick glass tube (9 cm in diameter) and then the tube was put into a Tedlar bag (DuPont, Wilmington, WA, USA). The flower stalk was wrapped in absorbent cotton that had just been soaked in a 10% sucrose solution to keep the flowers fresh during scent collection. To detect background contamination, potential volatiles in ambient air were collected as a control. Volatiles were drawn from the bag into a glass tube with adsorbent Porapak Q (100 mg, mesh 80–100; Waters, Milford, MA, USA) using a pump at an inlet flow rate of 300 mL/min for 1 h. The adsorbed volatiles were eluted with 500 μL *n*-hexane. The extracts were analyzed by HP 6890 gas chromatography (Agilent Technologies, Palo Alto, CA, USA), with a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness), linked to an HP 5973 mass spectrometer (Agilent Technologies). We identified compounds by comparing the gas chromatography retention times and mass spectrometry spectra with those of the authentic compounds, or tentatively identified by mass spectrometry spectra in the Wiley 7n.1 mass spectral library and retention indexes reported elsewhere (e.g., NIST Chemistry WebBook [http://webbook.nist.gov] and RI database [Adams's RI database; Adams, 2001]). Kovats retention indices were calculated by the formula $I_x = 100_n + 100(t_x - t_n)/(t_{n+1} - t_n)$. I_x is the retention index of a compound, t_x is the retention time of the compound, t_n and t_{n+1} are the retention times of the *n*-alkanes (Sigma-Aldrich, St Louis, MO, USA) eluted immediately before and after the compound, and *n* is the number of carbon atoms in the *n*-alkane eluted immediately before the compound (van Den Dool & Kratz, 1963; Chen et al., 2012). Relative amounts of compounds with respect to aggregate peak areas (excluding contaminants) of the floral scents were calculated.

2.4 Pollination effectiveness and breeding system
To evaluate the effectiveness of different floral visitors to *L. sargentiae* and the breeding system of this plant, we carried out five pollination treatments and only one flower was treated on each plant.

1. Natural pollination. Twenty flowers from different plants were visited by flower-visiting insects freely and were not caged or hand-pollinated.
2. Cross-pollination. Twenty flowers from different plants were pollinated with pollen from plants at least 20 m away (cross-pollination).
3. Moth-exclusion pollination. Twenty flowers from different plants were caged in nylon net (apertures, 15 × 15 mm)

which allowed small visitors to pass freely but kept large insects away from flowers.
4. Single-visit pollination. Sixteen flower buds from different plants were caged in nylon net (apertures, 0.3 × 0.3 mm) and were exposed to large and agile floral visitors for one visit after sunset when the flowers had been open for 2 days; by that time their stigmas had curved upward and easily contacted the visitors trying to probe into the perianths for nectar. This treatment was aimed at measuring how effective the visitors were per visit and how the flowers morphologically match the visitors. Small visitors had never been seen contacting the stigmas so we did not undertake this treatment for these insects.
5. Self-pollination. Twenty flowers from different plants were caged in nylon net (apertures, 0.3 × 0.3 mm) and were self-pollinated.

All of the five treatments were carried out on approximately 12 July, 2016. The fruits from all of the above treatments were collected on 28 September, 2016. The seeds and ovules of these fruits were counted for the calculation of seed set.

2.5 Statistical analysis
Independent samples *t*-tests for which equal variances were assumed were used to compare the numbers of visiting beetles in caged and uncaged flowers and to compare the lengths of floral visitor tongues and perianths because variances between groups were equal for both comparisons. A generalized linear model with a binomial distribution (events occurring out of a set of trials) and a logit link function was used to compare the seed sets of different pollination treatments. All statistical analyses were undertaken in SPSS 20.0 (IBM, Chicago, IL, USA).

3 Results

3.1 Floral visitor observations
The long-tongued hawkmoth *Agrius convolvuli* was the only moth species observed visiting *Lilium sargentiae* during our observation sessions in 2015 (144 visits) and 2016 (161 visits). When the moths hovered in front of a flower (Fig. 1A), extending the tongue into the perianth to extract nectar, they contacted the stigmas or not. Moths sometimes entered the perianth and alighted on the style and stamens or alighted on one side of the perianth opening (Figs. 1B, 1C). Pollen was attached to the legs, tongues, and ventral surface of the body (Figs. 1A–1C). It took each moth a few seconds to visit one flower and the moth usually visited many different plants within several minutes. Moth scales could be found on stigmas (Fig. 1A). Moths seemed most likely to contact the stigmas and/or stamens if they tried to enter the perianth, whereas moths sometimes missed them if they only hovered in front of the flowers, and seldom contacted them if they alighted on one side of the perianth opening.

