POLLINATION BIOLOGY OF FOUR *PEDICULARIS* SPECIES (SCROPHULARIACEAE) IN NORTHWESTERN YUNNAN, CHINA[1]

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Abstract

Pedicularis L. (Scrophulariaceae) represents a classic example of floral morphological diversity for pollination biologists. In the species-rich areas of the Himalayas there have been few studies on the pollination syndromes of *Pedicularis,* especially of the long-tubed species. In the summer and fall of 2000, the pollination biology of four species of *Pedicularis* was studied in Zhongdian of northwestern Yunnan, China. The four species, foraged by three bumblebee species, possess two different corolla types: *Pedicularis lutescens* Franch. ex Maxim. subsp. *lutescens* and *P. densispica* Franch. ex Maxim. have a short-tubed, erostrate, and nectariferous corolla type and are pollinated exclusively by *Bombus friseanus* Skorikov and *B. lucorum* Latr. workers foraging flower nectar and pollinating nototribically; *P. integrifolia* Hook. f. and *P. tricolor* Hand.-Mazz. have long-tubed, rostrate, and nectarless corollas and are pollinated sternotribically by *Bombus festivus* Smith and *B. friseanus* workers hanging on the S-shaped beak or supported by the bowl-shaped lower lips. Cross-pollination by bumblebee workers is the most important reproductive mechanism observed for these *Pedicularis* in Yunnan. Nectar-foraging bumblebee queens and lepidopteran moths or butterflies were not found to pollinate any of these *Pedicularis* species. Otherwise, the interactions between *Pedicularis* and *Bombus* and pollination mechanisms in northwestern Yunnan are similar to those in Japan and North America. The present study provides new evidence that the Japanese species had evolved from Himalayan migrants in a pattern parallel to that of North American species.

Key words: *Bombus,* northwestern Yunnan, *Pedicularis,* pollen/ovule ratio, pollination mechanism, Scrophulariaceae.

Pedicularis (Scrophulariaceae) comprises some 600 to 800 species (Wang et al., 2003) and is one of the largest angiosperm genera in the Northern Hemisphere (Mabberley, 1987; Yang et al., 1998). The species of *Pedicularis* are generally perennial or annual (rarely biennial) herbs hemiparasitic on grasses. Floral morphology displays striking differences in the corolla shape and the length of the corolla tube. The corolla is strongly zygomorphic, consisting of an upper lip (galea) enclosing four introrse anthers and a style running the length of the galea, with the stigma exserted beyond its tip. The galea can be variously beaked, toothed, and crested, while the lower lip is variously trilobed, usually spreading, and external to the upper lip in bud. Of four general corolla forms in the genus described by Maximowicz (1888) and Li (1951)—i.e., beakless, toothless; beakless, toothed; beaked, short-tubed; beaked, long-tubed—only the last is absent from Japan and North America, and this corolla form appears in species endemic to the Himalayas (Macior, 1984). Because of its diverse corolla forms and large number of species, the origin and evolution of *Pedicularis* have long intrigued botanists. Limpricht (1924) considered the Himalayan region as a center of origin, although Prain (1890) earlier suggested that *Pedicularis* originated in the Holarctic and dispersed from a common boreal center. Hong (1983) identified a small area in the Himalayas, in western Sichuan and northwestern Yunnan, as containing ca. 300 species of *Pedicularis.* Pointing out the morphological diversity and high degree of endemism in this region, Macior (1982, 1988) also favored the Himalayan origin hypothesis.

Pedicularis displays enormous diversity of floral form (Pennell, 1948) and is a classic example of pollination syndrome diversification within a genus. Beginning in the late 19th century, pollination mechanisms of *Pedicularis* have been studied in

[1] Project supported by the National Natural Science Foundation of China (No. 39970060) and the national program for R & D Infrastructure and Facility Development (2004DKA30430). We thank Cao-Chang Hu and Hui-Fen Zhu (KUN) for valuable help during fieldwork. We also thank Jie Cai (Yunnan University, now KUN) for carrying out partial pollen analysis. We are grateful to Gary Hannan (Eastern Michigan University) for critical reading of an earlier version of the manuscript, and to Lazarus Walter Macior (Akron University, Ohio) for kind assistance with the literature.

