Taiwania **66**(3): 298–306, **2021**
DOI: 10.6165/tai.2021.66.298

Pollination ecology of *Impatiens uliginosa* (Balsaminaceae) endemic to China

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(Manuscript received 1 February 2021; Accepted 1 July 2021; Online published 6 July 2021)

ABSTRACT: China is one of the five major distribution regions of *Impatiens* in the world, with more than 270 species distributed all over the country, especially in limestone areas such as Yunnan, Guizhou and Guangxi. In order to explore pollination ecology of *Impatiens uliginosa*, we conducted the following experiments: observing the floral traits and floral phenology, measuring the nectar volume and sugar concentration, counting pollen viability, deposition and removal, carrying out pollination treatments and recording floral visitors and their behavior. Results show that the average life span of a single flower is 3.23±0.68 d. The male and female phase lasted for 1.70±0.06 d and 1.53±0.63 d. The amount of nectar per flower is 6.97±0.47 ul and sugar concentration of nectar is 36.58±0.82%, respectively. Stigma is receptive on the first day of anthesis. Pollen viability is strongest on the second day of anthesis. The pollen-ovule ratio (P/O) is 3974.28±105.07. We investigated the contributions of different floral visitors. *Bombus* sp., *Apis dorsata*, *Lobocla proxima*, *Ant* sp. and *Ephydra* sp. are diurnal visitors. However, *Bombus* sp. is the most effective pollinator of *I. uliginosa*. Pollination treatments indicated that *I. uliginosa* is self-compatible but completely relies on pollinators for pollination. There is no restriction on pollination in the field and *I. uliginosa* can avoid inbreeding depression. This study is of great significance to enrich the biological data of pollination and protect the biodiversity of *Impatiens*.

KEY WORDS: Breeding system, *Impatiens uliginosa*, pollination biology, pollination treatments.

INTRODUCTION

The flowering plants comprise approximately one-sixth of all species on earth and provide the most amazing and various biological features on our planet. Their interaction with other organisms is a dominant factor in community structure and function; they support all nutrient and energy cycles by providing food, most of which use animal pollinators to achieve reproduction (Christenhusz and Byng, 2016).

Since flowers promote and affect plant reproductive processes, they are central to life evolution in the terrestrial world. Pollination is a key mutualistic relationship between plants and animals, perhaps the earliest exchange of sex for food. When the plant gains reproductive success, the animal usually gains a food reward as it visits the plant (Willmer, 2011). Pollination ecology is the study of various biological characteristics and laws related to the processes of pollination, especially the reciprocal interactions between flowering plants and pollinators. It is one of the hot topics in plant reproductive ecology and evolutionary biology (Huang and Guo, 2000). A plant can be visited by different kinds of pollinators which have different pollination efficiency. The ultimate goal of the evolution of pollination systems is to form a stable, specialized relationship between pollinators and plants (Tong *et al.*, 2018). Effective access by pollinators is the basis of reproductive success for angiosperm. Within a certain group, the divergences of floral characters usually emerge through a variety of intricate biological processes especially mediated by pollinators (eg. Bradshaw and Schemske, 2003; Abrahamczyk *et al.*, 2021).

The family Balsaminaceae includes two genera, *Impatiens* and *Hydrocera*. *Impatiens* is a large genus in angiosperms, comprising over 1000 species, mainly distributed in the tropical and subtropical regions (Yu, 2012; Yu *et al.*, 2016). There are more than 270 species of *Impatiens* in China, widely distributed in limestone areas such as Yunnan, Guizhou and Guangxi (Chen, 2001; Yu *et al.*, 2016). *Impatiens* is known for hypervariable structure and rich color. The species in the genus display a wide range of floral syndromes associated with a variety of pollinators (Greywilson, 1980; Janssens *et al.*, 2012; Ruchisansakun *et al.*, 2021). Therefore, it has attracted the attention of many specialists on pollination ecology. Robertson observed pollinators of *I. capensis* and *I. pallida* in 1928. This is the earliest report on the pollination biology of *Impatiens*. Since then, scholars have made great contributions to the study of pollination ecology of *Impatiens* from North America, Japan, Africa, Sumatra and other regions. In temperate regions, the majority of *Impatiens* are pollinated by bumblebees and hummingbirds. In tropical regions, *Impatiens* are mostly pollinated by butterflies, birds and bees (Rust, 1977;