Some beetles (Nitidulidae) were also common floral visitors but they were too small (not longer than 3 mm) and too sedentary within flowers to be clearly counted by the criteria of visit numbers. There were 11.2 ± 2.1 (mean ± standard error [SE]) and 5.8 ± 1.4 (mean ± SE) beetles in each flower of *L. sargentiae* in 2015 and 2016, respectively.

Fig. 1. Flower visitors to *Lilium sargentiae*. **A,** *Agrius convolvuli* hovering around the perianth opening. **B,** *A. convolvuli* in the perianth, with its abdomen contacting the anthers. **C,** *A. convolvuli* on one side of the perianth opening. (A–C) Moth scales on the stigma and pollen on the leg, tongue, and the ventral surface of the moths are magnified and marked by red arrowheads. **D,** Small beetles, indicated by red arrowheads.

They crawled around flowers and seemed to stay for a long time in one flower, occasionally feeding on the pollen (Fig. 1D). They were not observed contacting stigmas, suggesting that they might not play a significant role in the pollination in *L. sargentiae*.

3.2 Trait measurements

The perianths ($n = 26$) measured 146.0 ± 1.7 mm (mean $\pm$ SE) and were significantly longer than the tongues of *A. convolvuli* ($n = 12$, 105.4 ± 3.3 [mean $\pm$ SE]) ($t = 12.057$, d.f. $= 36$, $P < 0.001$). This result, together with our observations of moth behavior, suggested that the flowers did not simply tightly match the moth tongues in length, although they did seem to be adapted to *A. convolvuli* for pollination. The mean volume of nectar per flower ($n = 20$) was 15.2 ± 0.4 μL (mean $\pm$ SE), and the nectar solute concentration ($n = 20$) was $27.0\% \pm 0.9\%$ (mean $\pm$ SE). The nectar volume and concentration did not deviate from the ranges of those traits of flowers pollinated by *A. convolvuli* in Africa (Johnson & Raguso, 2016).

The inner and outer surface of *L. sargentiae* tepals are white and dark reddish brown, respectively. Correspondingly, the inner surface had a rather flat reflectance curve in the visible light region, whereas the outer surface showed relatively low reflectance with an increase in reflectance towards the red region (Fig. 2). A total of 19 compounds were detected from the scent extracts of *L. sargentiae*. Methyl benzoate was the dominant compound and this compound, together with 1,8-cineole and linalool, accounted for more than 95% of the total volatiles (Table 1).

3.3 Pollination effectiveness and breeding system

Ovaries of the self-pollinated flowers did not expand, indicating that *L. sargentiae* is fully self-incompatible and dependent on flower visitors for pollination. This treatment was therefore excluded from the statistical analysis of pollination treatments. The remaining four treatments differed significantly in seed set (Wald $\chi^2 = 42.036$, d.f. $= 3$, $P < 0.001$) (Fig. 3). Pairwise comparisons showed that natural
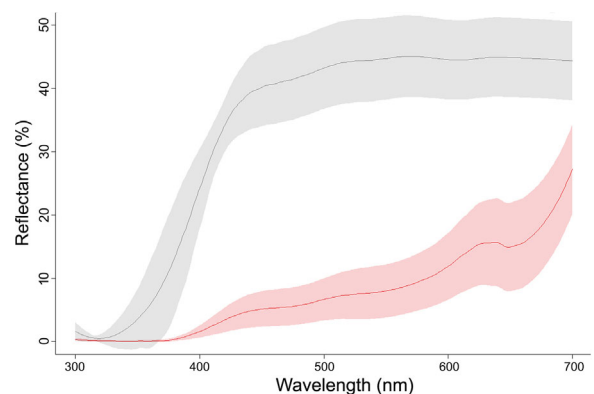

Fig. 2. Tepal reflectance spectra of *Lilium sargentiae*. Gray area indicates the inner white surface ($n = 12$). Red area shows the outer dark surface ($n = 8$). The line represents the mean and the shading represents the standard deviation.