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North America and Europe. Müller (1881), Weed (1884), and Kunth (1898–1905) primarily considered the floral morphology and the foraging behaviors of the pollinators. Since then, Macior (see 1982 for a summary of earlier studies; Macior, 1984, 1986) made a systematic and thorough investigation of the pollination ecology, endemic adaptation, and evolution of pollination mechanisms in *Pedicularis,* primarily in the North American species. Meanwhile, Sprague (1962), Faegri and van der Pijl (1966), MacInnes (1972), and Kwak (1979) demonstrated similar patterns of pollination ecology in this genus as well as co-adaptations between *Pedicularis* and its pollinators. Earlier studies revealed that the evolution of *Pedicularis* has been closely co-adapted to its pollinators, principally bumblebees (*Bombus*). Pollination has been noted for a solitary bee (*Osmia tristella*) in *P. semibarbata* L. (Macior, 1977) and by hummingbirds in *P. densiflora* Benth. (Macior, 1986), with these seen as more recent adaptations. Such phenomena have not yet been observed in Chinese *Pedicularis,* but there are hundreds more taxa to observe. The pollination ecology of *Pedicularis* in Asia remained completely unknown until Macior (1988) made his first study in Japan. Interestingly, his Japanese observations revealed a close parallel between pollination mechanisms in Japan and North America. Subsequently, some Asian species were observed primarily from the western and Chinese Himalayas (Macior, 1991; Macior & Sood, 1990, Macior & Tang, 1997, 1999; Wang & Li, 1998; Macior et al., 2001). This study was extended to the Himalayan species, especially those bearing flowers of the unique long-tubed corolla type, found only in the Himalayas. It was suggested that the species with long-tubed beaks were adapted to moth pollination (Pennell, 1943). However, Macior's preliminary observations revealed that the long, nectarless corolla tube was probably a floral adaptation of these plants, which are dwarf-sized at high elevations, for attracting other pollinators. Obviously, more case studies are needed to test Pennell's hypothesis and to compare Macior's results in Japan and the western Himalayas. There are 352 species of *Pedicularis* in China, of which some 272 species are endemic (Yang et al., 1998), with some transboreal species of extensive geographic disjunctions (Macior, 1988), e.g., *P. verticillata* L. In southwestern China, the province of Yunnan contains 151 species of *Pedicularis,* including all four corolla forms, with about half of the endemic species in China and 16 long-tubed species and varieties (Wang & Li, 1999; Li et al., 2002). In the summers of 1993 and 1997, we made a preliminary study of the pollination biology of *Pedicularis* species in northwestern Yunnan (Wang & Li, 1998; Wang, 1998) and provided evidence in support of Macior's (1991) explanation of the function of the long-tubed species. Meanwhile, through insect bagging studies in Sichuan, Macior and Tang (1997, 1999) reported pollinator dependence of three *Pedicularis* species. However, knowledge of the pollination syndromes of *Pedicularis* in the Himalayas is far from adequate. Only the pollination syndromes of 2 (e.g., *P. siphonantha* D. Don var. *delavayi* (Franch. ex Maxim.) Tsoong and *P. longiflora* Rudolph var. *tubiformis* (Klotzsch) Tsoong) of the 16 long-tubed species in Yunnan have been documented. These, plus the other 2 species (*P. megalantha* D. Don and *P. punctata* Decne.) observed in the western Himalayas, were the only 4 long-tubed species studied by pollination biologists. This study aimed mainly to test the controversial explanations about the function of the long-tubed flowers and to compare it with the function of other types of corollas. One interesting question is whether the long-tubed species are pollinated by lepidopteran insects or by bumblebees and the evolutionary consequences. It is believed that more studies on the pollination biology in the species-richest area will provide more light on the cause of diversification of *Pedicularis.*

Table 1. Distribution of the four studied *Pedicularis* species.

Taxon	Distribution
Pedicularis densispica	Northwestern Yunnan, western Sichuan, southern and southeastern Xizang
P. integrifolia	Northwestern Yunnan, southwestern and western Sichuan, southeastern Xizang; Bhutan, Nepal, Sikkim
P. lutescens subsp. *lutescens*	Northwestern Yunnan
P. tricolor	Northwestern Yunnan

Materials and Methods

In the summer and fall of 2000, pollination investigations were conducted in alpine areas of the county of Zhongdian, northwestern Yunnan, China. We here report the pollination biology of four *Pedicularis* species in northwestern Yunnan, including two long-tubed species, *P. integrifolia* and *P. tricolor,* and two short-tubed, erostrate, and nectariferous species, *P. lutescens* subsp. *lutescens* and *P. densispica* (Tables 1–6).

Table 2. Flowering phenology of the four *Pedicularis* species at the study sites.

Taxon	Study site	Altitude (m)	First flowers–last flowers (2000)
P. lutescens subsp. *lutescens*	Tianshengqiao	3150	13 July–28 July
P. densispica			9 July–28 July
P. tricolor			19 July–15 August
P. integrifolia			27 June–21 July
P. integrifolia	Wufeng Mountain	3500	24 June–25 July
P. densispica	Jiantang	3300	7 July–26 July
P. tricolor		3150	15 July–9 August

Three sites were chosen for field observation, i.e., Tianshengqiao, Wufeng Mountain, and Jiantang. The first site, Tianshengqiao, featured dense populations of *Pedicularis,* with all four species in this study found in the open meadow. One population of *P. integrifolia* was relatively isolated on the north-facing slope of the second site in the Wufeng Mountains. *Pedicularis densispica* and *P. tricolor* were found in the third site at Jiantang.

Techniques in this study follow those of Macior (1968, 1977, 1988; Macior & Sood, 1990). Observations of bumblebee behaviors in the different populations of *Pedicularis* species were made at three sites of approximately 15 × 15 m, respectively. Records were made directly by close-range stereo-photography. Pollen analysis followed Erdtman (1969) and Wang et al. (2003) in designating pure, mixed, and foreign pollen.

All four *Pedicularis* species in the study sites were tested by bagging experiments to test dependence of these species upon insect pollinators. Ninety-eight pre-anthesal inflorescences of unopened buds for the four *Pedicularis* species were covered with galvanized window screen enclosures; another 68 flower inflorescences were not bagged as a control for open pollination. Fifty fresh flowers of each species were randomly selected to measure corolla tube length for each population of all four species at the three study sites. These flowers were examined by using light microscopes (60×) to check for the possibility of any existent nectar. The pollen/ovule ratio (p/o) was calculated after counting under a stereoscope as detailed by Cruden (1977) (see Table 6). Seed set was estimated in the late fall by opening fruit set of the 98 bagged and 68 control inflorescences (Table 5). A record of the blooming phenology of each species of *Pedicularis* in the three populations was maintained (Table 2). Voucher specimens of plants and insect pollinators from study sites were deposited at the herbarium of the Kunming Institute of Botany (KUN) (see Appendix 1).

Results

Plant community constituents and floral morphology, phenology

The four *Pedicularis* species from the study sites are confined to the Himalayas (see Table 1). At the Tianshengqiao site, all four *Pedicularis* species were in bloom June–August 2000 in a rich, diverse, east-facing alpine meadow community (Table 2). *Pedicularis lutescens* subsp. *lutescens* was abundant there, while the other three *Pedicularis* species grew sparsely in open wet places. They were associated with *Drosera peltata* Smith subsp. *lunata* (Buch.-Ham. ex DC.) C. B. Clarke (Droseraceae), *Primula secundiflora* Franch. (Primulaceae), *Cynoglossum glochidiatum* Wall. ex Benth. (Boraginaceae), and *Ligularia lapathifolia* (Franch.) Hand.-Mazz. (Compositae). The meadow was close to a small hill dominated by *Quercus aquifolioides* Rehd. & Wils. (Fagaceae), *Populus szechuanica*

Table 3. Corolla tube length of the four studied *Pedicularis* species in northwestern Yunnan (based on 50 fresh flower measurements per population).

