

Research Article

Plant sexual systems correlated with morphological traits: Reflecting reproductive strategies of alpine plants

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Abstract Plant sexual systems can be affected by various environmental factors, and thus show a range of morphological differences. The associations between plant sexual systems and morphology are unique, for example, the subnival belt of the Hengduan Mountains has harsh climatic conditions, but hosts remarkable biodiversity. However, these associations have received surprisingly little attention. We analyzed the associations in this region, and compared the pattern of sexual systems with that of other floras, and we also reviewed the breeding system of 36 alpine species. Information on plant sexual systems and these traits were mainly based on published information, herbarium collections, and field observations. The results showed that there was a high proportion of hermaphroditic species and a low proportion of dioecious and monoecious species. Hermaphroditism was strongly associated with showy floral displays, specialist entomophily, and perennial forbs. Dioecy was strongly associated with inconspicuous, pale-colored flowers, anemophily, and shrubs, whereas monoecy was strongly associated with inconspicuous, pale-colored flowers, generalist entomophily, and perennial grasses. Although no association was found between sexual systems and fruit type, plants with dry fruits (98.1%) were predominant in our study region. With respect to the plant breeding systems in the alpine region of southwest China, we predicted that pollination service might be particularly low; most of the plants lacked enough outcrossing pollinators. Our data revealed that 97.1% of the hermaphroditic species were self-compatible, and 88.2% of them showed autonomous or facilitated selfing. The plant sexual systems identified might serve to optimize the probability of pollination and thus ensure reproductive success.

Key words alpine subnival belt, floral display, fruit types, Hengduan Mountains, pollination syndrome, sexual systems.

Plant sexuality encompasses the wide variety of sexual reproduction systems found across the entire plant kingdom. Sexual systems have an unrivalled diversity and functional significance in flowering plants (Barrett, 2002). Selective forces promoting the evolution of sexual systems have been studied intensively in recent years (reviewed in Bawa, 1980, 1982; Renner & Ricklefs, 1995; Matallana et al., 2005; Chen & Li, 2008a; Vamosi & Queenborough, 2010). Hermaphroditism is the predominant condition in flowering plants (Renner & Ricklefs, 1995), and it provides potential opportunities for selfing, especially in areas

where pollinators may be either absent or in short supply (Zhang & Li, 2008; Duan et al., 2010). However, selfing reduces the opportunity for outcrossing, resulting in pollen discounting and inbreeding depression, thus reducing the fitness of inbred offspring (Barrett, 1998). In contrast, dioecious species avoid inbreeding depression (reviewed in Sakai et al., 1995), but suffer reproductive failure when pollinators are not available.

The evolution of plant sexual systems has received more attention in tropical communities (reviewed in Matallana et al., 2005; Machado et al., 2006; Chen & Li, 2008b). Tropical communities are known to contain a relatively high percentage of dioecious species (Sakai et al., 1995; Matallana et al., 2005; Chen & Li, 2008b). A number of attributes have also been correlated with dioecy, and many authors have summarized the attributes that relate to dioecy, including woodiness, anemophily, small and pale-colored flowers, and fleshy

Received: 2 April 2013 Accepted: 28 June 2013

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fruits (reviewed in Renner & Ricklefs, 1995; Sakai et al., 1995; Matallana et al., 2005; Vamosi & Queenborough, 2010). However, plant sexual systems have received comparatively little attention in other regions, particularly in alpine regions; many of them are biodiversity hotspots and have great species diversity (Körner, 2003).

Unlike tropical (or lowland) regions, climatic conditions in alpine regions are harsh, with lower mean temperatures and atmospheric pressure, longer snow cover, usually stronger winds, higher solar radiation, and a shorter growing season (Körner, 2003); furthermore, it has often been reported that insect pollinator diversity, abundance, and activity decrease dramatically with increasing elevation (Arroyo et al., 1982; Bingham & Orthner, 1998; Medan et al., 2002), all of which adversely affect plant growth and reproduction. For these factors, alpine plant communities always consist of herbs and shrubs.

