

## Research Article

# Highly efficient pollination by bumblebees ensures seed production in *Pedicularis lachnoglossa* (Orobanchaceae), an early-flowering Himalayan plant

<sup>1,2</sup>Wen-Bin YU† <sup>1</sup>De-Zhu LI <sup>1</sup>Hong WANG\*

<sup>1</sup>(Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China)

<sup>2</sup>(Graduate University of Chinese Academy of Sciences, Beijing 100049, China)

**Abstract** *Pedicularis* (Orobanchaceae) is a common high altitude genus of the Himalayas that may be affected by pollination limitation. Using *Pedicularis lachnoglossa* from Yulong (Jade Dragon) Snow Mountain in Lijiang (Yunnan Province, southwest China), we investigated the effects of high altitude habitats on the process of pollination and seed production. Floral biology, pollinator foraging behavior, breeding system, and pollination efficiency were examined using observation and exclusionary techniques. *Pedicularis lachnoglossa* was found to be entomophilous and exclusively pollinated by *Bombus friseanus* and *B. yunnanicola*. Our results indicated that pollination limitation in *P. lachnoglossa* was not significant. Under open pollination, approximately 80% of flowers were successfully pollinated and developed to fruits, and about 38% of ovules developed to mature seeds. Bumblebee pollination is highly precise and efficient in *P. lachnoglossa*, because its flowering phenology and floral characters enhance the foraging of bumblebees on flowers. This study supports that animal pollination plays a crucial role in the outbreeding of the early flowering *Pedicularis*. The evolution of floral specification in *Pedicularis* has the advantages of adaptation to bumblebee pollination in adverse high altitude habitats.

**Key words** alpine plant, bumblebee pollination, floral specialization, *Pedicularis lachnoglossa*, pollen dispensing mechanism, pollinator limitation.

Alpine environments are characterized by extremely low temperatures with frequent strong winds, and short and unpredictable growing seasons (Billings, 1974a; Korner, 1999), however, alpine flora shows extraordinary diversity, with approximately 8000–10 000 species of higher plants (Korner, 1999). Some studies have documented that the abundance and activity of pollinators decrease with increasing altitudes (Arroyo et al., 1982, 1985, 2006; Yumoto, 1986; Totland, 1994, 1997; Bergman et al., 1996; Bingham & Orthner, 1998). Thus, reproduction of anthophilous plants in high altitudes may be compromised by weather (Molau, 1993; Garcia-Camacho & Totland, 2009), suggesting that the number of autogamous and wind-pollinated plant species could increase with altitude (Berry & Calvo, 1989; Totland & Sottocornola, 2001; Arroyo et al., 2006; Garcia-Camacho & Totland, 2009). However, field investigations show that animal pollination plays a crucial

role in outbreeding for many alpine plants (e.g., Galen & Stanton, 1989; Kudo, 1993; Macior et al., 2001; Kudo & Suzuki, 2002; Wang & Li, 2005; Duan et al., 2007).

Delayed selfing provides reproductive assurance for some alpine plants under unpredictable pollinator environments (e.g., Sun et al., 2005; Zhang & Li, 2008; Duan et al., 2010), but evolutionary reduction of inbreeding depression may promote outbreeding in extreme alpine environments (Harder & Barrett, 1996), especially for early flowering species (Molau, 1993). Wirth et al. (2010) and Ai (2010) showed that outcrossing rates increased with altitude, using molecular techniques. Therefore, alpine plants have developed series of strategies to achieve outbreeding in their harsh environment (Billings, 1974a; Korner, 1999). For example: floral heliotropism provides the benefit of warmth for visiting pollinators (Zhang et al., 2010); large and showy flowers enhance pollination attractiveness (Billings, 1974a); prolonged stigma receptivity and high pollination efficiency can complement lower flower visitation rates (Bingham & Orthner, 1998; Blionis & Vokou, 2001); and floral traits are evolved to adapt to generalized pollinators (Kearns, 1992; McCall & Primack, 1992).

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† Present address: Department of Botany, Field Museum of Natural History, Chicago, Illinois 60605, USA

\* Author for correspondence. E-mail: wanghong@mail.kib.ac.cn; Tel.: 86-871-5223534; Fax: 86-871-5217791.