Table 1 Average relative amounts of floral scent compounds from *Lilium sargentiae* ($n = 6$)

| Compound	Percentage[†]
Fatty acid derivatives	
Alcohols	
Hexanal	0.19 ± 0.10
Alkanes	
Tetradecane	0.02 ± 0.02
Benzenoids	
Benzaldehyde	0.05 ± 0.05
Benzeneacetaldehyde	0.03 ± 0.03
Benzyl alcohol	0.08 ± 0.06
Methyl benzoate	56.92 ± 2.88
Esters	
Methyl laurate	0.03 ± 0.03
Isoprenoids	
Monoterpenoids	
1,8-Cineole	18.71 ± 2.89
β-Fenchyl alcohol	0.08 ± 0.08
Limonene	0.53 ± 0.19
Linalool	19.55 ± 3.90
Linalool oxide	0.13 ± 0.06
Myrcene	0.22 ± 0.07
Neryl acetate	0.02 ± 0.02
Ocimene	0.13 ± 0.10
α-Pinene	1.23 ± 0.26
β-Pinene	0.06 ± 0.06
Sabinene	0.62 ± 0.11
α-Terpineol	1.40 ± 0.96

[†]Mean $\pm$ standard error.
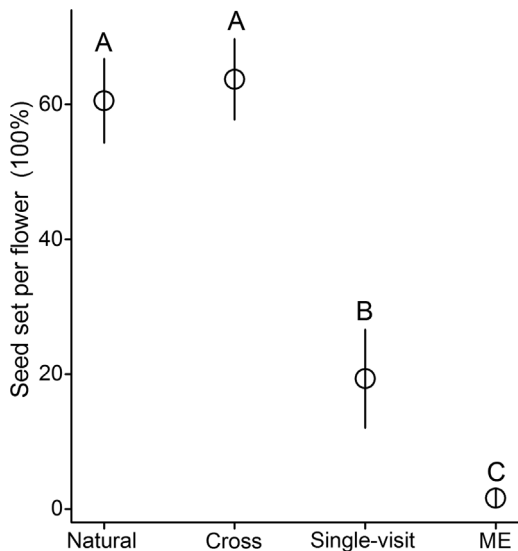
Fig. 3. Mean $\pm$ standard error of seed set from four pollination treatments of *Lilium sargentiae*. The sample size is 16 for the moth-exclusion (ME) treatment and 20 for other treatments. Each sample was the seed set of a fruit from a plant. Different letters above error bars indicate significant differences between groups.

pollination and cross-pollination were not significantly different in seed set ($P = 0.969$) and they both resulted in seed set that was much greater than the seed set from the moth-exclusion pollination ($P < 0.001$ for both comparisons). Thus, *L. sargentiae* did not show pollen limitation in 2016 and it primarily depended on *A. convolvuli* for pollination. The dependence on *A. convolvuli* for pollination was further confirmed by the finding that single-visit pollination by *A. convolvuli* also resulted in higher seed set than the moth-exclusion pollination ($P = 0.002$). Nine of the 16 ovaries from the single-visit pollination turned out to produce no seeds and the average seed set was significantly lower than that from natural and cross-pollination ($P < 0.001$ for both comparisons), suggesting that *A. convolvuli* did not always contact the stigmas when it was visiting *L. sargentiae* as we observed or it did not always deposit enough pollen to initiate fruit development. Thus, multiple visits to one flower were necessary for sufficient pollination. Beetles were not significantly fewer in caged flowers ($n = 20$, 9.9 ± 1.8 [mean $\pm$ SE]) (i.e., the moth-exclusion pollination treatment) than in uncaged flowers ($n = 20$, 11.2 ± 2.1, mean $\pm$ SE) ($t = 0.465$, $d.f. = 38$, $P = 0.645$). The caged flowers produced very few seeds and the uncaged flowers had much higher seed set, as discussed above. This suggests that beetles did not play a significant role in the pollination.