Zimmerman and Cook, 1985; Wilson and Thomson, 1991; Wilson, 1995; Walters and Stiles, 1966; Schemske, 1978; Waller, 1980; Schmitt and Ehrhardt, 1990; Randall and Hilu, 1990; Greywilson, 1980; Kato, 1988; Kato *et al.*, 1991; Twasuda and Yahara, 1994; Sato, 2002). The study by Tang *et al.* (2020) found that the formation of the long spur of *I. macrovexilla* may reflect adaptation to diurnal hawkmoth pollinators despite diversity of floral visitors. Nowadays, Chinese scholars pay more attention to taxonomy and molecular biology of *Impatiens*, whereas pollination biology of this genus was only reported on *I. reptans*, *I. chinensis*, *I. yaoshanensis*, *I. lateristachys*, *I. oxyanthera*, *I. hainanensis* and *I. macrovexilla* (Tian *et al.*, 2004; Xiao and Liu, 2009; Xiao, 2009; Mao *et al.*, 2011; Li and Wang, 2014; Zhong *et al.*, 2014; Tang *et al.*, 2020). According to these reports, the pollinators of *Impatiens* were difference among areas, which provides a basis for verifying the floral characteristic evolution with pollinator divergence. We research on the pollination of *Impatiens uliginosa*, an endemic species to China. We addressed two following questions: (1) Who is the most effective pollinator of *I. uliginosa*? (2) What's the breeding system of *I. uliginosa*?

MATERIALS AND METHODS

Study species and study area

I. uliginosa is an annual herbal species blooming from September to December, an endemic species in China. Flowers are deep pink with yellow dish patches. It is mainly distributed in Yunnan and Guangxi, China. *I. uliginosa* is hermaphrodite, with both male and female roles. Our study site is in Zhongba Village, Malipo County, Wenshan City, Yunnan Province, China (104°46'58"E, 23°13'13"N, 1,500 m altitude), where located a large wild population of *I. uliginosa*. They grow alongside a stream. The average annual temperature at the study site is 18.4℃. The annual average rainfall is 1,187.8 mm and the annual rainfall is about 2.8 billion cubic meters. Field observations were made and experimental samples collected from 11 Oct. to 24 Dec. 2019 and from 22 Oct. to 18 Dec. 2020, respectively.

Floral traits and floral phenology

A total of 30 plants of *I. uliginosa* were randomly selected from the sampling area. We measured plant height with a metric ruler (0.1 cm) and upper petal length, upper petal width, lateral united petals length, lateral united petals width, lower sepal length, lower sepal width, lateral sepal length, lateral sepal width, nectar spur length and stamens length of *I. uliginosa* were measured with a vernier caliper (0.05 mm). We labeled one floral bud on each of 30 different plants in 2019. When they bloom 24 hours later, recorded the life span of a single flower and duration of the male and female phase of a flower at 9 a.m. every day. We used the glass capillary tubes (0.3 mm in diameter) to measure the volume of accumulated nectar (one flower from each of 30 different individuals). Nectar sugar concentration was measured on a Hand-held Sucrose Refractometer (0–50%, g solute per 100 g solution; Bellingham and Stanley Ltd., London, UK).

To access pollen viability, we selected 6 anthers on the first, second and third days of anthesis respectively. These anthers of different phase were stained by MTT method (Dafni, 1992). The total number and the number of stained pollen grains within five separate areas on each slide were counted under a light microscope. From these figures, the percentage of stained pollen grains was calculated. To examine the pollen grains and ovules per flower, we dissected 30 floral buds from 30 different plants with a dissecting needle in field, and removed these anthers and stigmas with a tweezer. They were fixed in a centrifuge tube (1.5 ml) filled with 75% alcohol respectively. We brought back them to the laboratory. Mash each anther into 1 ml of pollen suspension. The liquid in each centrifuge tube was sucked 3 times with a 10 ul pipette gun and observed under a microscope to record the number of pollen grains per flower (P). The stigmas were dissected under stereoscope and the number of ovules per flower (O) was recorded.