Pedicularis species	N	Mean length (mm)	Range (mm)
P. densispica	50	4.4	3.5–5.5
P. lutescens subsp. *lutescens*	50	8.0	7–10
P. integrifolia	50	32.7	31.0–35.0
P. tricolor	50	58.0	56.5–58.5

Table 4. Number of bumblebees observed on different species.

Taxon	Sites	*Bombus friseanus*	*Bombus lucorum*	*Bombus festivus*	Subtotal
Scrophulariaceae					
Pedicularis lutescens					
subsp. *lutescens*	Tianshengqiao	34 (81.0%)	8 (19.0%)		42
P. densispica		17 (94.4%)	1 (5.6%)		18
P. integrifolia		3 (100%)			3
P. tricolor		6 (100%)			6
Boraginaceae					
Cynoglossum glochidiatum		29			29 (29.6%)
P. integrifolia	Wufeng Mountain	5 (10.9%)		41 (89.1%)	46
P. densispica	Jiantang	287 (96%)	12 (4%)		299
P. tricolor		44 (96%)	2 (4%)		46
Fabaceae					
Medicago edgeworthii		218 (96%)	9 (4%)		227
Subtotal of all populations of four *Pedicularis* species		396 (86.1%)	23 (5%)	41 (8.9%)	460
Total		643	32	41	716

Schneid. (Salicaceae), and *Rhododendron axillaries* Franch. (Ericaceae).

Pedicularis integrifolia was the only *Pedicularis* species present at the Wufeng Mountain site. The dominant species associated with *P. integrifolia* here were *Caragana franchetiana* Kom. (Papilionaceae), *Pinus densata* Mast. (Pinaceae), and *Gymnadenia souliei* Schltr. (Orchidaceae).

At the Jiantang site, *Pedicularis densispica* and *P. tricolor* grew together in a dry sandy location with some *P. tricolor* along a dry sandy path. That site was close to farmland with cultivated *Brassica campestris* L. var. *oleifera* DC. (Brassicaceae), *Avena sativa* L. (Poaceae), and *Raphanus sativus* L. (Brassicaceae). Nearby, *Medicago edgeworthii* Sirj. (Fabaceae) and *Anaphalis contorta* (D. Don) Hook. f. (Compositae) were also in bloom in the summer of 2000.

In the three study sites, the four species of *Pedicularis* were in bloom from late June through early August 2000 (Table 2). *Pedicularis integrifolia* was the earliest-flowering of the four species in June. *Pedicularis tricolor* also opened 4 days earlier at Jiantang than at Tianshengqiao. In the Wufeng Mountains, flowering of the isolated population of *P. integrifolia* was also three days earlier than at Tianshengqiao in June.

The flower color is pale yellow in *Pedicularis tricolor* and in *P. lutescens* subsp. *lutescens,* while corollas are pale pink in *P. densispica* and purple in *P. integrifolia. Pedicularis lutescens* subsp. *lutescens* and *P. densispica* fall into the short-tubed, erostrate corolla type while *P. integrifolia* and *P. tricolor* are of the long-tubed, rostrate type, the upper corolla lip being extended into an S-shaped beak in *P. integrifolia* and a circular crest in *P. tricolor.* The

Table 5. Pollination-related fertility of the four *Pedicularis* species in study sites.

		Bagged			Open-pollinated		
Taxon	Site	Inflorescence	Flower	Fruits	Inflorescence	Flower	Fruits
P. lutescens subsp. *lutescens*	Tianshengqiao	25	201	6*	6	53	39
P. densispica		18	138	0	15	165	86
P. integrifolia		12	63	0	13	75	57
P. tricolor		9	80	0	2	13	8
P. integrifolia	Wufeng Mountain	7	39	0	7	43	32
P. densispica	Jiantung	9	113	5*	8	79	64
P. tricolor		18	252	3*	17	139	70
Total		98	886	14*	68	567	356

* Following destruction of floral enclosures by yaks.

Table 6. Ovule and pollen numbers and seed set.*

Pedicularis species	Capsule no.	Pollen grain/ flower (P)	Ovule number/ flower (O)	Pollen-ovule ratio	Seed set open pollination	Seed set bagged
P. lutescens subsp. *lutescens*	8	86,000 ± 38	116.7 ± 174.9	1484.9 ± 325.6	59.75 ± 13.15	0
P. densispica	15	182,850 ± 52	32 ± 2	5755.9 ± 906.8	9.6 ± 3.8	0
P. integrifolia	9	501,400 ± 138	64.5 ± 2.6	7765.3 ± 338.0	40.6 ± 41.4	0
P. tricolor	15	54,800 ± 55	31.75 ± 1.71	1908.1 ± 323.6	7.8 ± 4.4	0

* Values are means ± s.d.

lobes of the lower lip in *P. tricolor* are expanded into a broad bowl-shaped form subtending the beak (Fig. 1). The length of the corolla tube ranges from 32.7 mm in *P. integrifolia* to 58 mm long in *P. tricolor* to only 4.4 mm in *P. densispica* to 8 mm in *P. lutescens* subsp. *lutescens* (Table 3).

POLLINATOR BEHAVIOR AND FREQUENCY

The collection of 205 foraging bumblebees on the four species of *Pedicularis, Cynoglossum glochidiatum* Wall. ex Benth. (Boraginaceae), and *Medicago edgeworthii* (Fabaceae) was carried out with 86 man-hours in the three study sites. The insect specimens were used for taxonomic identification, frequency assessment, tongue length measurement, and analysis of corbicular pollen. A total of 205 corbicular pollen loads from foragers on the four species of *Pedicularis* was examined microscopically. Three bumblebee species, *Bombus friseanus, B. lucorum,* and *B. festivus,* were observed to forage on and pollinate the four observed *Pedicularis* species as well as other adjacent plants (Table 4).