Community-level investigations show that fly and bee pollination are the dominant pollination systems at high altitudes (Arroyo et al., 1982; Yumoto, 1986; Kearns, 1992; McCall & Primack, 1992; Blionis & Vokou, 2001). Bumblebees (*Bombus* Latr.) are the primary pollinators for most tubular and/or zygomorphic flowers in the high altitudes of mountains in the Northern Hemisphere (Billings, 1974b; Macior, 1974; Yumoto, 1986; Galen & Stanton, 1989; Bergman et al., 1996; Korner, 1999; Blionis & Vokou, 2001). Some studies have documented that bumblebees have high pollination efficiency (Galen & Stanton, 1989; Wilson & Thomson, 1991; Bergman et al., 1996), and their sophisticated foraging behavior favors complex, especially zygomorphic, flowers (Neal et al., 1998; Kalisz et al., 2006; Rodriguez et al., 2004).

*Pedicularis*, with more than 600 species, mainly grow in cold, high altitude, and high latitude or montane habitats throughout the Northern Hemisphere. The greatest morphological diversity and species richness of this genus are found in the Himalayan region (Hong, 1983). More than half of the *Pedicularis* species are narrowly concentrated in this small region at an altitude above 2500 m, mostly between 3000 m and 4000 m (Li, 1948, 1949; Tsoong, 1956; Yamazaki, 1988). There is striking diversity in the shape of corolla galea and the length of corolla tube of *Pedicularis* species (Li, 1951; Ree, 2005; Yu et al., 2008). The beaked galea is very peculiar in flowering plants. It resembles a glass funnel that controls pollen dispersal and enhances pollination success (Harder, 1990; Kawai & Kudo, 2009).

*Pedicularis* are almost exclusively pollinated by bumblebees, and their floral characteristics are closely associated with the foraging behavior of bumblebees (Macior & Tang, 1997; Wang & Li, 1998, 2005; Tang & Xie, 2006; Yu et al., 2008). Pollination co-adaptation between *Pedicularis* and bumblebees has been investigated in many regions around the Northern Hemisphere (e.g., Kwak, 1973; Macior, 1982, 1990; Macior & Tang, 1997; Wang & Li, 2005; Tang & Xie, 2006). However, we know less about the specific contribution of bumblebee pollination on the reproductive output of *Pedicularis*. Some bagged experiments show that seed production of *Pedicularis* is entirely dependent on pollinators (e.g., Williams & Battzli, 1982; Eriksen et al., 1993; Macior et al., 2001; Wang & Li, 2005; Huang & Fenster, 2007; Xia et al., 2007), and autogamous selfing only occurs in a few beakless species under unpredictable pollinator conditions (Eriksen et al., 1993; Sun et al., 2005). In the Arctic regions of North America and Europe, studies show that the seed set of early-flowering *Pedicularis* species is lower than that of later-flowering species (Williams & Battzli, 1982; Eriksen et al., 1993; Philipp

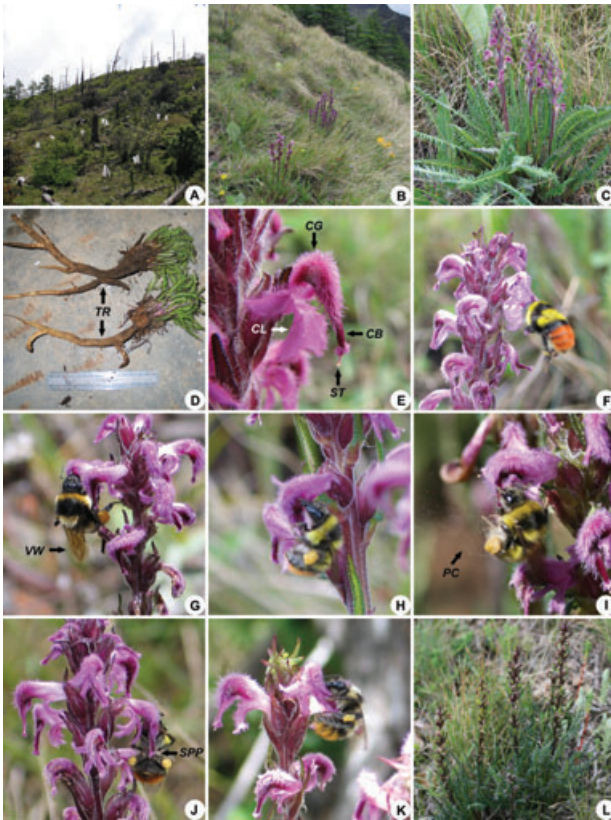
et al., 1996), and the seed set of high elevation species is lower than that of lower elevation species (Eriksen et al., 1993). In the Himalayan region, bumblebees effectively pollinate some mid- and later-flowering *Pedicularis* in mid-altitude meadows (Macior et al., 2001; Wang & Li, 2005; Yang et al., 2005; Xia et al., 2007). To date, there is no information about the reproductive biology of early-flowering *Pedicularis* in the Himalayan region. In this study, therefore, we chose an early-flowering lousewort, *Pedicularis lachnoglossa* Hook. f., to evaluate the contribution of bumblebee pollination to reproductive output under natural pollination. The main aims were to: (i) evaluate whether *P. lachnoglossa* experienced pollination limitation, and if not, which factor(s) enhanced pollination success; and (ii) estimate the pollination efficiency of bumblebees.