Pollination treatments

In order to study the breeding system of *I. uliginosa*, we conducted five treatments in 2020. The treatments started when the floral buds were about to open and finished after anthesis. Each treatment included 30 flowers and all flowers used in this study were from different individuals. (1) Control: flowers were always exposed. (2) Autogamy (autonomous self-pollination): flowers were always bagged. (3) Apomixis: dissect the floral buds with a dissecting needle and remove the stamens. (4) Selfing (self-pollination): flowers were bagged and hand-pollination with pollen from itself. (5) Outcrossing (cross-pollination): flowers were bagged and hand-pollination with another plants (mixed pollens) more than 10 m away. Each of the five treatments was conducted on one flower per plant. After each treatment is finished, different color lines are tied to distinguish them. Fruits were collected 50 days later, when the perianth had wilted; then seeds and ovules per fruit were counted to calculate seed set.

Floral visitors and their behavior

To observe floral visitors and their behavior, we selected a certain number of blooming flowers, then both diurnal and nocturnal observation were taken in 2019 to 2020. In 2020, diurnal observations were conducted randomly from 8:00 to 18:00 h and nocturnal observations were conducted from 20:00-23:00 h in October to December respectively, from the 10th to 17th. Visitor identity and the number of flowers it visited were recorded. To obtain visitation rate of *I. uliginosa*, the visits by all visitors of

Fig. 1 The natural habitat of ***Impatiens uliginosa*** and their floral architecture. **A.** habitat **B.** flower (front view) **C.** flower (lateral view) **D.** bud **E.** capsule **F.** parts of a flower. **A-F.** All photographed by Zhao-Feng Li & Bai-Zhu Li.

different categories were recorded during one observation period (half an hour) in the sunny days. The visitation rate (per flower per hour) was estimated as the number of visit per observation period doubled and divided by the number of observed flowers (Liu and Huang, 2013). A small flashlight covered with thick red plastic film was used during nocturnal observation. We captured floral visitors and made them into specimens. All insects were first confirmed by consulting the Chinese Insects Illustrated (Zhang and Li, 2011). Furthermore, these insects were sent to insect taxonomy experts to further identify the species, but a few species were only identified to genus only.

Pollen deposition and removal

To estimate the contributions of different diurnal visitor categories to pollination, we calculated the number of pollen grains deposit per visit and pollen grains carryover per visit. Meantime, we also calculated pollen transfer efficiency to determine effective pollinators (Reynolds *et al.*, 2012). Flowers were bagged before bloom and then exposed to visitors after the stigmas matured. The stigmas were collected once one insect visits and deposits pollen grains on each stigma. We removed the stigmas from flowers with tweezers and put them into centrifuge tubes (1.5 ml) with 75% alcohol. Take them back to the laboratory and count pollen grains under a

Table 1 Test of breeding system.

	Autogamy	Apomixis	Selfing	Outcrossing	Control
Seed set（%）	0 （n=30）	0 （n=30）	49.08±6.40 （n=30）	76.21±4.91 （n=30）	72.36±2.27 （n=30）

microscope. Conspecific pollen could not be distinguished from heterospecific pollen but the quantity of pollen deposited by different visitor categories could be estimated (Liu and Huang, 2013).

Data analysis

To analyse floral traits, visitation rate, the average life span of a single flower, male and female phase, pollen viability, the amount of nectar per flower and sugar concentration of nectar, we used a generalized linear model (GLM) with normal distribution and constant function. A generalized linear model (GLM) with poisson distribution was used to analyse the amount of pollen grains and ovules per flower. Binomial distribution logistic correlation function in a generalized linear model (GLM) was used to analyze and compare the seed set of *Impatiens uliginosa* under different pollination treatments; whereas pollen carryover and deposition were analyzed with a logit link function (Wang *et al.*, 2019; Zhang *et al.*, 2021). All the analysis and mapping were conducted in SPSS 23.0 (IBM, Armonk, NY, USA).

RESULTS

Floral traits and floral phenology

I. uliginosa is an annual herb (Fig. 1). Plant height was 53.74±1.91 cm. Flowers deep pink with yellow dish patches. Upper petal length was 10.66±0.22 mm and width was 12.68±0.23 mm. Lateral united petal length was 19.58±0.54 mm and width was 7.76±0.19 mm. Lower sepal length was 27.15±0.51 mm and width was 15.95± 0.54 mm. Lateral sepal length was 7.17±0.13 mm and width was 5.58±0.08 mm. Nectar spur length was 24.14± 0.71 mm. Stamen length was 4.36±0.07 mm and width was 2.39±0.06 mm.