For alpine plants, sexual systems may have evolved in two distinct routes, thereby potentially mitigating the negative effects of the reduced abundance of insect pollinators. First, as most diclinous species are obligate outcrossers and many rely on insect visitors, the occurrence of anthophilous dioecious species should decrease in the alpine region. In this case, hermaphrodites may be favored in alpine vegetation, because hermaphroditic species can guarantee reproductive success by autonomous or facilitated selfing (Sun et al., 2005; Duan et al., 2007a, 2010; Zhang & Li, 2008).

Second, it has been suggested that wind pollinated species are proportionally more abundant in alpine habitats, because wind pollination is more likely to offer reproductive success for plants at high elevations (reviewed in Totland & Sottocornola, 2001). Harsh environments tend to select for longevity, and dioecy is linked with anemophily and long-lived (woody) life forms (reviewed in Vamosi & Queenborough, 2010). If this is the case, selection could favor long-lived, wind pollinated dioecious species, and the relative level of dioecy may increase in alpine regions.

The subnival belt is located at altitudes between alpine meadows and the alpine nival zone, which is the highest land ecosystem. Vegetation in the subnival belt is found at low densities, and scattered groups of plants are predominant. There are very highly specialized life forms (e.g., greenhouse, cushion, and woolly plants; Yang, 2008) and abundant endemic plants in the subnival belt of the Hengduan Mountains (Xu et al., 2013a). Because of the large number of highly specialized and endemic plants confined to the stressful subnival conditions, the functional ecology of these

plants is of particular interest to ecologists (Tsukaya et al., 2002; Yang et al., 2008, 2010; Yang & Sun, 2009). The patterns of floral traits in this region were reported in Peng et al. (2012), however, the relationship between plant sexual systems and flower morphological traits was not examined in that study.

To address these issues, we reinvestigated the distribution of sexual systems and flower morphology based on the latest and more comprehensive plant checklist (Xu et al., 2013b). In addition to the categories used by Peng et al. (2012), we added pollination syndrome and fruit type as additional features, and the categories of life form and floral color were modified for this study. The main aims of the study were to: (i) determine the frequency distribution of hermaphroditic, monoecious, and dioecious sexual systems; (ii) compare the frequency distribution of sexual systems with that in other floras; and (iii) analyze the associations between sexual systems and plant morphological and ecological traits.

1 Material and methods

1.1 Study site

The Hengduan Mountains, which lie in SW China (area ca. 364 000 km², 24°84'–34°80' N, 96°82'–104°83' E; Li, 1987), have large areas of alpine region. This survey was carried out at the subnival belt (alpine scree) of the Hengduan Mountains. The subnival belt is the transition zone between alpine meadow and the nival belt, which is the highest ecosystem in the world; its average altitude is usually above 4300 m. Borders between snowfields, moraines, dry scree slopes, rocky outcrops, and rock fissures are common in this region (Wu, 1987). Usually, snow cover persists from late October to mid-May, and active glaciers are common in the upper mountain belts. Consequently, the flowering season lasts from mid–late May to early–mid-September. Based on records from 1982 to 1984 (Zhang, 1998), annual precipitation at the nearest meteorological station (28°23' N, 99°01' E, 4290 m) was 680–790 mm in the Baima Mountains, NW Yunnan. The annual average air temperature was –1.0 °C, and the difference in mean monthly temperature between the coldest and the warmest months was approximately 15 °C; the mean wind speed was 2.9–3.1 m/s (Wang, 2006). The zone supports a number of prostrate shrubs and perennial herbs, including *Rhododendron*, *Juniperus*, *Berberis*, *Salix*, *Chesneya*, *Meconopsis*, *Gentiana*, *Saxifraga*, *Cremanthodium*, *Primula*, *Saussurea*, and *Delphinium*. Of the species in this region, 295 (31.32%) are endemic to the subnival

belt and 151 (16.03%) strictly endemic to the subnival belt of the Hengduan Mountains (Xu et al., 2013a). Bumblebees are the dominant pollinators of alpine plants in this region (reviewed in Meng et al., 2012).

1.2 Data collection

The dataset used for this study was mainly based on the latest checklist of seed plants from the subnival belt of the Hengduan Mountains (Xu et al., 2013b). The original species list included 942 seed plants. We excluded 10 species of gymnosperm, so a total of 932 angiosperm species belonging to 46 families and 166 genera were involved in our survey.