All pollinators recorded on the four *Pedicularis* species were workers of three *Bombus* species, principally *B. friseanus* (86.1%; cf. Table 4), while queens were not observed. The short-tubed, erostrate flowers of *P. lutescens* subsp. *lutescens* and *P. densispica* were regularly visited by *Bombus friseanus* and *B. lucorum* workers for nectar and pollen in two sites. These workers scraped pollen from four anthers concealed within the galea (corolla upper lips) in an erect position. Pollen was dropped and deposited on the dorsal side of the bee's head and thorax. In *P. densispica, B. friseanus* workers approached the galea with antennae extended toward it (Fig. 2A) and grasped the lower lip with the forelegs (Fig. 2B). While foraging for nectar, the workers scraped and deposited pollen on the dorsal side of the thorax (Fig. 2C). A similar situation was observed in *P. lutescens* subsp. *lutescens*: the stigma protruding from the galea tip contacted pollen deposited on the bee's dorsal thorax (Fig. 2D, E). Both long-tubed and rostrate species (*P. integrifolia* and *P. tricolor*) were pollinated sternotribically by bumblebee workers; the bees grasped the flower while hanging in an inverted position with the ventral side of the thorax aligned with the opening in the galea through which pollen was released (cf. Macior, 1991). On Wufeng Mountain, workers of *B. friseanus* were also found foraging pollen on *P. integrifolia,* as at the Tianshengqiao site, but *B. festivus* workers were the principal pollinators at Wufeng Mountain (constituting 41 of 46 *Bombus* recorded, cf. Table 4). The workers of *B. festivus* grasped the long, S-shaped beak of *P. integrifolia* with all six legs (Fig. 2F), scraped pollen onto their ventral thorax and abdomen by hanging from the beak and vibrating the flower (Fig. 2G) with a buzzing sound while the stigma contacted residual pollen that was not transferred to the insects' corbicula. *Pedicularis tricolor* was mostly pollinated by *B. friseanus* workers (44 of 46 observations, or 96%). Pollinators grasped the lower part of the corolla beak first with forelegs, gradually with all six legs, hanging inverted from the corolla while being supported by the extended lower lip and scraping pollen, while the ventral thorax and abdomen directly contacted the stigma protruding from the tip of the galea (Fig. 2H).

Bumblebees began to visit and forage on *Pedicularis* species and adjacent plants at about 0800 hours, through 1700–1800 (Fig. 3). Peak visitation was between 1200 and 1500. *Bombus friseanus* appeared at about 0800 with an activity peak around 1400, while peak vistation activity for *B. festivus* was at 1600. *Bombus lucorum* appeared later at about 0900, with a peak at 1500 (cf. Fig. 3).

At each site the frequency of bumblebee species on the four studied *Pedicularis* species differed. Across all *Pedicularis* populations, *Bombus friseanus* formed 86.1% of the total followed by *B. festivus* (8.9%) and *B. lucorum* (5%). At Tianshengqiao, *Bombus friseanus* was the most abundant species on *P. lutescens* subsp. *lutescens* (34 of 42, or 81%) and *P. densispica* (17 of 18, or 94.4%) foraging for