I. uliginosa usually blooms in the early morning. The average life span of a single flower is 3.23±0.68 d. The male and female phase lasted for 1.70±0.06 d and 1.53± 0.63 d. The peak flowering occurs during September to November. Reproduction success of *I. uliginosa* is entirely dependent on pollinators. The amount of nectar per flower is 6.97±0.47 ul and nectar sugar concentration per flower is 36.58±0.82%, respectively. The number of pollen grains per flower is 57436.67±1325.56 and ovules grains per flower is 14.60±0.35. According to the field observation, the stamen existed for about 3 days after flowering. Most stamens shed after 3 days. Pollen viability was highest on the second day of anthesis (Fig. 2). On the first day of anthesis, pollen viability is 58.23± 2.32% and on the third day of anthesis, pollen viability is 85.61±3.21%.

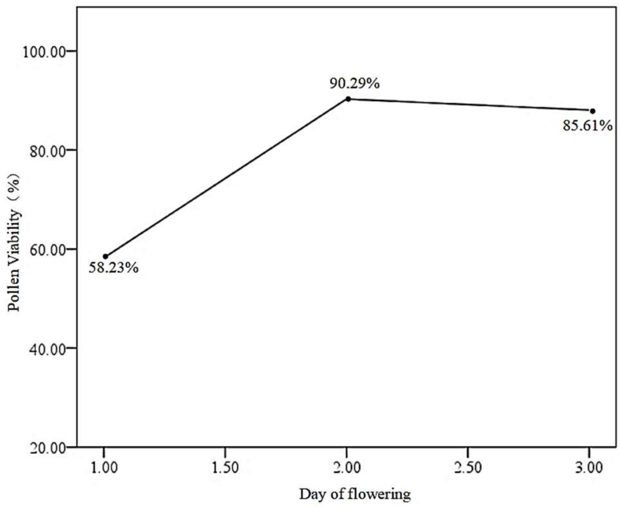

Fig. 2. Pollen viability of ***Impatiens uliginosa***.

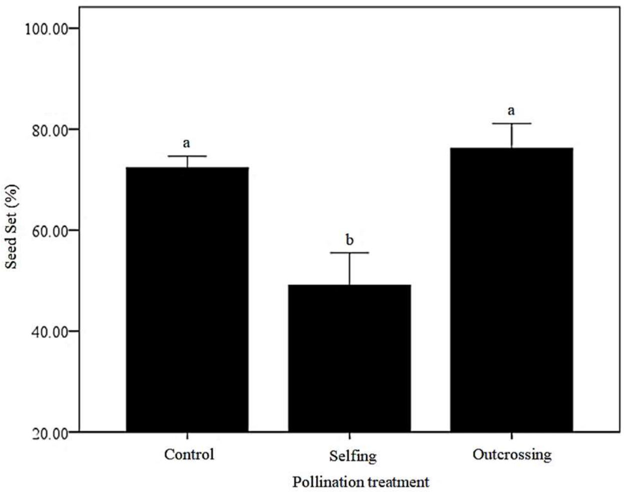

Fig. 3. Proportion seed set per flowers under different pollination treatments of ***Impatiens uliginosa*** in 2020; different letters above bars indicate significant differences between treatments within species.

Pollination treatments

Results from the pollination systems experiments are shown in Table 1 and Fig. 3. The results of the autogamy and apomixis treatments show that the ovaries of the flowers did not swell and soon dropped, so no fruits were produced. Each of the flowers used in the natural pollination (control), self-pollination (selfing) and cross-pollination (outcrossing) treatments produced a fruit,

Fig. 4 Floral visitors of ***Impatiens uliginosa***. **A. *Bombus*** sp. **B. *Apis dorsata***. **C. *Lobocla proxima***. **D. *Ephydra*** sp. All photographed by Zhao-Feng Li & Bai-Zhu Li.

suggesting that *I. uliginosa* may be self-compatible and no pollen limitation. The self-pollination (selfing) compared to natural pollination (control) and cross-pollination (outcrossing) with a significant difference ($P<0.05$), indicating that *I. uliginosa* is a self-compatible species and prefer to cross-pollination.