We used information from the *Flora of China* at <http://www.efloras.org/>, an online database at <http://hengduan.huh.harvard.edu/fieldnotes/>, field investigations, personal communications with taxonomists, and examination of specimens (herbarium KUN). Plant morphological traits considered in this study are listed in Table 1. The classification method of sexual systems, floral clustering, size, symmetry, and shape followed Peng et al. (2012) exactly, and life form and floral color were slightly modified. Floral clustering, size, and color were considered to be floral display traits.

We calculated the level of flower specialization (F_S , hereafter) for showy petal species according to the formula: $F_S = N_s/N_i$, where N_i and N_s were the numbers of investigated and specialized floral traits, respectively. The specialized floral traits included solitary flowers, large floral size (large and medium), bright color (blue-purple and pink-red), zygomorphic, and tubular

morphologies (Gong & Huang, 2011; Peng et al., 2012). According to the value of F_S , we classified pollination syndrome as specialist entomophily or generalist entomophily. If the F_S of a species was more than 0.5, we defined the species as showing specialist entomophily, otherwise the species was classified as showing generalist entomophily. Other species were placed in the anemophily category, and they included the species of Poaceae, Cyperaceae, and Juncaceae.

In order to gain a full understanding of the correlations between plant sexual systems and morphology in the alpine region of SW China, we compiled information from the 32 available studies on the reproductive biology of 36 species belonging to 15 families and 24 genera that mainly occur in the alpine region of the Hengduan Mountains or the Qinghai–Tibet Plateau, including information on life form, pollinator limitation, self-compatibility, sexual segregation, and any forms of selfing.

1.3 Data analysis

To identify any correlation between sexual systems and life form, floral traits, pollination syndrome, and fruit type, we analyzed data pertaining to the plant traits with sexual systems using χ^2 -tests. The observed frequency was the actual frequency that was obtained from the investigations; the expected frequency was a theoretical predicted frequency obtained from automatic generation by statistical software. The null hypothesis showed that no variation exists between variables, that is, that the frequency distribution of sexual systems in the various plant trait categories was not significantly different from the distribution for the entire flora. All analyses were carried out using SPSS version 11.5 (SPSS, Chicago, IL, USA).

2 Results

2.1 Sexual systems and comparison with other floras

The frequency of hermaphrodite species was 85.2% (794 species); only 6.4% (60) of the species were dioecious, and 8.4% (78) were monoecious. Of 166 genera recorded in this survey, 143 (86.1%) were hermaphroditic, 7 (4.2%) were dioecious, and 11 (6.6%) were monoecious. Five genera showed two types of sexual systems. Of 46 families recorded in the survey, 35 (76.1%) were represented by exclusively hermaphroditic species. Unisexual species were present in 23.9% of the families: three were represented by exclusively dioecious species and one by exclusively

Table 1 Plant traits and the categories used to classify them in this study

Plant trait	Category
Life form ^a	Shrub, perennial forb, perennial grass, annual herb
Sexual systems	Hermaphrodite, dioecious (including androdioecy, gynodioecy, and trioecy), monoecious (including andromonoecy, gynomonoecy, and polygamonoecy)
Floral clustering	Single, inflorescence (a cluster of flowers arranged on a stem)
Floral color ^b	Blue-purple, white, yellow, pink-red, green-brown
Flower size ^c	Flower diameter: large, >1 cm; medium, 0.5–1 cm; small, <0.5 cm
Floral shape	Tubular, open (including open inflorescences)
Floral symmetry	Actinomorphic, zygomorphic
Pollination syndrome	Generalist entomophily, specialist entomophily, anemophily
Fruit type ^d	Dry fruit, fleshy fruit

^aA forb was a herb that is not a grass (Poaceae, Cyperaceae, and Juncaceae). Annual herbs included annual (biennial) forbs/grasses; ^bIf flowers included two or three colors, the most prominent color was coded; ^cClassification followed Chen & Li (2008a, 2008b); ^dFleshy fruit included berry, drupe, and aggregate fruits. Others were classified as dry fruits.

monoecious species. The remaining seven families showed two or three types of sexual systems. Doc. S1 provides a full list of species names and their respective families, sexual systems, floral traits, pollination syndromes, fruit types, and life forms.

