Postprint: Floral Syndrome and Breeding System of the Spring-Flowering Plant *Iris bloudowii*

Authors: Li Qi, Ma Hanze, Janet Khan Mamut

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Abstract

Iris scariosas is a perennial spring-flowering plant belonging to the genus Iris of the family Iridaceae, possessing significant ornamental value. To investigate the floral syndrome and breeding system characteristics of this species, and to promote its hybrid breeding and germplasm resource exploitation and utilization, this study employed Iris scariosas as research material and utilized a combination of field observations and controlled experiments to investigate its flowering phenology, floral syndrome, breeding system, and pollination characteristics. The results demonstrated that: (1) The species entered the initial flowering period in early May, the full flowering period in mid-May, and the final flowering period in late May, with a flowering duration of 16 days. (2) The flowers are blue-purple, possess a special odor and a small quantity of nectar, with individual flower longevity of 2.5–3.0 days. (3) Artificial pollination experimental results indicated that this species belongs to an obligate outcrossing breeding system, lacking apomixis and autonomous self-pollination capacity. (4) This species belongs to a generalized pollination system, with Apis mellifera ligustica, Apis cerana, and Lasioglossum spp. being the primary pollinators, with visitation frequencies of (0.57 ± 0.05), (0.42 ± 0.04), and (0.19 ± 0.03) visits $\cdot$ flower^{-1} $\cdot$ h^{-1}, respectively. The characteristics of Iris scariosas flowers, including their showy coloration, large floral display, coincidence of insect visitation peak with the period of maximum pollen viability and optimal stigma receptivity, and yellow beard-like appendages on the midrib of the outer perianth segments, are of significant importance for ensuring successful pollination completion and promoting outcrossing reproductive success. These research results provide important theoretical data for the resource utilization and germplasm innovation of Iris scariosas.

Full Text

Floral Syndrome and Breeding System of the Spring-Flowering Plant *Iris scariosa* (Iridaceae)

LI Qi[1], MA Hanze[1], and Jannathan MAMUT[1],[2]* [1]College of Life Science, Xinjiang Agricultural University, Urumqi 830052, China [2]Ministry of Education Key Laboratory for Western Arid Region Grassland Resources and Ecology, College of Grassland Sciences, Xinjiang Agricultural University, Urumqi 830052, China

Abstract: *Iris scariosa* is a perennial spring-flowering Iridaceae species with important ornamental value. To explore its floral syndrome and breeding system characteristics and promote its hybrid breeding and germplasm resource utilization, this study investigated the flowering phenology, floral syndrome, breeding system, and pollination traits of *I. scariosa* using field observations and controlled experiments. The results were as follows: (1) The species entered initial flowering in early May, peak flowering in mid-May, and final flowering in late May, with a flowering duration of 16 days. (2) Flowers were blue-purple, with a distinctive scent and small amount of nectar, and individual flower duration was 2.5–3.0 days. (3) Artificial pollination experiments indicated that this species has an obligate outcrossing breeding system, with no apomixis or autonomous self-pollination capacity. (4) The species has a generalist pollination system, with *Apis mellifera*, *A. cerana*, and *Halictus* sp. as the main pollinators, with visitation frequencies of (0.57 ± 0.05), (0.42 ± 0.04), and (0.19 ± 0.03) visits $\cdot$ flower^{-1} $\cdot$ h^{-1}, respectively. The floral traits of *I. scariosa*, including bright coloration, large floral display, synchronization of peak insect visitation with the period of highest pollen viability and optimal stigma receptivity, and the yellow beard-like appendages on the midrib of outer tepals, are important for ensuring successful pollination and promoting outcrossing reproductive success. These results provide important theoretical information for the resource utilization and germplasm innovation of *I. scariosa*.