Floral visitors and their behavior

It is well known that the flowers of *Impatiens* have enormous diversity and different pollinators. There are five species of insects belonging to Hymenoptera, Lepidoptera and Diptera visiting *Impatiens uliginosa* in 2019 and 2020 (Table 2; Fig. 4). *Bombus* sp., *Apis dorsata*, *Lobocla proximain*, *Ephydra* sp. and *Ant* sp. are diurnal visitors of *I. uliginosa*. No nocturnal visiting insects were seen. Among these visitors, *Bombus* sp., *Apis dorsata and Lobocla proximain* can contact anthers and stigmas as they visited *I. uliginosa*; pollen attached to the head, proboscis or thorax. Bumblebees and bees are the most frequent pollinators of *I. uliginosa*. Normally they stay on the lower sepal and use their long proboscis insert into nectar spur to consume nectar. They contacted the stigma or pollen with their head and back. When they visit next flowers, the pollen will deposit on other stigmas. They also stay on leaves and grooming pollen from their bodies to the corbicula on legs. Sometimes bumblebees bite

Table 2. Floral visitors and their behavior of ***Impatiens uliginosa***.

Order	Family	Pollinator	Aim	Behavior of floral visitor	Pollen carrying site
Hymenoptera	Apidae	*Bombus* sp.	Nectar/Pollen	Pollination/Nectar robbing	Head/Proboscis/Back
		Apis dorsata	Nectar/Pollen	Pollination/Collect pollen	Head/Back
	Formicidae	*Ant* sp.	Nectar	Nectar robbing	
Lepidoptera	Hesperiidae	*Lobocla proxima*	Nectar	Pollination	Head/Proboscis
Diptera	Ephydridae	*Ephydra* sp.	Pollen	Pollen robbing	

Table 3. Pollen carryover and pollen deposition on stigmas of virgin flowers of ***Impatiens uliginosa*** after one visit by the three categories of pollinators.

Treatment	*Bombus* sp.	*Apis dorsata*	*Lobocla proxima*
Pollen carryover	18431.22±2109.87	12356.56±1523.89	3433.28±240.36
Pollen deposition	7125.84±103	3649.15±50.47	394.27±18.62
Pollen transfer efficiency (%)	38.66	29.53	11.48

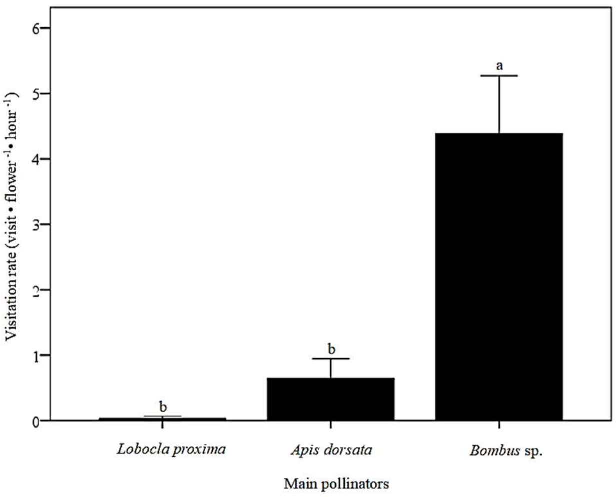

Fig. 5 Visitation rates of three pollinator categories of ***Impatiens uliginosa*** in 2020.

nectar spur to consume nectar. In the process of visiting flowers, butterflies carry pollen on their proboscis and rarely on their heads. The water-born *I. uliginosa* is more likely to be visited by butterflies than the land-born. Almost all floral visitors seem not to distinguish flowers between male and female phase, continuously visiting many flowers on neighbouring branches within a patch. It is similar to the behavior of floral visitors of *I. macrovexilla* (Tang *et al.*, 2020). These three insect species were considered to play a role in pollination; therefore they should be regarded as pollinators. Flies (*Ephydra* sp.) often gather in flowers to feed on pollen. Sometimes ants enter the nectar spur to consume nectar.

Pollen deposition and removal

Any animal to be an effective pollinator must have the ability to passively pick up pollen from the anthers of a flower to another stigma when its body moves between flowers. *Bombus* sp. deposited significantly more pollen grains on *Impatiens uliginosa* per visit than did by *Apis dorsata* and *Lobocla proximain* (Table 3). The results combining pollen deposition and visitation rate ($P<0.0001$) (Table 3; Fig. 5) indicated that *Bombus* sp. is the most effective pollinator of *I. uliginosa*.