The frequency distribution of the sexual systems of the subnival flora was comparable to other floras. The six alpine communities considered showed similar patterns: hermaphroditic species were present in high proportions, with low proportions of dioecious and monoecious species (Table 2). Subarctic and arctic floras had similar proportions of dioecy as alpine regions. The relative frequency of dioecy in alpine floras was lower than in tropical floras, and hermaphroditism was more frequent. Alpine floras had similar proportions of hermaphrodites as temperate floras, whereas monoecy was more frequent (Table 2).

2.2 Association of sexual systems with pollination traits

Among 932 species, 301 (32.3%) produced solitary flowers and 631 (67.7%) produced inflorescences. In total, 482 (51.7%) species produced large flowers, 208 (22.3%) medium flowers and 242 (26.0%) small flowers. With respect to color, 348 (37.3%) species produced blue-purple flowers, 163 (17.5%) white flowers, 249 (26.7%) yellow flowers, 77 (8.3%) pink-red flowers and 95 (10.2%) green-brown flowers. The analysis of the associations between floral display and sexual systems indicated that floral display traits are correlated with the species' sexual systems (Table 3).

Flowers produced by the hermaphroditic species are most commonly solitary, large (or medium) and blue-purple. Dioecious species produce relatively higher proportions of inflorescences, and small and green-brown flowers, than expected, whereas monoecious species produce relatively higher proportions of inflorescences, and large and yellow or green-brown flowers, than expected.

Of the species studied, 512 (54.9%) used generalist entomophily, 338 (36.3%) used specialist entomophily, and 82 (8.8%) used anemophily (Table 3). The most frequent pollination syndrome of hermaphroditic species was generalist entomophily; however, there were more hermaphroditic species using specialist entomophily than expected. Diclinous species using anemophily were present in higher proportions than expected, whereas generalist entomophily was the most common syndrome amongst the monoecious species, primarily because of the large numbers of monoecious Asteraceae which rely on generalist entomophily for pollination.

2.3 Fruit type and life form in the whole flora

A total of 914 (98.1%) species produced dry fruits and 18 (1.9%) produced fleshy fruits. Perennial forbs accounted for 80.3% (748) of the flora in the area. Perennial grasses represented 5.5% (51), annual herbs represented 5.9% (55), and shrubs represented 8.4% (78) of the flora. The hermaphroditic species included a higher proportion of perennial forbs and annual herbs than expected, whereas monoecious species included a

Table 2 Percentage occurrence of hermaphrodite (H), dioecious (D), and monoecious (M) systems among all plant species in the subnival belt of the Hengduan Mountains, SW China, compared to distributions in other floras

Plant communities	No. species	Sexual systems (%)		
		H	M	D
Alpine region				
Subnival belt of the Hengduan Mountains, SW China	932	85.2	8.2	6.4
	850 ^a	89.4	7.1	3.5
Alpine belt of the Teton Range, USA (Spence & Shaw, 1981)	209 ^b	74.1	20.6	5.3
Alpine community on Mount Olympus, Greece (Makrodimos et al., 2008)	61 ^a	90.2	9.8	0
Alpine belt of the Ruby Mountains, USA (Hartman & Rottnia, 1987)	213 ^b	74.2	22.5	3.3
Alpine belt of the Tushar Mountains, USA (Taye, 1995)	166 ^b	78.3	19.3	2.4
Tropical high-altitude grassland, Brazil (Freitas & Sazima, 2006)	124	82.3	3.2 ^c	8.9
Subarctic and arctic region				
Subarctic-alpine tundra of the northernmost high mountains, Sweden (Molau, 1993)	135 ^b	76.3	13.3	10.4
Alaska (subarctic), USA (Fox, 1985)	1343 ^c	—	—	3.9
Alaskan arctic slope (Fox, 1985)	367 ^c	—	—	5.8
Temperate region				
SW of W. Australia (Mccomb, 1966)	3886	90.0	2.6	7.4 ^f
S. Australia (Mccomb, 1966)	2102	88.9	5.8	6.4 ^f
British Isles (Mccomb, 1966)	1594	80.7	8.7	10.6 ^f
Tropical ^d (Machado et al., 2006)	—	73.5	14.9	11.6

—, No data; ^aSpecies with large showy petals are considered to be pollinated by animals; ^bWe examined the sexual systems based on the species list in these references; ^cIncludes gymnosperms; ^dAverage values of nine tropical floras that include species from all life forms; ^eDoes not include gynomonocious species; ^fIncludes dioecious and mixed species.