Keywords: *Iris scariosa*, spring-flowering plant, floral syndrome, breeding system, outcrossing

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Introduction

Plant breeding systems have become one of the most active research areas in ecology and evolutionary biology, typically referring to all sexual characteristics that influence the genetic composition of offspring (Wyatt, 1983). Floral morphological characteristics, display patterns, longevity of floral parts, pollinator types and visitation frequencies, self-compatibility levels, and mating systems are all closely related to breeding systems (He & Liu, 2003; Cao et al., 2022). Through long-term evolution, angiosperms have evolved diverse floral features to adapt to suboptimal pollination environments and ensure reproductive success (Spigler & Kalisz, 2013). These features not only affect pollinator attraction, pollen dispersal, pollinator foraging behavior, and the degree of self- versus cross-pollination, but also influence male and female fitness, ultimately shaping plant mating patterns and population dispersal capacity (Barrett & Harder, 1996; Zhang, 2004). Previous studies have shown that breeding systems, serving as a bridge connecting plant sexual reproduction, play important roles in determining plant genetic diversity and genetic structure (Loveless & Hamrick, 1984) and are crucial for phenotypic variation and evolutionary trajectories (Wyatt, 1983; Zhang, 2004; Cao et al., 2022). Therefore, research on plant breeding systems and their diversification is significant for revealing evolution across plant taxa, exploring species formation, population expansion, and environmental adaptation (Dai et al., 2013).

Iridaceae is a large family in the monocotyledonous angiosperms, comprising 77 genera and over 1,630 species, primarily distributed in tropical, subtropical, and temperate regions, southern Africa, tropical America, and the Mediterranean region of Europe (Goldblatt, 1990; Huang, 2014). China has 11 genera and 71 species, with Xinjiang containing 5 genera (Cui et al., 1996). Most species in this family are excellent perennial flower resources with high ornamental value and important medicinal, edible, and economic significance (Rahmani et al., 2017; Weng et al., 2018). Additionally, Iridaceae species inhabit complex environments, with most growing in arid or semi-arid desert environments, forest margins in mountainous areas, wetlands, and islands (Scott & Panetta, 1993; Guo et al., 2011), resulting in complex and diversified breeding systems. Currently, international research on the reproductive ecology of this family has focused on flowering biology (Tarasjev, 1997; Shang & Wang, 2014), floral syndrome (Ascough et al., 2011; Huang, 2014), pollinator types and pollination mechanisms (Goldblatt & Manning, 2006; Watts et al., 2013), and mating systems (Ishii & Sakai, 2001; Zhang & Tan, 2009; Paula et al., 2018). These studies have shown that Iridaceae species are characterized by long flowering periods, special floral morphology, diverse pollination strategies, and varied mating systems, making them ideal taxa for studying plant reproductive characteristics. Research on the reproductive characteristics of these taxa can not only explain life history traits and environmental adaptations but also provide important theoretical foundations for the cultivation, breeding, and conservation of Iridaceae species in China.

Iris scariosa is a perennial spring-flowering species in the genus *Iris* (Iridaceae), mainly distributed in Kazakhstan, Russia, Siberia, and Xinjiang, China (Cui et al., 1996). In Xinjiang, this species primarily occurs in the Altay, Tacheng, Hami, and Yining regions at elevations of 520–1,500 m, inhabiting arid rocky slopes in front of mountains, forest grasslands, and alluvial gravel piles in mountainous areas (Cui et al., 1996). Its flowers are relatively large, with six perianth segments arranged in two whorls of three, displaying radial symmetry. The three stamens are milky white, fused at the base with the outer perianth segments, with anthers positioned outside the style branches and closely adhering to them. These distinctive floral morphological features and ornamental values make *I. scariosa* an important germplasm resource for breeding superior ornamental flowers. Current research on *I. scariosa* has primarily focused on anti-inflammatory activity (Bian et al., 2018; Chang et al., 2020), chemical constituents (Yang et al., 2013, 2020), and seed dormancy and germination characteristics (Zhang et al., 2016), while studies on its reproductive biological characteristics are rare (Ma et al., 2017). Therefore, this study conducted detailed investigations on the flowering phenology, floral syndrome, pollination characteristics, and breeding system of this species to address the following questions: (1) What are the characteristics of its flowering phenology? (2) What are its floral morphological characteristics and flowering process? (3) What type of breeding system does this species have? (4) What are its pollination characteristics, and how do the beards on perianth segments affect pollinator visitation? Through this research, we aim to provide a scientific basis for the artificial propagation and industrial production of this species.