DISCUSSION

Floral architecture and floral visitors

Various animal species must have been interacting with plants both for shelter and to find food for a long time (Willmer, 2011). According to our observations, the flies *Ephydra* sp. stay on the surface of the bud, waiting until the bud blooming; once the bud opening, they entered into the flower simply for food. The flies did not consume nectar but feeding on pollen. Hence, they have no effect on other pollinators consume nectar rewards, but causes pollen discounting. They may be regular visitors to *I. uliginosa* but are unlikely to be effective pollen carriers; in effect, they act as cheaters and pollen robbers as far as the flower is concerned. Meantime, we found their white eggs when dissected the anthers of the blooming *I. uliginosa*. So we figured *I. uliginosa* flowers are sometimes visited just as convenient habitats, simply because they offer an equable sheltered microclimate for *Ephydra* sp. The flies can rest in the flowers where protected against bad weather or predators. Abrahamczyk's field observations on *I. rivularis* confirmed that this species is regularly and exclusively visited by a range of small flies, which were observed to carry pollen. These groups of flies are known to lay eggs on the fruiting bodies of fungi (Abrahamczyk *et al.*, 2021; Tuno *et al.*, 2019) and have been observed as pollinators of spawning mimicry orchids and Aristolochiaceae (Melo *et al.*, 2011; Johnson and Schiestl, 2016). Further, their autonomous and self-pollination treatments revealed that *I. rivularis* and many other *Trimorphopetalum* species only set very few seeds in the absence of pollinators (Abrahamczyk *et al.*, 2021). Therefore, their results suggest that a sophisticated form of brood-site deception and fungus mimicry has evolved within *Impatiens* section *Trimorphopetalum.* Whether such a mechanism is persistent in *I. uliginosa* remains to be investigated. Weather change affects the activity of butterfly. Butterflies are occasionally seen to visit flowers on sunny days. However, they were completely inactive on rainy or cloudy days. Our results are similar to Tian's (Tian *et al*., 2004).

The most effective pollinator principle (MEPP) suggests that the evolution of the floral architecture (size, color, scent, height of inflorescences) is adapted to the most effective pollinators' visits (Huang, 2007; Huang, 2014). Floral architecture of *Impatiens uliginosa* is very suitable for pollinators' visits. *I. uliginosa* is a symmetrical flower with deep pink floral color, a long nectar spur and light fragrance. The above characteristics are consistent with the characteristics of melittophily flowers (Faegri and Pijl, 1966). According to our field observation and the results of pollen transfer efficiency, we hold the opinion that *Bombus* sp. is the most effective pollinator of *I. uliginosa*. We speculate that the long proboscis of *Bombus* sp. may play a selective role in length of nectar spur of *I. uliginosa* to form a mutually beneficial co-evolution. They play a decisive role in the shaping of floral architecture.

In addition to pollination, some bumblebees also used their long proboscis to make holes on the nectar spur of flowers to eat nectar and then fly away. They don't carry pollen on their bodies and touch the stigma when visiting *I. uliginosa.* Hence, they are typical nectar robbers. Nectar robbers are frequently described as cheaters in the plant-pollinator mutualism, because it is assumed that they obtain a reward (nectar) without providing a service (pollination). Nectar robbing is one of the most effective predatory strategies of short-proboscis bees against flowers with long nectar spur (Zhang *et al.*, 2006). In our opinion, bumblebees only get food in illegitimate way because of the mismatch of the morphologies of bumblebees' mouthparts and floral structure. The other point of view argues that nectar robbing is a relatively more efficient, thus more energy-saving way for bumblebees to get nectar from flowers. Previous studies had shown that the behaviors of nectar robbers are innate, but choosing where to make a hole on the nectar spur are learned and acquired (Olesen, 1996; Arizmendi, 2001). Researches on other different species show that: Bumblebees fly further to visit the next one after visiting a low-nectar flower (Miller and Travis, 1996; Zimmerman and Cook, 1985; Maloof, 2001). After visiting a high-nectar flower, they will fly a short distance to visit the next flower. Nectar robbing increasing the flying distance of legitimate pollinators. When the amount of nectar in the flowers falls below a certain critical value, pollinators rarely visit the flowers or inflorescences (Pyke, 1982; Hodges, 1985). Thus, bumblebees may play a role in regulating the amount of nectar in flowers and inflorescences. If the amount of nectar is reduced as a result of nectar robbing, legitimate pollinators may quickly leave the inflorescence and could result in improved reproductive fitness of plants through increased pollen flow and outcrossing. We consider it is especially important for *Impatiens uliginosa* with self-compatible breeding systems and other plants with many flowers in a single inflorescence.