Table 3 Associations between sexual systems (hermaphroditism (H), dioecy (D), and monoecy (M)), floral display, and pollination syndrome in the subnival belt vegetation of the Hengduan Mountains, SW China

Pollination traits	No. species	Hermaphroditism		Monoecy		Dioecy	
		No. obs.	No. exp.	No. obs.	No. exp.	No. obs.	No. exp.
Floral display							
Floral clustering		$\chi^2 = 67.6, P < 0.001, n = 932$					
Inflorescence	631	496	537.6	78	52.8	57	40.6
Solitary	301	298	256.4	0	25.2	3	19.4
Floral size		$\chi^2 = 93.4, P < 0.001, n = 932$					
Large	482	410	410.6	57	40.3	15	31.0
Medium	208	205	177.2	0	17.4	3	13.4
Small	242	179	206.2	21	20.3	42	15.6
Floral color		$\chi^2 = 203.0, P < 0.001, n = 932$					
Blue-purple	348	326	296.5	17	29.1	5	22.4
Yellow	249	213	212.1	34	20.8	2	16.0
White	163	143	138.9	7	13.6	13	10.5
Pink-red	77	69	65.6	1	6.4	7	5.0
Green-brown	95	43	80.9	19	8.0	33	6.1
Pollination syndrome		$\chi^2 = 211.3, P < 0.001, n = 932$					
Generalist entomophily	512	425	436.2	59	42.8	28	33.0
Specialist entomophily	338	335	288.0	1	28.3	2	21.8
Anemophily	82	34	69.9	18	6.9	30	5.3

No. obs., number of observed frequencies; No. exp., number of expected frequencies.

higher proportion of perennial grasses, and dioecious species a higher proportion of shrubs than expected (Table 4). Although no association was found between sexual systems and fruit type, dry fruits were the most common across the whole flora (Table 4).

2.4 Plant breeding system

Reliable information was available for 36 species, of which 34 were hermaphrodites and two were polygamodioecious or gynodioecious (Table 5); 26 species (72.2%) were pollinator limited. Among the hermaphroditic species, only one was self-incompatible (2.9%), and 30 (88.2%) showed some level of autonomous or facilitated selfing. The other two species showed obligate autogamy (*Comastoma pulmonarium*) and xenogamy (*Delphinium caeruleum*). All the annual plants were hermaphrodites, and they had a large proportion of autonomous selfing (57.1%), however, the proportion only was 20.7% in long-lived plants (Table 5). All species (100%) used sexual segregation (dichogamy or herkogamy).

3 Discussion

3.1 High incidence of hermaphroditism in alpine floras

The subnival flora of the Hengduan Mountains contained a high proportion of hermaphrodites and a low proportion of dioecious species, and similar patterns were identified in other alpine floras (Table 2). This might be explained by two facts. First, alpine floras have a relatively low proportion of woody species, for example, only 8.4% of all species were woody (shrub) in our study; this figure is similar to that reported for other alpine areas (9.7%, Spence & Shaw, 1981; 9.0%, Collins et al., 1983). Some studies have suggested that woody species are strongly associated with dioecy (Bawa, 1980, 1982; Matallana et al., 2005; Chen & Li, 2008a). In this case, the relatively low proportion of dioecious species in alpine floras was to be expected because of the relatively low proportion of woody species. In contrast, woody species are common in tropical floras (45.0%,

Table 4 Fruit type and life form of the subnival belt vegetation in the Hengduan Mountains, SW China

Parameters	No. species	Hermaphroditism		Monoecy		Dioecy	
		No. obs.	No. exp.	No. obs.	No. exp.	No. obs.	No. exp.
Fruit type		$\chi^2 = 1.1, P > 0.05, n = 932$					
Dry	914	777	778.7	78	76.5	59	58.8
Fleshy	18	17	15.3	0	1.5	1	1.2
Life form		$\chi^2 = 116.5, P < 0.001, n = 932$					
Perennial forb	748	660	637.2	59	62.6	29	48.2
Perennial grass	51	31	43.4	18	4.3	2	3.3
Annual herb	55	54	46.9	1	4.6	0	3.5
Shrub	78	49	66.5	0	6.5	29	5.0

No. obs., number of observed frequencies; No. exp., number of expected frequencies.