Materials and Methods

1.1 Study Site and Materials

The study site was located at the experimental field of Xinjiang Agricultural University in the western suburbs of Urumqi, Xinjiang, with geographic coordinates of 87°32 24.7 E, 43°48 55.4 N, and an elevation of 850 m. This area belongs to the alluvial fan plain of the Tianshan Mountains on the southern edge of the Junggar Basin. The annual minimum temperature (January) is -32.8°C, maximum temperature (July) is 40.5°C, mean annual precipitation is 234 mm, and mean annual evaporation reaches 2,219 mm (Mamut et al., 2018).

Iris scariosa plants consist of underground rhizomes, basal leaves, inflorescences, and flowers. The species generally sprouts in mid-March each year, with initial flowering from late April to early May, peak flowering in mid-May, final flowering in late May, fruit maturation from June to July, and capsules as fruits.

1.2 Experimental Methods

1.2.1 Flowering Phenology and Temperature-Humidity Monitoring During Flowering Three 1 m × 1 m quadrats were randomly established at the observation site. Starting from the first day of flowering, the total number of open flowers in each quadrat was recorded daily to calculate the start time and duration of initial, peak, and final flowering periods. Simultaneously, an EL-USB-2 temperature and humidity recorder (Lascar, China) was used to monitor environmental temperature and humidity during the flowering period of *I. scariosa.*

1.2.2 Floral Syndrome and Flowering Process Ten plants at peak flowering were randomly selected, and one fully open flower was marked on each plant to observe color, scent, and nectar presence. Floral dimensions were measured using an electronic digital caliper (Guilin Guanglu Digital Measurement and Control Co., Ltd., Guilin, China), including flower size, inner and outer perianth segment dimensions, and pistil and stamen lengths (including filament, anther, ovary, and style length and width). Additionally, on ten plants at peak flowering, one flower bud about to open was marked on each plant (total of 10 flowers). Undehisced anthers from each flower were placed in a 5 mL EP tube, crushed, and fixed with FAA solution to a final volume of 5 mL. Each time, 5 L was taken for slide preparation and pollen grains were counted under an Olympus BH-2 optical microscope (Olympus Corp., Tokyo, Japan), with five replicates per flower. The total pollen number per flower (N) was calculated as n × 1000. While measuring pollen number, the ovary was dissected with a needle and ovule number was counted under a dissecting microscope.

During peak flowering, ten flower buds about to open were randomly marked to observe the single-flower opening process, daily opening and closing times, and individual flower duration (floral longevity).

1.2.3 Pollen Viability and Stigma Receptivity Pollen viability was assessed following Dafni et al. (2005). During peak flowering, flowers about to open were marked and bagged until the end of anthesis. Starting from flower opening, anthers from 10 flowers were collected from different plants every 12 h. After thorough mixing, a small amount of pollen was placed on a slide, heat-inactivated as a control, and stained with 0.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The remaining pollen was directly stained with 1 L MTT at room temperature for 10 min and observed under an Olympus BH-2 optical microscope. Purple-black stained pollen grains were considered viable, while yellow unstained grains were non-viable. Six random fields of view were selected per slide to count stained and total pollen grains, and the percentage of viable pollen was calculated. Simultaneously, style branches from each flower were immersed in MTT stain to test stigma receptivity; stained stigmas indicated receptivity, while unstained stigmas indicated loss of receptivity.

1.2.4 Artificial Pollination Experiments During peak flowering, 25 healthy plants were randomly selected, and three flowers were marked on each plant. The 75 flowers were divided into five groups (15 flowers per group) and subjected to the following treatments: Natural pollination, to assess fruit and seed set under natural conditions (control, NP); Bagging without emasculation, to test for autonomous self-pollination (FB); Bagging after emasculation, to test for apomixis (BE); Artificial self-pollination after emasculation, to test self-compatibility (ASP); Artificial cross-pollination after emasculation, to test outcrossing (ACP). After pollination was completed or fruit set began, all bags were removed, and fruit and seed set were measured when fruits matured but had not yet dehisced.

1.2.5 Pollination Characteristics During three sunny days in the peak flowering period of *I. scariosa*, three flower buds about to open were marked daily on each of ten plants (30 flowers total). Continuous observations were made from 10:00 to 18:00 each day to record visitor insect species and foraging behavior, and to calculate visitation frequency (visits · flower^{-1} · hour^{-1}). To avoid observer disturbance, all observations were conducted from a distance of at least 1 m. Simultaneously, a portable temperature-humidity meter was used to record temperature and humidity at approximately 50 cm above ground (matching plant height), and daily weather conditions were documented to analyze relationships between insect activity and weather changes. After observations, five individuals of each visitor insect type were captured and brought to the laboratory for specimen preparation and species identification.