## 1 Material and methods

### 1.1 Plant material and study sites

*Pedicularis lachnoglossa* is a semiparasitic perennial herb that starts to bloom in late May, reaches peak blooming between middle June and early July, and rarely extends to early August (Yu, 2007). Plants are usually 10–25 cm tall with 2–15 cespitose stems and numerous permanent leaves at the base (Fig. 1: C). Taproots are thick and long with loose xylem and marrow (Fig. 1: D). Inflorescences are raceme at the ends of stems, and can produce approximately 10–50 flowers per inflorescence, blooming from the bottom to top. A monosymmetric flower consists of a short corolla tube and a beaked galea (upper lip) covered with long reddish-purple jointed hairs, especially on the corolla galea and beak (Fig. 1: E).

Field observations were carried out at two sites on Yulong (Jade Dragon) Snow Mountain (27°01'N, 100°11'E), Lijiang county (northwest Yunnan, China), from May to August, for two consecutive years (2006, 2007). Site Cikeba (CKB) is located in a shrub habitat at altitude 3690 m (Fig. 1: A), and site Maoniupo (MNP) is located in an alpine meadow habit at altitude 3980 m (Fig. 1: B). The size of each sample site was approximately 100 m × 50 m. In 2006, the flowering density of CKB was on average 1.93 (standard deviation (SD), 0.62, hereafter) plants m<sup>-2</sup>, and that of MNP was on average 1.76 (0.37) plants m<sup>-2</sup> (Yu, 2007). The field air temperature and humidity at MNP were automatically recorded using a temperature data logger (AZ8829; AZ Instrument, Taichung City, Taiwan, China) from May 22 to July 22, 2006 (Yu, 2007). The mean ambient temperature began to increase at 07:00 h, and decreased after 17:00 h. During the recording period, the highest



**Fig. 1.** *Pedicularis lachnoglossa* and bumblebees. **A**, Site Cikeba (CKB), a shrub habitat. **B**, Site Maoniupo (MNP), an alpine meadow habitat. **C**, Habit and inflorescences. **D**, Root. **E**, Flower. **F**, A bumblebee approaching flowers. **G–K**, Bumblebees foraging with a sternotribic pattern. **L**, Infructescences. **F–J**, *Bombus friseanus* Skorikov. **K**, *Bombus yunnanicola* Bischoff. **CB**, corolla beak; **CG**, corolla geala; **CL**, corolla lower lip; **PC**, pollen cloud; **SPP**, strategic pollination position; **ST**, stigma; **TR**, taproot; **VW**, vibrating wings.

temperature reached 27.5 °C, and the lowest temperature was only 1.4 °C.

## 1.2 Floral biology and pollinator observations

To examine the timing of floral development under natural conditions, 20 randomly selected flowers from 10 plants at each site were marked, and the onset of stigma receptivity, anther dehiscence, and floral longevity were observed. Floral longevity of bagged flowers was recorded. A solution of benzidine–hydrogen peroxide (1% C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>:6% H<sub>2</sub>O<sub>2</sub>:H<sub>2</sub>O = 4:11:22) was used to test stigma receptivity (Kearns & Inouye, 1993; Jiang et al., 2010). To examine anther dehiscence, we dissected one side of the galea to check whether the introversive anther wall was cracked. Single flower longevity was counted from the lower lip unfolded to the whole flower withered. Open pollination and bagged treatments investigated 20 flowers each. To evaluate the effect of pollination on floral display of inflores-

cence, we compared differences in floral display size between the two treatments (open vs. bagged). Thirty-five open inflorescences and 24 bagged inflorescences were marked, and the number of open flowers on an inflorescence were recorded from the beginning of flowering to the peak of flowering (i.e., 12–27 June 2006) at MNP.