Table 5 Compilation of available information regarding the reproductive biology of species in the alpine belt, SW China

Species	Family	Sexual segregation	Selfing	Self-compatible	Pollinator limitation [†]	References
<i>Aconitum gymnandrum</i> [‡]	Ranunculaceae	D, H	F	Yes	Yes	Zhang et al. (2006)
<i>Adenophora jasionifolia</i>	Campanulaceae	H	F	Yes	No	Liu (2011)
<i>A. khasiana</i>	Campanulaceae	H	F	Yes	No	Liu (2011)
<i>A. capillaris</i> subsp. <i>leptosepala</i>	Campanulaceae	H	F	Yes	Yes	Liu (2011)
<i>Anemone rivularis</i>	Ranunculaceae	D	F	Yes	No	Zhang (2010)
<i>Anisodus carniolicoides</i>	Solanaceae	D, H	F	Yes	Yes	Wang et al. (2009)
<i>A. luridus</i>	Solanaceae	D, H	A, F	Yes	Yes	Wang et al. (2009)
<i>A. tanguticus</i>	Solanaceae	D, H	A, F	Yes	Yes	Duan et al. (2007b)
<i>Comastoma pulmonarium</i> [‡]	Gentianaceae	D	A	Yes	No	Zhang et al. (2011a)
<i>Cyananthus delavayi</i>	Campanulaceae	–	Gynodioecy	–	Yes	Niu (2011)
<i>C. hookeri</i> [‡]	Campanulaceae	D	A	Yes	No	Niu (2012, unpublished data)
<i>Delphinium caeruleum</i>	Ranunculaceae	D, H	No	Yes	Yes	Zhang et al. (2012)
<i>Eriophyton wallichii</i>	Lamiaceae	D, H	F	Yes	No	Peng (2012, unpublished data)
<i>Gentiana lawrencei</i> var. <i>farreri</i>	Gentianaceae	D, H	F	Yes	Yes	Hou et al. (2009)
<i>G. siphonantha</i>	Gentianaceae	D, H	F	Yes	Yes	Hou et al. (2008)
<i>G. straminea</i>	Gentianaceae	D, H	F	Yes	Yes	Duan et al. (2007c)
<i>Gentianopsis barbata</i> [‡]	Gentianaceae	H	A	Yes	Yes	Duan et al. (2007a)
<i>G. paludosa</i> [‡]	Gentianaceae	H	A	Yes	Yes	Duan et al. (2010)
<i>Incarvillea sinensis</i> var. <i>sinensis</i> [‡]	Bignoniaceae	H	F	Yes	Yes	Qu et al. (2007)
<i>Mandragora caulescens</i>	Solanaceae	D, H	No	Yes	Yes	Wan et al. (2011)
<i>Megacodon stylophorus</i>	Gentianaceae	D, H	F	Yes	Yes	Meng et al. (2012)
<i>Neottia listeroides</i>	Orchidaceae	H	F	Yes	Yes	Wang et al. (2008)
<i>Parrya nudicaulis</i>	Brassicaceae	D	No	Yes	Yes	Fulkerson et al. (2012)
<i>Pedicularis densispica</i> [‡]	Orobanchaceae	H	F	Yes	Yes	Sun (2005)
<i>P. dumiana</i>	Orobanchaceae	D, H	A	Yes	Yes	Sun et al. (2005)
<i>P. lachnoglossa</i>	Orobanchaceae	D, H	F	Yes	No	Yu et al. (2012)
<i>Phaius delavayi</i>	Orchidaceae	H	F	Yes	Yes	Li et al. (2010)
<i>Rheum nobile</i>	Polygonaceae	D	F	Yes	No	Song (2011, unpublished data)
<i>Rhodiola fastigiata</i>	Crassulaceae	–	Polygamodioecy	–	Yes	Ding (2008, unpublished data)
<i>Roscoea cauleoides</i>	Zingiberaceae	H	F	Yes	Yes	Zhang et al. (2011b)
<i>R. humeana</i>	Zingiberaceae	H	F	Yes	Yes	Zhang et al. (2011b)
<i>R. schneideriana</i>	Zingiberaceae	H	A	Yes	Yes	Zhang & Li (2008)
<i>Saussurea involucrata</i>	Asteraceae	D, H	F	Yes	No	Dai (2008, unpublished data)
<i>Sinopodophyllum hexandrum</i>	Berberidaceae	H	A	Yes	Yes	Xu et al. (1997)
<i>Stellera chamaejasme</i>	Thymelaeaceae	H	No	No	Yes	Zhang et al. (2011c)
<i>Swertia przewalskii</i>	Gentianaceae	D, H	A	Yes	No	Duan & Liu (2007)