4 Discussion

This study shows that flowers of *Lilium sargentiae* are visited by the widespread long-tongued hawkmoth *Agrius convolvuli* as well as small beetles, but *A. convolvuli* is the primary visitor that contributes to the pollination in this self-incompatible species, as we first hypothesized. This is the first evidence for hawkmoth pollination in the genus *Lilium* in its natural range and for the use of long-tongued hawkmoths as a pollinator niche in Asia. *Lilium sargentiae* displays a floral syndrome characteristic of hawkmoth pollination, including perianth length, nectar traits, tepal reflectance spectra, and scent composition.

Flowers are commonly visited by animals that are not predicted on the basis of floral syndromes (Waser et al., 1996; Ollerton et al., 2009). This holds true for *L. sargentiae*, which was visited by both the predicted hawkmoth *A. convolvuli* and the unpredicted small beetles. However, the moth-exclusion pollination treatment resulted in a low seed set, so compared with *A. convolvuli*, these unpredicted beetles were ineffective flower visitors. We even suspected that the seeds in the fruits from the moth-exclusion pollination treatment might not be due to beetle visitation because those small beetles were never seen contacting the stigmas. They might be due to occasional visits by hawkmoths that tried to visit the caged flowers, with its tongue extending though the net and into the perianths (i.e., this treatment cannot completely deter hawkmoths). The use of floral syndromes as indicators of pollinators has received considerable criticism because flowers are often visited by varied animals, including those that seem surprising, judging from the floral traits (Waser et al., 1996; Waser, 2006; Ollerton et al., 2009; Waser et al., 2011). Our findings added evidence for the hypothesis that, for angiosperms, flower visitors that do not coincide with what

the floral syndrome predicts may not be as effective in pollination as the visitors predicted by the floral syndrome (see Fenster et al., 2004; Liu & Huang, 2013). The absence of pollination limitation, together with the complete self-incompatibility, suggests that *A. convolvuli* could not only carry a certain amount of pollen but also sufficiently transfer the pollen among plants of *L. sargentiae*, although it did not lead to sufficient pollination on every visit to the flowers. *Lilium sargentiae* might have originated relatively recently from an ancestor pollinated by short-tongued insects, so it might not have evolved floral organs precisely matching *A. convolvuli*, therefore the visiting moths often failed to contact the stigmas. In fact, one of its closely related species, *Lilium henryi* Backer (Gao et al., 2013) has very short flowers that are probably adapted to short-tongued insects.

The hawkmoth *A. convolvuli* is common in many countries in the Old World (Pittaway & Kitching, 2018). A guild of plant species in Africa has evolutionarily converged for pollination by long-tongued hawkmoths and *A. convolvuli* was found to be the dominant or sole pollinator of those species (Martins & Johnson, 2013; Johnson & Raguso, 2016). Since the geographical distribution of pollinators is important for biogeographical patterns of floral evolution (Stebbins, 1970) and *A. convolvuli* is not confined to Africa, the pollinator niche of long-tongued hawkmoths is also supposed to occur and to be used by plants elsewhere, some of which may also have evolved to specialize on *A. convolvuli* and other hawkmoths similar in tongue length. This study confirmed this hypothesis for the first time. *Lilium sargentiae* shows a group of floral traits in common with those African plants pollinated by long-tongued hawkmoths, including long floral tubes (>8 cm), abundant nectar, fragrance, and pale perianths (Johnson & Raguso, 2016). In particular, *L. sargentiae* resembles the African species *Crinum delagoense* I. Verd. in the color pattern of perianths, whose flowers also have a dark outer surface and a white inner surface, and their tepal reflectance spectra are also similar (Fig. 2; Johnson & Raguso, 2016). The three most abundant compounds are common in moth-pollinated flowers (Knudsen & Tollsten, 1993; Dudareva & Pichersky, 2006). We could further predict that a guild of Asian plants in addition to *L. sargentiae* might also be pollinated by long-tongued hawkmoths such as *A. convolvuli* because China alone has some plants with floral tubes that are comparable to *L. sargentiae* in length (pers. obs., 2016, 2017). For example, fragrant and long-tubed flowers of *Hosta plantaginea* Asch. have also been seen being visited by *A. convolvuli* (A master dissertation: Study on the variation of floral scents and adaptation of floral traits to pollinators in the genus *Hosta*, Qian Liu, University of Chinese Academy of Sciences, 2015). Interestingly, there are four plant species that were introduced to Africa and pollinated by *A. convolvuli* in their African populations (Rodger et al., 2010; Johnson & Raguso, 2016). Two of the four species are *Lilium formosanum* A. Wallace and *Hedychium gardnerianum* Sheppard ex Ker Gawl., which are both endemic to Asia and have natural ranges overlapping with that of *A. convolvuli*. These facts strongly suggest the existence of a guild of Asian plant species that are adapted to long-tongued hawkmoths such as *A. convolvuli* and resemble their African counterparts in floral syndromes. Further work is required to elucidate the pollination biology of these plants.