Pollinator observations were carried out at both sites at 10:00 h–15:00 h on five sunny days between 10 and 30 June (2006 and 2007). Observers systematically walked through the population to track potential pollinators and recorded the number of pollinators visiting flowers (Dafni et al., 2005). Foraging behavior of pollinators included how visitors approached flowers and collected pollen, where pollen was deposited, and where the stigma contacted the body of the visitors. At least three individuals of each pollinator were captured for identification. Voucher specimens of insect pollinators were deposited at the Insect Collection of the Kunming Institute of Botany, Chinese Academy of Sciences.

At the MNP site, two approaches were used to measure the floral constancy of different pollinators (Dafni et al., 2005). First, pollinators were captured, and at least 15 corbicular pollen loads per species were collected. Each pollen load was dissolved in a 0.5 mL centrifugal tube in 0.1 mL 70% ethanol. Five microliters of solution was extracted and mounted in glycerin jelly on a microscopic slide, which was used to identify pollen types under a light microscope. Each pollen load was examined using five slides, 100 pollen grains per slide. In addition, at least 15 individuals for each pollinator species were tracked on a foraging trip within the sampled population. We counted the number of flowers visited successively by an insect within an inflorescence and/or a plant.

## 1.3 Breeding system

To evaluate the contribution of insects and wind in pollen transportation, we carried out three experimental treatments using 15 plants for each type of pollination at both sites for 2 years. The treatments were: (i) self-pollination, in which flowers buds were covered for exclusion using plastic bags before anthesis, which excluded anemophily and entomophily; (ii) anemophilous pollination, in which flower buds were bagged using nylon nets before anthesis, which excluded entomophily; and (iii) open pollination, which acted as the control and marked inflorescences were kept accessible to pollinators. All infructescences were harvested before fruit dehiscence. The seed production was recorded for 60 mature fruits that were randomly selected from 15 plants (four for each plant, at each site in both years).

## 1.4 Pollination efficiency

Pollination efficiency was estimated by counting the number of pollen grains deposited on a stigma after a single visit. Inflorescences were bagged by nylon nets before anthesis. When flowers opened, the nylon nets were taken off. The flowers were immediately harvested after the first pollinator visit and fixed in the formalin-aceto-alcohol solution. At least 15 flowers for each pollinator were used to count stigmatic pollen loads.

During the peak flowering time, 50 mature flowers per day (replicated five times at each site per year) were randomly collected and fixed into formalin-aceto-alcohol solution. Pollination rates were estimated as the number of flowers bearing at least one pollen grain on the stigma divided by the total number of examined flowers. Fifty pollinated flowers of each site were used to measure stigmatic pollen loads. Stigmas were dyed using Fast Green FCF for approximately 5 s, then placed on a slide and pressed with a glass cover. The number of pollen grains per stigma was counted under a light microscope. The ovary was carefully dissected and placed into a drop of water on a slide. The entire placenta with attached ovules was removed through a longitudinal slit in the ovary wall. The ovules were carefully loosened from the placenta and spread in the drop of water to be counted under a dissecting microscope.

## 1.5 Data analysis

All data were analyzed using SPSS version 13.0 for Windows (Gaur & Gaur, 2006). Two-way ANOVA was used to analyze the difference in floral longevity among treatments and sites, and that of fruit-sets, seed production, pollination rates, and visitation rates among sites and years. An independent-sample *t*-test was used to analyze the difference in stigmatic pollen loads between virgin and open pollinated flowers.

## 2 Results

### 2.1 Floral biology and pollinator observations

The stigma became receptive and the anthers dehisced at the first day of blooming. Ovule production per flower varied from 11 to 22, and the two sites had no significant difference (Table 1). Under open pollination conditions, flowers bloomed on average 3.55 (0.64) days, however, the bagged treatment significantly prolonged floral longevity (Tables 1, 2). The altitude had no effect on the flower longevity of open pollination or bagged flowers (Tables 1, 2). At the beginning of flowering, the inflorescence floral display size of open and bagged treatments was equal, but the floral display of

**Table 1** Observations of floral biology and pollinator behavior in *Pedicularis lachnoglossa* made over two years and under different treatments at Cikeba (CKB) and Maoniupo (MNP) sites in Yunnan, China