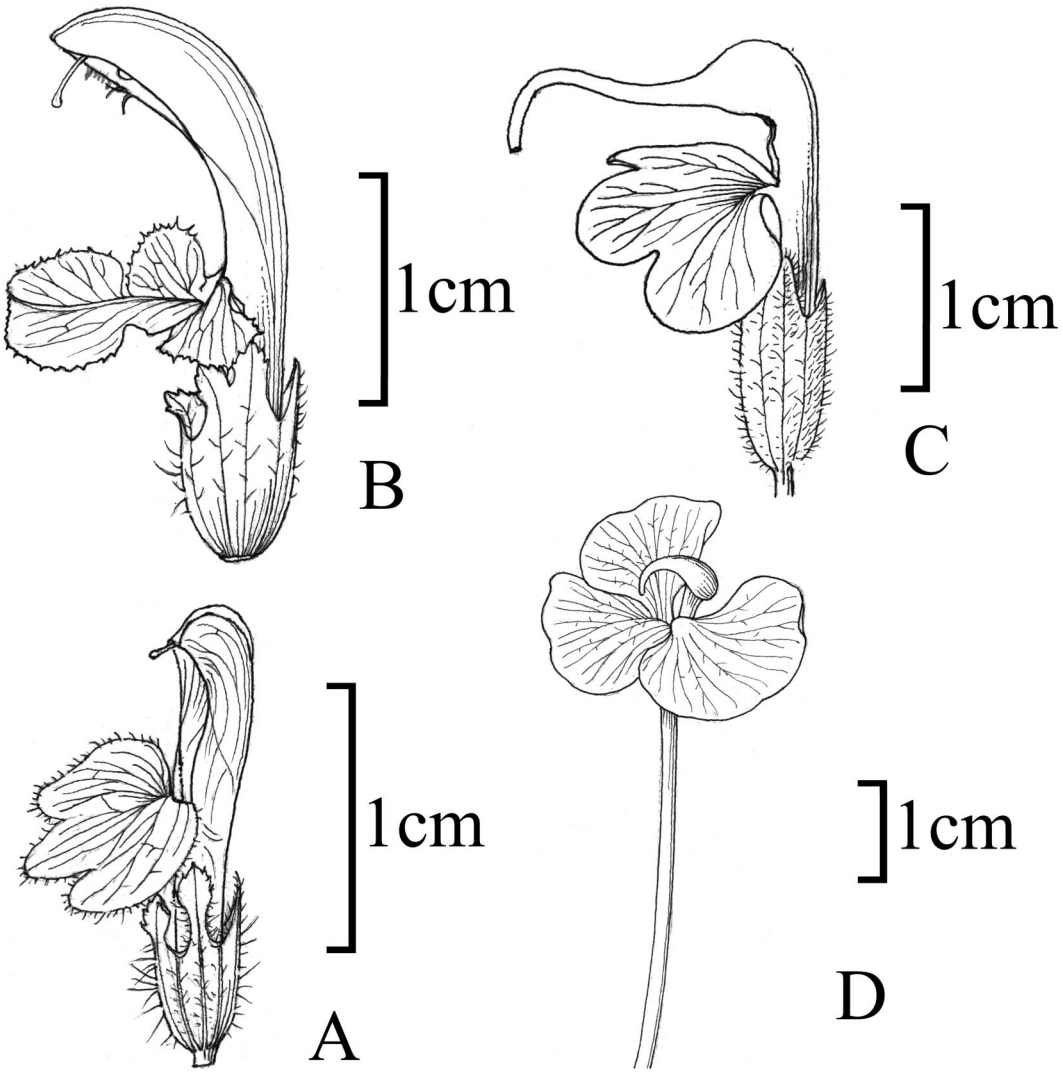

Figure 1. Corolla types of the four studied *Pedicularis* species. —A. *P. densispica,* corolla short-tubed, erostrate, and toothless. —B. *P. lutescens* subsp. *lutescens,* corolla short-tubed, erostrate, and toothed (with 4–10 teeth near the apex of each side of the galea). —C. *P. integrifolia,* corolla long-tubed, S-shaped beak. —D. *P. tricolor,* corolla long-tubed, upper corolla with a circular crest.

nectar and pollen (Table 4). Foragers alternately foraged on other flowers growing in the same site, notably *Cynoglossum glochidiatum.* A similar situation happened at Jiantang where the pollinators of *B. friseanus* (287 of 299, or 96%) appeared more frequently than *B. lucorum* (12 of 299, or 4%) on *P. densispica* and *Medicago edgeworthii* (also 96%) for nectar and pollen. For the two nectariferous *Pedicularis* species, *P. densispica* and *P. lutescens* subsp. *lutescens,* our field observations indicated that one flower of an inflorescence could be visited 6 to 10 times by different individuals of the same *Bombus* species; on nectarless species, a single flower was visited only 3 to 5 times by different individuals of the same *Bombus* species.

Corbicular pollen load analysis indicated 51.5% of *Bombus friseanus* carried *Pedicularis* pollen mixed with pollen from other species. *Pedicularis integrifolia* pollen was found in all loads of *Bombus festivus,* and the proportion of *P. integrifolia* pollen averaged 69.6%. *Bombus lucorum* carried 28% *Pedicularis* pollen (pers. obs., unpublished). The tongue lengths of the three observed *Bombus* species range from short (6.5–8.0 mm) in *B. lucorum* to long (9.0–11.0 mm) in *B. festivus.* Between them fell *B. friseanus* (7.0–8.8 mm) (Wang & Li, 1998).