Lilium sargentiae is not widespread and only occurs along great rivers in southwestern Sichuan Province; our study was carried out near Dadu River, along which *L. sargentiae* is distributed most densely. We still might not have documented all floral visitors to *L. sargentiae*, and floral visitor composition commonly shows geographical variation (e.g., Herrera, 2005; Liu & Huang, 2013). *Agrius convolvuli* is also likely to be the pollinator of *L. sargentiae* elsewhere because the range of this moth fully covers the range of this lily and *A. convolvuli* is one of the most common hawkmoth visitors to flowers in various communities in China (pers. obs., 2014–2017). The long trumpet-shaped flowers of *Lilium* such as *L. sargentiae* might also be visited by hawkmoths with relatively short tongues in other sites. However, the long perianth should be primarily adapted to long-tongued hawkmoths like *A. convolvuli* and a long perianth would be wasteful if only short-tongued visitors are involved. *Megacorma obliqua* is the only hawkmoth species in Asia, of which we are aware, that has a tongue of comparable length to that of *A. convolvuli* (Kawahara, 2007) but its range does not overlap the range of *L. sargentiae* (see Pittaway & Kitching, 2018). This species probably visits tropical Asian flowers with long perianths in its range and constitutes a long-tongued hawkmoth pollinator niche with *A. convolvuli* in tropical Asia.

Until now, hawkmoth pollination in natural habitats has not been experimentally tested in the widespread and ornamentally and economically important genus *Lilium*. It has been confirmed in *L. formosanum* in Africa (Johnson & Raguso, 2016), where this lily was introduced, and hawkmoth visitation (rather than pollination) has been reported in three other *Lilium* taxa (Brantjes & Bos, 1980; Ollerton et al., 2009; Yokota & Yahara, 2012). The trumpet-shaped (or funnel-form) flowers have evolved in different lineages in this genus (Gao et al., 2013) and many are reported to be fragrant (http://foc.eflora.cn/), suggesting that species from different *Lilium* lineages might have evolved for hawkmoth pollination in parallel. As we observed here and Yokota & Yahara (2012) observed in *Lilium japonicum* Thunb., trumpet-shaped lilies do not seem to be adapted to long-tongued hawkmoths simply by perfectly matching the tongues of hawkmoths in length. Instead, they tend to be longer, probably because this can induce hawkmoths to go into the perianth to exploit the nectar completely. If a hawkmoth enters the perianth deeply enough, the narrow space will prohibit it from hovering, and it has to alight and make solid contact with the anthers and/or the stigma (Fig. 1B). Such a floral mechanism might also occur in other species with trumpet-shaped hawkmoth flowers, such as some Cactaceae (Walter, 2010; Eggli & Giorgetta, 2015).

Tepal-reflexed flowers are also common in *Lilium* and occur in more lineages of *Lilium* than trumpet-shaped flowers (Gao et al., 2013), is highly reflexed tepals. *Lilium* flowers of this form are usually brightly colored and may be pollinated by butterflies (e.g., *Lilium philadelphicum* L. in Edwards & Jordan, 1992). Some *Lilium* species distributed in the Hengduan Mountains have small campanulate flowers that are possibly pollinated by bees (pers. obs., 2017). Moreover, flowers of several species with reddish scentless tepals in North America are apparently adapted to hummingbirds for pollination and mixed pollination systems could also occur in a few taxa (Grant, 1994; Yokota & Yahara, 2012). In general, it seems that

Lilium has undergone remarkable adaptive radiation and floral phenotypic diversification mediated by pollinators. More work on the floral ecology and adaptive speciation of *Lilium* is desirable.

Acknowledgements

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