Traits	CKB (mean ± SD)	MNP (mean ± SD)
Ovule production per flower	15.66 ± 2.67	15.50 ± 2.74
Pollinator frequency per day		
2006	97.60 ± 9.21	91.80 ± 8.67
2007	97.20 ± 9.09	93.80 ± 7.15
Total	97.40 ± 8.63	92.80 ± 7.47
Pollination rate per day		
2006	0.82 ± 0.09	0.80 ± 0.07
2007	0.82 ± 0.09	0.78 ± 0.06
Total	0.82 ± 0.08	0.79 ± 0.06
Stigmatic pollen load	36.84 ± 9.44	35.16 ± 9.18
Fruit-set per inflorescence		
2006	0.84 ± 0.07	0.79 ± 0.11
2007	0.87 ± 0.06	0.83 ± 0.08
Total	0.86 ± 0.07	0.81 ± 0.10
Seed production per capsule		
2006	5.98 ± 1.92	5.95 ± 1.85
2007	6.37 ± 1.83	5.98 ± 1.48
Total	6.18 ± 1.88	5.97 ± 1.67
Floral longevity (days)		
Control	3.50 ± 0.61	3.60 ± 0.68
Bagged	6.25 ± 0.97	6.40 ± 0.94

bagged inflorescences increased as the peak flowering arrived (Fig. 2).

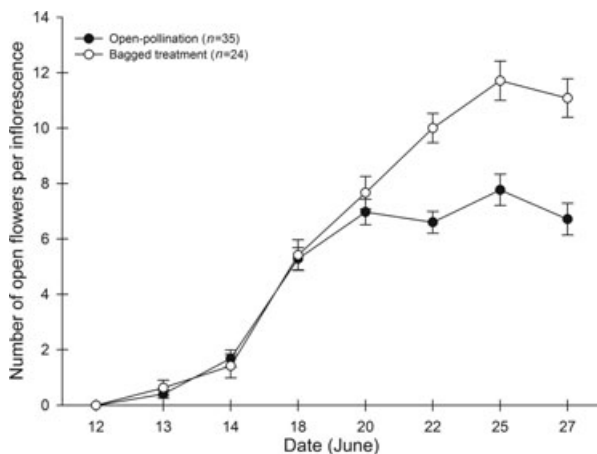
*Pedicularis lachnoglossa* was exclusively pollinated by two bumblebee species, *Bombus friseanus* Skorikov and *Bombus yunnanicola* Bischoff (Fig. 1: F–K). During peak flowering, we recorded 82–111 bumblebee workers of *B. friseanus*/*B. yunnanicola* visiting flowers in a sunny day (Table 1). The flowers did not have nectararies (Wang et al., 2006, unpublished data), thus pollinators were rewarded with pollen only. Bumblebees collected pollen in a sternotribic pattern (i.e., the bees grasped the corolla beak while hanging in an inverted position) (Fig. 1: G–K). Due to *P. lachnoglossa* having beaked galea (Fig. 1: E), bumblebees collected pollen using wing vibrations to dislodge pollen (Fig. 1: G). Buzz pollination is usually associated with poricidally dehiscent anthers (see Buchmann, 1983, for a review). The anthers of *P. lachnoglossa* are longitudinally dehiscent, but the beaked galea resembles a glass funnel and its tip restricts the pollen removal. Generally, the visiting bumblebee (Fig. 1: F) approached the flower from the right side (Fig. 1: G), and the mouthpart bit the inferior margin of the galea and the forelegs grasped the beak (Fig. 1: H). While the bumblebee was vibrating its wings, pollen grains were released from the tip of the corolla beak onto the anterior ventral abdomen of the bee's body with a pollen cloud around the flower and bee (Fig. 1: I). Simultaneously, the corolla beak forced the stigma to contact the same body location (Fig. 1: J). Both own and other plants' pollens could be simultaneously deposited on the stigma.