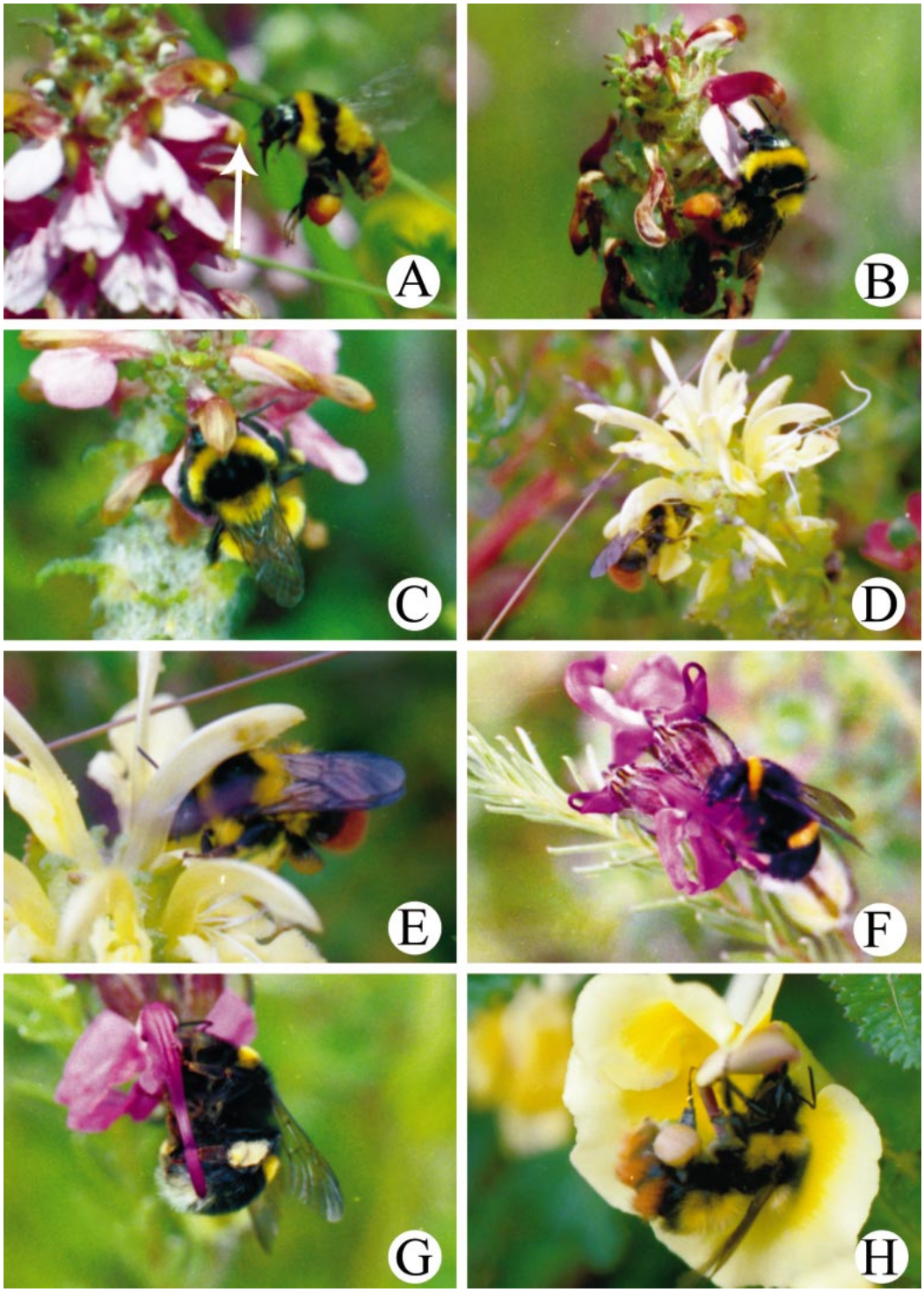

Figure 2. *Bombus* foraging on *Pedicularis* flowers. A–C. *Bombus friseanus* worker pollinating *P. densispica*.—A. Bumblebee approaching flowers with antennae extended. —B. Grasping the lower corolla lip with the forelegs. —C. Nototribically contacting stigma. D, E. *Bombus friseanus* contacting pollen deposited on the dorsal side of the thorax on *P. lutescens* subsp. *lutescens*. F, G. *Bombus festivus* worker on *P. integrifolia*.—F. Grasping the S-shaped corolla beak with all six legs scraping pollen. —G. Hanging on the corolla beak and vibrating the flower. —H. *Bombus friseanus* worker supported by extended lower lip as a platform and scraping pollen on *P. tricolor*.

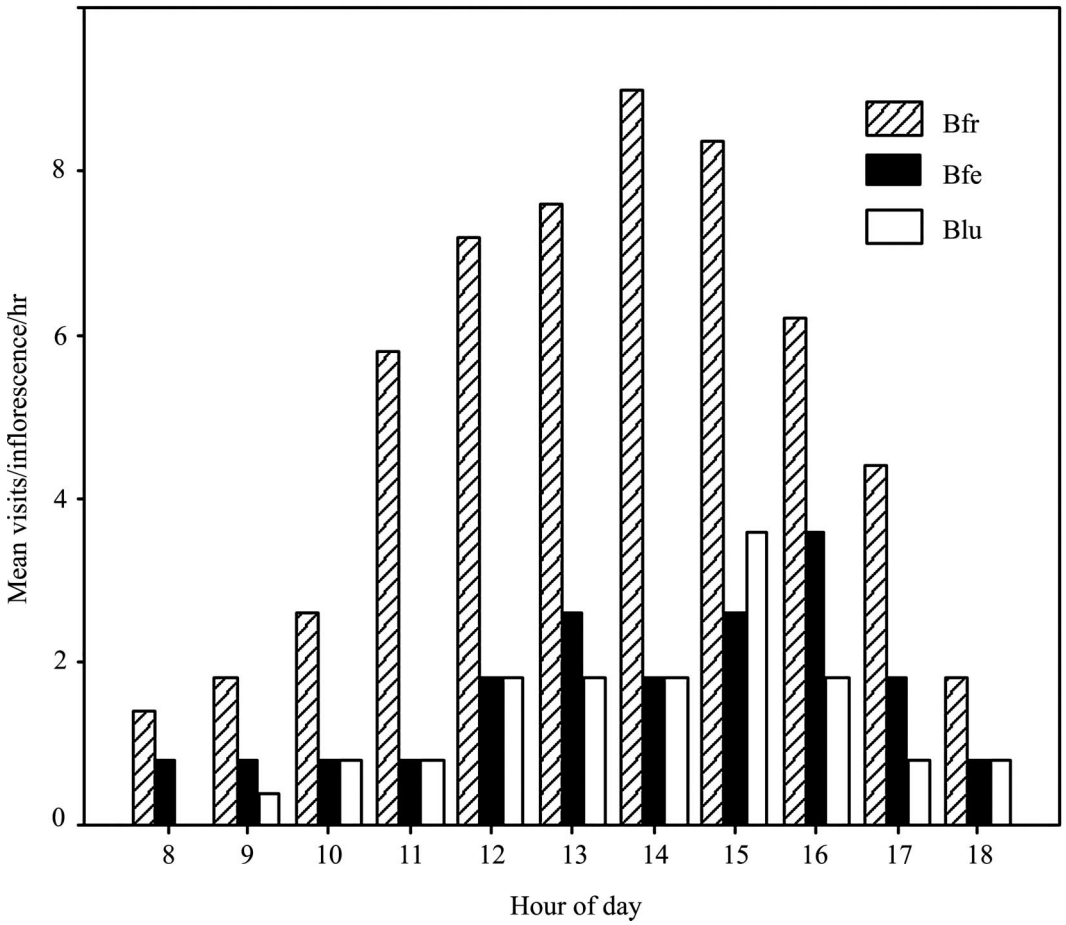

Figure 3. The visitation pattern of pollinators. Bfr = *Bombus friseanus*; Bfe = *B. festivus*; and Blu = *B. lucorum.*

It was noticed that three *Bombus* species with different tongue lengths might visit both *P. tricolor* (with a 58 mm long tube) and *P. densispica* (with a 4.4 mm long tube), as well as other flowering plants in the same population. Tongue length of *Bombus* pollinators and length of the corolla may not be correlated, as had been previously reported (Wang & Li, 1998).

floral biology and mating system

Our study demonstrated that the four investigated *Pedicularis* species were incapable of unassisted self-fertility and were dependent on *Bombus* pollination for the production of seed. The only bagged inflorescences that produced fruits resulted from yaks destroying the plant enclosures (Table 5).

Pollen-ovule ratios may be used as a predictor of a plant's breeding system rather than floral size and morphology (Cruden & Miller-Ward, 1981). The highest p/o ratio was found in *P. integrifolia* with purplish pink flowers, while the lowest was *P. lutescens* subsp. *lutescens* bearing yellow corollas (Table 6). Similarly, the other yellow-flowered species, *P. tricolor,* also had a lower p/o ratio. Fruits were not developed in the bagged group, which suggested that the production of fruits in all four *Pedicularis* species was dependent upon pollen vectors in natural populations. In the open pollination group, the seed set of *P. lutescens* subsp. *lutescens* was the highest of the four species.