1.2.6 Effect of Beard on Pollinator Visitation During peak flowering, one flower bud about to open was marked on each of 15 plants (15 flowers total). Without damaging the outer perianth segments, the beards on the outer perianth were gently scraped off. Over three sunny days, starting from morning flower opening, pollinator species and their visitation behavior were continuously observed, and the number of visits per flower per hour was recorded and visitation frequency calculated.

1.3 Data Analysis

Data were analyzed using SPSS 22.0 statistical software. A normal distribution model with an identity function in the Generalized Linear Model (GLM) was used to compare the effect of beards on pollinator visitation frequency. A binomial distribution with a Logit link function in GLM was used to compare fruit and seed set rates among different pollination treatments. All statistical data are presented as mean ± standard error, and SigmaPlot 14.0 software was used for graphing.

Results

2.1 Flowering Phenology and Temperature-Humidity During Flowering

At the observation site, *Iris scariosa* entered initial flowering on May 5, peak flowering on May 10, and final flowering on May 17, with a total flowering duration of 16 days [Figure 1: see original paper]. The species concentrated daily flowering in the early morning from 08:00–12:00, with peak anthesis at 09:00–10:00, and wilting concentrated at 17:00–19:00. Environmental temperature and humidity at initial flowering were (12.44 ± 0.28)°C and (58.73 ± 2.49)%, respectively. As temperature increased and relative humidity gradually decreased, the daily number of open flowers increased, reaching peak flowering on day 6, with the peak flowering period lasting 4 days and showing synchronized flowering. At this time, temperature was (21.05 ± 0.82)°C and relative humidity was (40.89 ± 1.99)% [Figure 1: see original paper]. Thereafter, the flowering rate decreased significantly.

2.2 Floral Characteristics and Flowering Process

Flowers of *Iris scariosa* are bisexual, blue-purple [FIGURE:2: A, B], with a distinctive scent and small amount of nectar. Corolla diameter is (53.97 ± 2.20) mm, with outer perianth segments obovate and bearing yellow beard-like appendages on the midrib, inner perianth segments oblanceolate, and style branches pale purple, making it a wild ornamental flower of considerable value. Floral morphological parameters are shown in .

Individual flower pollen number was (59,042 ± 3,184) grains, ovule number was (52 ± 4), and the pollen-to-ovule ratio (P/O ratio) was 1,035.42.

On sunny days, *I. scariosa* began opening at 08:00. Before opening, buds swelled with no obvious separation between anthers and stigma. During opening, one outer perianth segment began to unfold first, followed simultaneously by the other two outer segments, which bent downward, while inner perianth segments remained wrapped around the style branches before gradually opening [Figure 3: see original paper]. The single-flower opening process required (2.03 ± 0.10) h, and individual flower duration was (2.50 ± 0.20) days. During anthesis, stamens remained tightly pressed against the outer side of the style. When pollinated flowers closed, both inner and outer perianth segments lost water and withered toward the center, and the stigma dried up [Figure 3: see original paper].

2.3 Pollen Viability and Stigma Receptivity

Pollen viability in *Iris scariosa* lasted approximately 60 h during anthesis. Pollen viability at initial dehiscence reached (84.20 ± 0.55)%, remained above 50% viable at 48 h after release, then gradually declined to (28.26 ± 3.79)% at 60 h when flowers wilted [Figure 4: see original paper].

MTT staining results showed that stigmas were dark purple from initial opening to 36 h, indicating optimal receptivity; at 48 h, stigmas were light purple, indicating reduced receptivity; and at 60 h when flowers wilted, stigmas were unstained, indicating loss of receptivity.

2.4 Artificial Pollination Experiments

No fruit set occurred in *Iris scariosa* under bagging without emasculation, bagging after emasculation, or artificial self-pollination after emasculation treatments. Fruit and seed set rates under natural pollination and artificial cross-pollination treatments were significantly higher than in the other three treatments (fruit set: Wald-^{2} = 1,018.33, d.f. = 4, $P < 0.001$; seed set: Wald-^{2} = 2,338.44, d.f. = 4, $P < 0.001$), but no significant difference existed between natural pollination and artificial cross-pollination [Figure 5: see original paper]. These results indicate that *I. scariosa* has a typical outcrossing breeding system.