**Table 2** Two-way ANOVA of floral longevity (days), fruit-set, seed production, pollination rate, and pollinator visitation rate in *Pedicularis lachnoglossa* showing the effect of study site and year/treatment, and the interaction between site and year/treatment

Source of variation	Type III SS	df	MS	F-value	P-value
<b>Pollinator frequency per day</b>					
Corrected model	116.20 <sup>a</sup>	3	38.73	0.53	0.670
Site	105.80	1	105.80	1.46	0.250
Year	3.20	1	3.20	0.05	0.840
Site × Year	7.20	1	7.20	0.10	0.760
Error	1161.60	16	72.60		
<b>Pollination rate per day</b>					
Corrected model	0.01 <sup>b</sup>	3	0.00	0.39	0.760
Site	0.01	1	0.01	1.04	0.350
Year	0.00	1	0.00	0.17	0.700
Site × Year	0.01	1	0.00	0.08	0.780
Error	0.10	16	0.01		
<b>Fruit-set per inflorescence</b>					
Corrected model	0.49 <sup>c</sup>	3	0.16	2.37	0.080
Site	0.03	1	0.03	4.42	<0.050
Year	0.02	1	0.02	2.61	0.110
Site × Year	0.00	1	0.00	0.81	0.780
Error	0.39	56	0.01		
<b>Seed production per capsule</b>					
Corrected model	7.05 <sup>d</sup>	3	2.35	0.74	0.530
Site	2.60	1	2.60	0.83	0.370
Year	2.60	1	2.60	0.83	0.370
Site × Year	1.84	1	1.84	0.58	0.450
Error	746.75	236	3.16		
<b>Floral longevity (days)</b>					
Corrected model	154.34 <sup>e</sup>	3	51.45	77.65	<0.001
Site	0.31	1	0.31	0.47	0.490
Treatment	154.01	1	154.01	232.47	<0.001
Site × Treatment	0.01	1	0.01	0.02	0.890
Error	50.35	76	0.66		

a,  $R^2 = 0.09$ ; b,  $R^2 = 0.07$ ; c,  $R^2 = 0.11$ ; d,  $R^2 = 0.01$ ; e,  $R^2 = 0.75$ . *df*, degrees of freedom; *MS*, mean squares; *P*, significance;  $R^2$ , coefficient of multiple determination; *SS*, sum of squares.

Analysis of corbicular pollen loads showed that both bumblebee species had a high degree of foraging fidelity on flowers of *P. lachnoglossa*. Of corbicular pollen loads from *B. friseanus* and *B. yunnanicola*, 88.9% (16 of 18) and 93.3% (14 of 15) were com-

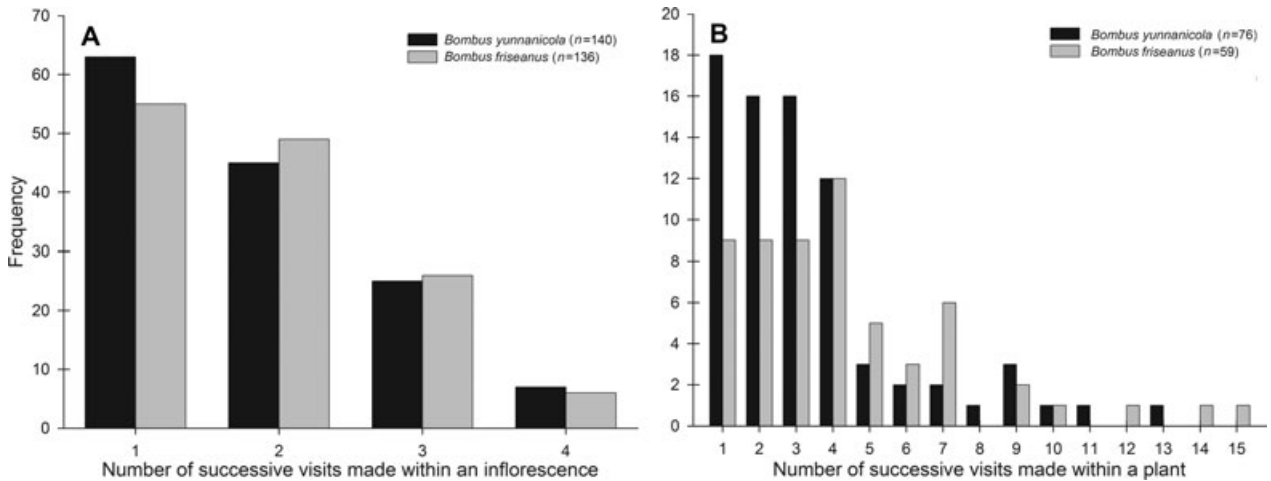


**Fig. 2.** Comparisons of the floral display size of *Pedicularis lachnoglossa* between open and bagged inflorescences from the beginning of flowering to the peak of flowering. *n*, number of studied inflorescences. Error bar = mean  $\pm$  SE.

posed entirely of *P. lachnoglossa*, respectively, and more than 90% of pollens in the mixed loads were from this species. Pollinator tracking showed that both species of bumblebee workers favored flowers of *P. lachnoglossa* to other plants flowering at the same time. Only three of 15 individuals of *B. friseanus* and two of 15 individuals of *B. yunnanicola* left *P. lachnoglossa* to visit *Aster batangensis* Bureau & Franch., *Ranunculus yunnanensis* Franch., or *Veronica piroliformis* Franch., all of which reward bumblebees with nectar. In addition, most bumblebees visited more than two flowers on an inflorescence and multiple flowers on a plant before leaving (Fig. 3).