[†]Pollinator limitation was defined as lack of enough pollinators for outcrossing; [‡]Annual plant (including biennial species), whereas others were long-lived; –, No data; A, autonomous selfing, where the plant had a well-developed capacity for selfing by themselves rather than rely on pollinators; D, dichogamy; F, facilitated selfing, where selfing could occur within flowers (autogamy) or among flowers on the same plant (geitonogamy), which required the action of a pollinator or other outside force (e.g., *Incarvillea sinensis* var. *sinensis*, wind-dragged corolla enhances self-pollination); H, herkogamy.

Matallana et al., 2005; 72.0%, Chen & Li, 2008b), consequently, tropical floras contain many dioecious plants.

Second, the harsh alpine environment could result in pollinators being rare or unreliable (Arroyo et al., 1985). For this reason, hermaphroditism would be very advantageous for alpine plants to achieve reproductive assurance by autonomous selfing (Sun et al., 2005; Duan et al., 2007a, 2010; Zhang & Li, 2008) or wind-facilitated selfing (Qu et al., 2007). Most of the annual herbs were hermaphroditic species in our study region (Table 4), moreover, we found that many of them could reproduce successfully through autonomous selfing (Table 5). Consequently, for annual plants, hermaphroditism would help to compensate for the reduced mate chance by autonomous selfing. Furthermore, it has been suggested that selfing hermaphroditic plants are favored during colonization

(Stebbins, 1957), and are most likely to establish new populations in alpine areas. In contrast, dioecious species depend on vectors for cross-pollination. Although some dioecious species are wind-pollinated (e.g., *Salix* spp., Totland & Sottocornola, 2001; *Rhodiola* spp., Ding, 2008, unpublished data), the low density and small size of alpine plants can be obstacles to the success of this method.

3.2 Relationship between sexual systems and morphological traits

Floral morphological traits were significantly correlated with sexual systems in our study region, and the associations seem to be the result of a strong selection for pollination. Dioecious species depend on vectors for cross-pollination, however, insects are not reliable pollinators in alpine areas. Moreover, most dioecious species in this area had inconspicuous floral

displays (small and pale-colored flowers), which could not attract pollinators effectively. The result was that dioecious species were unlikely to be pollinated successfully. In contrast, hermaphroditic species contain both pistils and stamens, and most of them can achieve reproductive success by autonomous or facilitated selfing (Table 5). However, selfing can lead to inbreeding depression, thus reducing fitness as a maternal parent (reviewed in Barrett, 1998). In our study, we found that hermaphroditic species had a number of mechanisms that reduced selfing and promoted more efficient pollen dispersal (Tables 3, 5). First, specialized and showy flowers: blue-purple flowers tend to be more attractive to bumblebee pollinators than flowers of other colors (Gong & Huang, 2011). A showy and large floral display is likely to attract more pollinators and increase pollination probability (Arroyo et al., 2006) and, thus, is beneficial for pollen dispersal and pollen capture. In addition, bumblebees are efficient pollinators at high elevations (Bingham & Orthner, 1998). Second, solitary flowers: a solitary flower with a large display can reduce geitonogamous self-pollination and pollen discounting through more efficient pollen dispersal to other plants (Barrett, 1998). Finally, sexual segregation (herkogamy or dichogamy): sexual structures can be spatially or temporally separated in a flower, thus reducing the likelihood of self-pollination; this is a strategy that promotes cross-pollination (Barrett, 2002).