Breeding system

I. uliginosa did not set seeds by autogamy and apomixis, which indicates that the reproduction of *I. uliginosa* completely relies on pollinators. Both self-pollination (selfing) and cross-pollination (outcrossing) can produce fruit. There was no significant difference between the numbers of seed set by cross-pollination (outcrossing) and natural pollination (control). But the seed production of self-pollination (selfing) was significant lower than natural pollination (control) and cross-pollination (outcrossing) ($P<0.05$). The results indicated *I. uliginosa* is self-compatible and prefer outcrossing. The breeding system of self-compatible of *I. uliginosa* was retained. When there is no pollinator in the wild, the reproductive assurance can be achieved even if *I. uliginosa* fails to accept pollen from other plants. At the same time, outcrossing of *I. uliginosa* can avoid the phenomenon of inbreeding depression. Our pollination experiments were consistent with many other studies on *Impatiens* species (eg: Tian *et al.*, 2004; Xiao, 2009; Li and Wang, 2014; Zhong *et al.*, 2014; Tang *et al.*, 2020). The results can be interpreted as the reason for the high morphological diversity of *Impatiens*, including *I. uliginosa*. In the wild, *Impatiens* relies on pollinators and tends to outcrossing, which not only provides reproductive assurance, but also maintains certain variation. It provides the impetus for the differentiation of *Impatiens.*

The pollen sheaths formed by the stamens of *I. uliginosa* encase the stigma. Pollen does not touch the stigma until the stamen has fallen off. On the first day of anthesis, we pollinate the pistil after removing the stamens and the pistil can still bear fruit. It indicated that the stigma had receptivity in male phase. Stigma is receptive on the day of anthesis. The results of our study are consistent with those of *Impatiens chinensis*, *I. hainanensis*, *I. capensis* and *I. pallida* (Rust, 1977; Xiao, 2009; Zhong *et al.*, 2014). Hence *I. uliginosa* is a hermaphrodite flower, its male and female functions through spatial separation. Numerous studies have shown that inbred offspring are less fit than outbred offspring and this inbreeding depression is generally recognized as one of main selective forces that shape the evolution of plant mating strategies (Barrett, 2002; Charlesworth and Charlesworth, 1987; Webb and Lloyd, 1986). We consider that *I. uliginosa* (the self-compatible plants) are often protected from the harmful effects of self-fertilization by developing anti-selfing mechanisms including herkogamy.

The study of plant pollination ecology is helpful to understand the characteristics of species, and then to understand population survival and community evolution. *I. uliginosa* is a self-compatible species and prefer to cross-pollination, which can improve genetic diversity and promote plant evolution under the condition of ensuring successful reproduction. The long spur of *I. uliginosa* showed a high degree of adaptation to bumblebee, which is the most effective pollinator. Furthermore, the population of *I. uliginosa* grows in moist places (near streams). The humidity in Malipo is suitable for the growth of *I. uliginosa*, which indicates that *I. uliginosa* has adapted to the environment (soil, climate) in this area. However, the abundant rainfall in this area has a great influence on the reproduction of *I. uliginosa*, which will limit the activity of the pollinators. They also die easily during the frost. The self-compatible breeding system of *I. uliginosa* may be a guarantee mechanism to achieve successful sexual reproduction. Local peasants often cut the plants to feed their livestock and the land with *I. uliginosa* was reclaimed to grow crops. Therefore, a specialized habitat and habitat loss may be the reason for its limited distribution and being an endemic species.

ACKNOWLEDGMENTS

The project was supported by the Joint Fund of the National Natural Science Foundation of China and the Karst Science Research Center of Guizhou province (Grant No. U1812401), Natural Science Foundation of China (Grant Nos. 31901208, 31560184,31300317), Science and Technology Fund of Guizhou Province (2019 [1237]), Karst Mountain Ecological Safety Engineering Center [Qian Education Contract No. KY [2021] 007], Subsidizing Doctoral Research Projects in Guizhou normal university (GZNUD [2017] 1). We are grateful to Yun-Jing Liu, Bo Xu, Zhi-Wei Wang, Qing-Song Tu, Xiu-Xiong Hu and Cheng-Ying Pi of Guizhou Normal University for their help in the field experiment. We would like to thank Han-Qing Tang and Xi Dong of Guizhou Normal University for their suggestions on the first draft of this article. Thank you very much to Xi-Bing Guo and his family from Laoshan Nature Reserve in Malipo County, Yunnan Province for their care and support during the field experiment.

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