## 2.2 Breeding system

None of the bagged inflorescences bore fruit, whereas on average approximately 80% of open-pollinated flowers produced fruits (Table 1, also see Fig. 1: L). These results showed that *P. lachnoglossa* depended entirely on bumblebees for ovule fertilization, and self-pollination and anemophily did not occur. Flowers are herkogamous (referring to the spatial separation of stigmas and anthers within flowers), and the four anthers are tightly enclosed by the beaked galea (Fig. 1: E), thus



**Fig. 3.** Number of successive flowers visited by an individual pollinator within a foraging trip on *Pedicularis lachnoglossa*. **A**, An individual inflorescence as a unit, so that some plants may be divided into more than one unit. **B**, A plant with more than one inflorescence considered as a unit. *n*, number of subtotal inflorescences/plants visited by each species of bumblebee.

selfing is virtually impossible and wind is unlikely to dislodge pollen.

There were no significant differences between years in mean fruit-set of open pollinated plants at the two sites (Table 2), however, the mean fruit-set at the low altitude site (CKB) was significantly greater than the high altitude site (MNP) (Tables 1, 2). There were no significant differences in year or site effects for seed production (approximately six seeds per capsule).

### 2.3 Pollination efficiency

*Bombus friseanus* and *B. yunnanicola* deposited at least 21.87 (5.08) and 20.87 (4.78) pollen grains per visit to virgin, respectively. The pollination efficiency of the two bumblebees had no significant difference ( $t = 0.56$ ,  $P > 0.50$ ). Under natural conditions, on average more than 78% of flowers were pollinated by bumblebees (Table 1). The stigmatic pollen loads of open pollinated flowers showed no significant difference between the two sites ( $t = 0.90$ ,  $P > 0.10$ , Table 1).

## 3 Discussion

*Pedicularis lachnoglossa* was frequently visited by two species of bumblebee workers. Pollinator frequency and stigmatic pollen loads of pollinated flowers showed that *P. lachnoglossa* did not suffer from significant pollination limitation at either site. Under open pollination, on average approximately 80% of flowers were successfully pollinated and developed to fruits, and approximately 38% of ovules developed to mature seed, even though 78% of ovules were fertilized (Yu, 2007).

Fruit-set and seed:ovule (S/O) ratios of this species are at a middle level of investigated *Pedicularis* species in the Himalayan region (fruit-set, 64.5%–94%; S/O ratio, 15%–52%; Macior et al., 2001; Sun et al., 2005; Wang & Li, 2005; Xia et al., 2007), as well as other regions (fruit-set, 59%–99.5%; S/O ratio, 19%–60%; Kwak, 1973, 1979; Williams & Battzli, 1982; Macior, 1983; Eriksen et al., 1993; Macior, 1993; Philipp et al., 1996). The S/O ratio of *P. lachnoglossa* is between the mean values of early-flowering (30%) and mid-flowering (55%) species (Molau, 1993) indicating that the early flowering *P. lachnoglossa* favors outbreeding with selected abortion of zygotes.

Flowering phenology of *Pedicularis* matches the life-cycle of the bumblebee that has been stated by Macior and his colleagues (Macior, 1970, 1974, 1982; Macior & Sood, 1991), which may enhance pollination success of *Pedicularis*. Generally, the spring flowering species with beakless and nectariferous flowers are primarily pollinated by bumblebee queens, and the estival and autumn species with beaked and nectarless flowers are exclusively pollinated by bumblebee workers (Macior, 1982). *Pedicularis lachnoglossa* blooms in early summer, one of the earliest flowering *Pedicularis* species in the Himalayan region. For developing the colony, bumblebee workers could prefer pollen-rewarding species to nectar-rewarding species. Each flower of *P. lachnoglossa* produces on average 37 382 (4675) pollen grains (Yang & Guo, 2004), which are concealed and can be collected only by bumblebees using buzz pollination (Macior, 1982). The concealed anthers prevent ineffective visitors from consuming pollen, and enhances the pollination efficiency of the

system (Harder, 1990). In addition, favorable temperatures, high flowering density, and pollen-rich flowers of *P. lachnoglossa* enhance the visitation and pollination rates of bumblebees; thus, this species avoids the problem of pollinator limitation occurring in other early-flowering plants in high mountains (Kudo, 1993; Molau, 1993; Kudo & Suzuki, 2002).