However, diclinous species have also evolved some strategies adapting them to the harsh alpine conditions. We found that monoecy was strongly associated with perennial grasses. Spence & Shaw (1981) suggested that some of these species, particularly the *Poa* species, were probably partially or wholly apomictic. Some grasses can survive through clonal reproduction (Körner, 2003). We also found that dioecy was strongly associated with shrubs (Table 4). Shrubs show some adaptations to tolerate the harsh alpine conditions, including deep root systems, and leathery or robust leaves, which are unlikely to be damaged in strong winds and are resistant to herbivory (Collins et al., 1983). Moreover, most of the diclinous species used anemophily or generalist entomophily (Table 3), thus compensating for the reduced mate availability and potentially mitigating the negative effects of the reduced abundance of biotic pollinators expected at higher elevations (Arroyo & Squeo, 1990).

Although no statistically significant association was found, dry fruits (suggesting abiotic dispersal) were the most common type in the subnival region (Table 4). In contrast, Matallana et al. (2005) and Chen & Li (2008b) have found that fleshy fruits are common in

tropical floras. Fleshy fruits often have an attractive structure that encourages seed dispersal by birds and mammals (reviewed in Willson et al., 1989). However, in the subnival belt, fleshy fruits would not be efficiently dispersed by zoochory, because of the scarcity of birds and mammals. Therefore, species with fleshy fruits were unlikely to have improved fitness or disperse successfully in alpine regions. In contrast, capsula, capitulum, siliqua, and follicle fruit types were most abundant (produced by 47.5%, 15.7%, 7.6%, and 6.1% of the species, respectively) in our study region. They generally contain numerous seeds, are abiotically dispersed and may have numerous seeds initially dispersed ballistically or by wind and then biotically, for example, by ants (Renner & Ricklefs, 1995). Pellissier et al. (2010) also suggested that the proportion of plants with capsules increases with increasing altitude, and capsules may represent an efficient dispersal system because they contained large numbers of very small, light seeds. Further, as growing seasons become shorter with increasing altitude, production costs of dry fruits and accompanying structures may be lower, enabling faster ripening and dispersal (e.g., Tébar & Llorens, 1993). Thus, dry fruits were good for plant survival and dispersal in the subnival belt, explaining their dominance in subnival habitats.

To conclude, combined with our previous study (Peng et al., 2012), our results showed that hermaphrodite, showy flowers, and generalized floral traits were dominant in the subnival belt of the Hengduan Mountains. The current study also established a clear association between plant sexual systems and reproductive traits, for example, hermaphroditic species were strongly associated with specialized entomophily and showy flowers, and diclinous species with anemophily and inconspicuous flowers. The patterns of flower morphology and sexual systems represented the reproductive strategies used by plants growing in alpine areas. However, some of the patterns we observed may be the results of environmental influences. We did not investigate the effects of environmental factors on phylogeny, which was a limitation of our study. This issue should be addressed in further investigations of the patterns of sexual systems, with the phylogenetic effect examined at higher taxonomic levels (e.g., Renner & Ricklefs, 1995).

Acknowledgements We are grateful to B. SONG and Y. YANG for their help with the field study, and the department of botanical teaching and the research section of the School of Life Science, Yunnan Normal University as well as the alpine floral research group of the Kunming Institute of Botany for providing logistical

support. The first author is grateful to C.Q. LIU for his comments on an earlier version of the manuscript. We also thank three anonymous reviewers for valuable comments on earlier versions of this article. The study was supported by the National Natural Science Foundation of Area (Grant No. 30360049 to Z. M. Li), the National Natural Science Foundation of China (Grant Nos. 40930209 and 31100179), the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (Grant No. XDB03030112) and the Hundred Talents Program of the Chinese Academy of Sciences (Grant No. 2011312D11022 to H. SUN).

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Supplementary Material

The following supplementary material is available for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.12046/supinfo>:

Doc. S1. Data of reproductive traits of subnival plants.