Discussion

pollination mechanisms of the himalayan *pedicularis* species, especially the long-tubed ones

Pennell (1943) noted the exceeding diversity of corolla forms in *Pedicularis.* By attributing the differences in corolla form to the processes of pollination, he pointed out that this was a genus in

which field observations and the collecting of pollinators would be especially worthwhile. Earlier pollination studies on *Pedicularis* by Macior (1982, 1984, 1986) in North America, Japan (Macior, 1988), and the Kashmir Himalayas (Macior & Sood, 1990) revealed a general pattern of pollination in the genus, primarily associated with bumblebees. Pollination of the fourth floral form, i.e., the corolla with a beak and slender elongated tube, which occurs only in the Himalayas, was first reported by Macior & Sood (1990). He found that the magenta, nectarless, rostrate, long-tubed (to 2 cm) flower of *Pedicularis punctata* Decne. in the Kashmir Himalayas was pollinated by pollen-vibrating bumblebee workers. Our preliminary pollination observations on *Pedicularis* in northwestern Yunnan (Wang & Li, 1998) in the late summer of 1993 revealed that the yellow, nectarless, rostrate, exceptionally long-tubed (to 6 cm) flower of *Pedicularis longiflora* var. *tubiformis* was also pollinated by pollen-vibrating bumblebee workers. The present study demonstrates that the yellow, nectarless, rostrate, long-tubed (5.8 cm) flowers of *P. tricolor* are pollinated exclusively by *Bombus friseanus* and *B. lucorum* workers. As in *P. punctata, P. megalantha* (Macior & Sood, 1990), *P. longiflora* var. *tubiformis,* and *P. siphonantha* var. *delavayi* (Wang & Li, 1998), the long, nectarless corolla tube of *P. tricolor* appears to function in extending the rostrate vibration pollination mechanism beyond the plant's foliage and is not an adaptation for nectar-foraging lepidopteran pollinators, as suggested by Pennell (1943, 1948).

The pollen-vibration mechanism of the *P. tricolor* flower generally resembles the rostrate, nectarless, long-tubed floral mechanism as reported by Macior and Sood (1990) and Wang and Li (1998), but also differs markedly from them. In *P. punctata, P. siphonantha* var. *delavayi,* and *P. longiflora* var. *tubiformis,* the lower corolla lip is composed of three broad reflexed lobes and the corolla has a color contrast between the distal and proximal petal areas (purple vs. white in *P. punctata* and *P. siphonantha* var. *delavayi,* yellow vs. purple in *P. longiflora* var. *tubiformis*). In *P. tricolor,* the lower lip is composed of three much broader ascending lobes in a bowl-shaped form, which provides the *Bombus* pollinators a unique platform for scraping pollen. In *P. integrifolia,* the S-shaped beak is extremely long and robust, and the insects hang and scrape pollen without any support from the lower lip. Again, both *P. tricolor* and *P. integrifolia* are nectarless and do not appear to be pollinated by lepidopteran insects.

COMPARISON WITH THE POLLINATION SYNDROMES OF THE NORTH AMERICAN SPECIES

As pointed out by Macior (1988), a major determinant of pollination behavior is the form of flowers in *Pedicularis.* The erostrate species are pollinated by pollen-foraging bumblebee queens that vibrate or scrape pollen concealed within the galea (see Table 7). One exception is *Pedicularis furbishiae* S. Watson in Maine, U.S.A., and New Brunswick, Canada (Macior, 1978), which was previously thought to be the only nectariferous, erostrate, and short-tubed *Pedicularis* species that blooms in middle to late summer and is pollinated exclusively by bumblebee workers. Both *P. lutescens* subsp. *lutescens* and *P. densispica* are short-tubed, erostrate, and nectariferous species and were exclusively visited by *Bombus friseanus* and *B. lucorum* workers foraging for nectar and pollinating nototribically: they approached the tip of the galea and grasped the flowers; their heads were forced into the throat of the corolla and scraped pollen from four anthers concealed within the galea in the upright position. This is not in accordance with other erostrate and nectariferous species of *Pedicularis* vernally blooming in North America, but reveals a remarkable parallel to *P. furbishiae.*

Generally speaking, the floral form, floral color, and pollination behaviors on flowers of Chinese species of *Pedicularis* are comparable to those of North America and Japan. According to Macior (1978, 1982), *Pedicularis* species flowering in mid to late summer are nectarless, with the exception of the nectar-bearing *P. furbishiae.* Our study displayed a similar general pattern with the unique exceptions of *P. lutescens* subsp. *lutescens* and *P. densispica.*

Similar to the bagged experiments in *P. pulchella* Pennell of southwestern Montana and northwestern Wyoming, U.S.A. (Macior, 1986), as well as *P. palustris* L. and *P. sylvatica* L. in the northern Netherlands (Kwak, 1979), our bagged experiments and preliminary pollination controls showed that the production of seed in the four studied species of *Pedicularis* in northwestern Yunnan was dependent upon insect pollination, given the remarkably high difference in seed set between caged and open-pollinated flowers. Pollen/ovule ratio indicate that they are all incapable of unassisted self-fertility. Therefore, cross-pollination by bumblebee workers represents an important step in the reproductive biology of *Pedicularis.* Further work is needed with artificial cross- and self-pollination trials to examine their self-incompatibility.

As the bumblebee workers have a preference for

Table 7. Comparison of floral syndromes in *Pedicularis* in Yunnan, Himalayas, Japan, and North America.