2.5 Pollination Characteristics

During the flowering period, visitor insects of *Iris scariosa* included *Apis mellifera* [FIGURE:2: C], *A. cerana*, *Halictus* sp., flies (Muscidae), and *Pieris rapae*. Among these, *A. mellifera*, *A. cerana*, and *Halictus* sp. all carried *I. scariosa* pollen on their bodies, while flies and butterflies did not, indicating that the former three are pollinators. On sunny days, *A. mellifera* had the highest visitation frequency at (0.57 ± 0.05) visits $\cdot$ flower^{-1} $\cdot$ h^{-1}, followed by *A. cerana* at (0.42 ± 0.04) visits $\cdot$ flower^{-1} $\cdot$ h^{-1}, and *Halictus* sp. had the lowest at (0.19 ± 0.03) visits $\cdot$ flower^{-1} $\cdot$ h^{-1} [FIGURE:6: A].

At the observation site, daily activity periods of *A. mellifera* and *A. cerana* concentrated at 10:00–17:00, while *Halictus* sp. appeared at 11:00–16:00. *Apis mellifera* and *A. cerana* began frequent visits at 10:00, earlier than *Halictus* sp. which started at 11:00. Pollinator activity was most active at 12:00–15:00 when environmental temperature was higher, indicating that pollinator activity is closely related to environmental temperature and humidity [FIGURE:6: B].

2.6 Effect of Beard on Pollinator Visitation

Daily total visitation frequency was significantly higher on flowers with beards (intact flowers) than on beard-removed flowers at all time periods ($P < 0.05$) [Figure 7: see original paper]. Pollinators visited beard-removed flowers only between 11:00–16:00. Pollinators typically spent 5–15 s on a single visit to bearded flowers, sometimes up to 20 s, but usually less than 5 s on beard-removed flowers.

Discussion

Flowers are the reproductive organs of angiosperms, with sexual reproduction as their primary function (Liu et al., 2022). Flowering characteristics are important factors affecting plant fitness, mainly including flower number, flowering time, and flowering duration. These characteristics not only influence individual and population reproductive success (Wu et al., 2021) but also affect pollinator abundance, diversity, and foraging behavior (Weiss, 1991; Dai & Tan, 2011). Plant flowering characteristics are often influenced by abiotic factors such as light and temperature (He et al., 2005). In the arid environment of northern Xinjiang, most plants concentrate their flowering in spring or summer after snowmelt due to winter low temperatures and snowmelt timing. In this study, *Iris scariosa* flowered in early May after snowmelt, when temperature was above 10°C and relative humidity below 60%, providing favorable environmental conditions for flowering and fruiting. Before peak flowering, the daily number of open flowers in quadrats increased with rising temperature and gradually decreasing relative humidity, reaching peak flowering on day 6 with a 4-day duration, showing synchronized flowering. According to Herrera (1986), this flowering pattern is termed “mass-flowering pattern,” which facilitates rapid increase in floral display to attract more pollinators and enhance outcrossing opportunities during the relatively concentrated flowering period (Zhang & Qiu, 2017; Ye et al., 2022). This result is consistent with Szőllősi et al. (2011) on *Iris sibirica*. Therefore, the flowering characteristics of *I. scariosa* are more conducive to adaptation to harsh environments.