Bumblebees are the most effective pollinators for many alpine plants in the Northern Hemisphere (e.g., Billings, 1974a; Macior, 1974; Bauer, 1983; Galen & Stanton, 1989; Bergman et al., 1996; Bingham & Orthner, 1998). Many *Pedicularis* species are entirely dependent on bumblebees for seed production (Williams & Battzli, 1982; Macior, 1983; Macior et al., 2001; Wang & Li, 2005). Floral traits of *Pedicularis* are closely correlated to the bumblebees' physical form and behavior (Macior, 1982). For beaked and nectarless flowers, bumblebees collect the pollen using the vibration of their wings with nototribic or sternotribic patterns (Macior, 1982). For *P. lachnoglossa*, bumblebees had sternotribic foraging behavior, and the strategic pollination position was on the anterior ventral abdomen of the bee's body. This pollination mechanism is also found in other species with a straight beak, such as *P. racemosa* Douglas ex Benth., *P. resupinata* L., *P. integrifolia* Hook. f., and *P. tricolor* Hand.-Mazz. (Macior, 1970, 1988; Wang & Li, 2005). Some species with an S-shaped corolla beak, such as *P. davidii* Franch., *P. longiflora* Rudolph, and *P. siphonantha* D. Don, are also exclusively pollinated by bumblebees using the sternotribic pattern (Wang & Li, 1998; Tang & Xie, 2006; Yu et al., 2007, unpublished data).

The pollen of *P. lachnoglossa* was discharged along the funnel-shaped beak and deposited onto a specific position of the bumblebee's venter. The beak also forced the stigma to contact this position on the bumblebees' body (Harder, 1990). Because the diameter of the beak tip directly controls pollen removal rates, it is a type of pollen dispensing mechanism (Harder, 1990). As reported by previous field investigations, all beaked species are nectarless (Macior, 1982; Ree, 2005), and they reward pollinators with pollen only (Yu et al., 2008, for a review); thus, the pollen dispensing mechanism enhances pollination success of the beaked species of *Pedicularis* by increasing the visitation times (Kawai & Kudo, 2009). Stigmatic pollen loads of open pollinated flowers exceeded that achieved in the single visit, indicating that most open pollinated flowers of *P. lachnoglossa* receive multiple visits, and our observations of visitation rates suggest that is certainly the case.

Analyses of corbicular pollen loads and pollinator tracking showed that the two types of bumblebee

workers had high flower constancy to flowers of *P. lachnoglossa*. As documented by Macior (1970), bumblebees show higher fidelity to the pollen-rewarding species of *Pedicularis* than to the nectar-rewarding species. Thus, beaked flowers with complex shape could promote pollinator fidelity (i.e., flower constancy) in *Pedicularis*, because they require additional learning and handling time investment of the pollinator (Neal et al., 1998; Chittka et al., 1999; Gegear & Laverty, 2001). Bumblebees are the primary pollinators for many alpine plants. Thus, high flower constancy to *P. lachnoglossa* could not only reduce pollen wastage and interspecific pollen transportation, but also enhance pollination efficiency and accuracy of pollinators to flowers.

At the study sites, overcast and/or rainy days are frequent and continue for several days during the flowering time. Pollinator visitations and pollination rates are depressed. Unpollinated flowers enlarging floral display size could enhance pollination attractiveness to pollinators, and increase pollination success when the weather is sunny. Plasticity of floral longevity may be an advantage used to cope with adverse pollination conditions at high altitudes. However, the disadvantage of geitonogamy caused by large floral displays is still not clear. In *P. densispica* Franch. ex Maxim., *P. longiflora*, and *P. siphonantha*, Xia et al. (2007) and Yang et al. (2005) found that self-pollen sources reduced seed production because of inbreeding depression limiting the development of self-zygotes. Further investigations should test whether pollen quality or resources, or both factors, restrict seed production in this species.

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