Floral syndrome category in *Pedicularis*	Yunnan and Himalayan species	Japanese species	North American species*
1. Erostrate, nectariferous & nototribic	*P. lutescens* subsp. *lutescens* *P. densispica* aestival, by workers)	*P. apodochila* Maxim. **P. oederi* Vahl **P. verticillata* L.	*P. canadensis* L. *P. verticillata* (vernal, by *Bombus* queens) *P. capitata* Adams (early aestival, by queens and workers) *P. furbishiae* (aestival, by workers)
2. Erostrate, nectarless & nototribic or polleniferous/sternotribic	**P. rex* C. B. Clarke ex Maxim. **P. roylei* Maxim. **P. tsekousensis* Bonati	Lack of data	*P. bracteosa* Benth. ex Hook.
3. Erostrate, nectarless & sternotribic, by pollen-scraping	Lack of data	*P. chamissonis* Steven *P. resupinata* L. *P. yezoensis* Maxim.	*P. lanceolata* Michx.
4. Erostrate, nectarless & sternotribic, by vibration	Lack of data	Lack of data	*P. racemosa* Dougl. ex Hook.
5. Nectarless, short-tubed & nototribic, by vibration	Lack of data	Lack of data	*P. groenlandica* Retz.
6. Rostrate, nectarless, long-tubed & sternotribic, by vibration	**P. megalantha* **P. punctata* **P. siphonantha* var. *delavayi* **P. longiflora* var. *tubiformis* *P. integrifolia* *P. tricolor*	No such species available	No such species available

* Only examples are listed in this table (sources: Macior, 1988, 1991; Macior & Sood, 1990; Wang & Li, 1998; Wang, 1998).

foraging on the nectariferous flowers, as Macior (1978) suggested, nectar is a greater attractant than pollen. In California (Macior, 1977) and in the Himachal Himalayas, this competitive disadvantage for *Pedicularis* species was observed. In northwestern Yunnan, *Pedicularis* species were flowering with other sympatric, synchronous blooming, bumblebee-pollinated species, such as *Cynoglossum glochidiatum* and *Medicago edgeworthii*. However, the ecological or competitive consequences of such a situation need further investigation. The discovery of bumblebee workers as the exclusive pollinators of *P. tricolor* is in agreement with Macior (1991), who assumed that the mid-season flowering species represent a time when pollen foraging was emphasized by bumblebee brood-rearing with queens remaining in the nest.

DIVERSIFICATION OF POLLINATION MECHANISMS IN *PEDICULARIS* SPECIES

Maximowicz (1888) proposed the recognition of *Pedicularis* on the basis of four basic corolla types: (1) beakless, toothed, and short-tubed; (2) beakless, toothless, and short-tubed; (3) beaked, toothless, and short-tubed; and (4) beaked, toothless, and long-tubed. Li (1948, 1949, 1951) was the first to emphasize the importance of pollinator selection as a force in determining the diversification of floral forms in the genus. He emphasized that parallel evolution had occurred in corolla morphology by clearly defining the series according to the four corolla types proposed by Maximowicz. He assumed that in *Pedicularis* toothed corollas were derived from toothless, beaked from non-beaked, and long-tubed from short-tubed, under the pressure of pollination and adaptation to pollinator insects. Tsoong (1955, 1956) proposed another important classification for the genus, which was similar to Li (1948, 1949, 1951) in that all the species in each primary group had the same phyllotaxy. However, Tsoong did not place as much importance on phyllotaxy as Li did.

Based on pollination function and phenology of bloom, the floral syndromes of *Pedicularis* were

classified into six categories (Macior, 1991) (Table 7). No data were available for two major types, i.e., the erostate, nectarless, and sternotribic type and the nectarless, short-tubed type, of *Pedicularis* in Yunnan. Study of these pollination syndromes is needed for an understanding of the evolution of floral forms in *Pedicularis* in relation to their adaptation to pollination pressure, especially in this center of diversification for *Pedicularis* in the Himalayas (Hong, 1983).

As for the four recognized corolla types in the genus, the fourth type, the nectarless, rostrate, and long-tubed one is restricted to Yunnan, Sichuan, and the Himalayas. The presently available information for studied species in the Chinese Himalayas (Macior & Tang, 1997, 1999; Wang & Li, 1998; Macior et al., 2001) reveals a similar pattern of pollination syndrome description as in North American species. Further study in the early spring, and on the rostrate and short-tubed, nectarless species in Yunnan will fill in our gaps of knowledge of pollination syndromes in the entire genus. By studying the pollination ecology of Japanese *Pedicularis,* Macior (1988) proposed that the Japanese species evolved in a pattern parallel to that pursued by North American species from Himalayan migrants. In general, our present and previous studies provide evidence in support of this hypothesis. Further evidence is needed to understand the evolution of this important and interesting genus, e.g., pollen morphology and its relationships with pollination syndrome (Wang et al., 2003), chromosome numbers and karyotype morphology (Cai et al., 2004), and, in particular, molecular phylogeny (Ree, 2001).

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Appendix 1. A list of voucher specimens for pollination studies. All voucher specimens were collected in Zhongdian county, northwestern Yunnan, China, and were deposited in KUN.

Scrophulariaceae

Pedicularis densispica Franch. ex Maxim.: *Hong Wang 0021,* Tianshengqiao; *Hong Wang 0065,* Jiantang.

P. integrifolia Hook. f.: *Hong Wang 0022,* Tianshengqiao; *Hong Wang 0034,* Wufeng Mountains.

Pedicularis lutescens Franch. ex Maxim. subsp. *lutescens*: *Hong Wang 0023,* Tianshengqiao.

P. tricolor Hand.-Mazz.: *Hong Wang 0024,* Tianshengqiao; *Hong Wang 0066,* Jiantang.

Boraginaceae

Cynoglossum glochidiatum Wall. ex Benth.: *Hong Wang 0028,* Tianshengqiao.

Fabaceae

Medicago edgeworthii Sirj.: *Hong Wang 0071,* Jiantang.

Apidae (Insecta)

Bombus festivus Smith: *Hong Wang 115,* Wufeng Mountains.

Bombus friseanus Skorikov: *Hong Wang 63,* Tianshengqiao; *Hong Wang 127,* Wufeng Mountains; *Hong Wang 150,* Jiantang.

Bombus lucorum Latr.: *Hong Wang 68,* Tianshengqiao; *Hong Wang 147,* Jiantang.

Volume 92, Number 1, pp. 1–138 of the Annals of the Missouri Botanical Garden
was published on May 27, 2005.