Floral syndrome represents adaptive strategies to pollinators evolved by plants over long-term evolutionary processes (Goodwillie et al., 2010), mainly including floral design and floral display. Floral design refers to characteristics such as flower structure, color, scent, and rewards provided to visitors, while floral display refers to the number of open flowers at a given time, their arrangement on the inflorescence, opening rate, and floral longevity (Zhang, 2004; Halibunuer et al., 2022). In the pollination process, the sole purpose of flower color is to attract pollinators (Zhang, 2004). Brightly colored flowers are generally considered more attractive to pollinators (Sobrevila et al., 1989). Flower size, as a visual signal provided to pollinators, plays an important role in pollinator attraction (Zhang et al., 2019). *Iris scariosa* has blue-purple flowers, relatively large corolla diameter (approximately 6 cm), three style branches specialized into petal-like structures that further increase individual floral display and provide landing platforms for pollinators, along with scent and nectar rewards. Additionally, the beard-like appendages on outer perianth segments can attract pollinators and increase visitation frequency, thereby enhancing successful pollination opportunities. This is similar to the view of Zhang et al. (2019) that the special floral features of *Iris bulleyana* are important visual signals for attracting pollinators. Moreover, *I. scariosa* has an individual flower longevity of approximately 2.5–3.0 days, with pollen release after flower opening. Its pollen viability and stigma receptivity last relatively long, and the period of highest pollen viability coincides with the optimal stigma receptivity period, facilitating pollen export and stigma pollen reception to ensure successful pollination (Harder et al., 1994; Wang & Tan, 2011). In summary, the bright coloration, large floral display, special floral structure, rewards provided to pollinators, and highly synchronized pollen viability and stigma receptivity of this species represent a floral syndrome evolved to ensure reproductive success.

Outcrossing is a sexual reproductive strategy that can avoid inbreeding depression caused by selfing, thereby improving seed quality, offspring fitness, and genetic diversity (Peng et al., 2012). Artificial pollination experiments showed that *Iris scariosa* produced no fruit under bagging without emasculation, bagging after emasculation, or artificial self-pollination after emasculation treatments, indicating no apomixis or autonomous self-pollination mechanisms and self-incompatibility, belonging to an obligate outcrossing breeding system. Many species in the genus *Iris* are self-incompatible, such as *I. bismarckiana* (Segal et al., 2006) and species in the Oncocyclus section including *I. atropurpurea*, *I. hermona*, and *I. haynei* (Sapir et al., 2005). Self-incompatibility during sexual reproduction can avoid inbreeding depression and promote outcrossing to enhance female fitness (Fornoni et al., 2016; Fachinetto et al., 2018). This result is consistent with Watts et al. (2013) on the breeding system of *Iris atropurpurea*, but differs from the self-compatible species *Iris versicolor* in northeastern

North America, which has diverse pollinator insects and can perform both outcrossing and wind-mediated self-pollination (Kron et al., 1993). Additionally, no significant difference in fruit and seed set rates existed between natural pollination and cross-pollination treatments, indicating no pollen limitation in natural conditions. Thus, *I. scariosa* has evolved an outcrossing breeding system in its reproductive characteristics to ensure reproductive success, which may be related to its special floral features.

Pollination is a crucial step in the sexual reproduction process of flowering plants. Since flowering plants are sessile, pollen transfer requires certain pollination vectors, mainly including animals, wind, and water (Zhang, 2004). Studies have shown that approximately 87.5% of flowering plants on Earth rely on animals for pollen transfer (Ollerton et al., 2011). Most *Iris* species have typical animal pollination characteristics, including complex flower shapes, variable colors, and rich floral rewards. These features can attract more pollinators (Zhang et al., 2019) and promote outcrossing reproductive success. *Apis mellifera*, *A. cerana*, and *Halictus* sp. are the main pollinators of *I. scariosa*, indicating that this species has a generalist pollination system that can accept visits from multiple pollinators. This pollination strategy can increase the number of pollination events and avoid reproductive failure (Suzuki et al., 2007). This is consistent with the pollination systems reported for many *Iris* species (Zhang et al., 2019). Previous studies have shown that pollinator species, abundance, behavior, and frequency are closely related to environmental factors such as light, temperature, wind speed, and rainy weather (Pellissier et al., 2010; Hu et al., 2012). In this study, *A. mellifera* and *A. cerana* typically began visiting flowers at 10:00, while *Halictus* sp. started at 11:00, with the former beginning earlier than the latter, possibly related to pollinator life habits. Regarding daily activity patterns, the three pollinators of *I. scariosa* were most active at 12:00–15:00 on sunny days when environmental temperature was higher and humidity lower, indicating that pollinator activity is closely related to environmental temperature and humidity. Furthermore, this study showed that pollinator visitation frequency and residence time significantly decreased after beard removal, possibly because the yellow beard-like appendages on the outer perianth midrib provide more precise visual signals to guide pollinators into the floral corolla tube, thereby increasing visitation frequency (Olvera et al., 2008; Zhang et al., 2019).

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