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# Difference in Floral Traits, Pollination, and Reproductive Success between White and Blue Flowers of *Gentiana leucomelaena* (Gentianaceae) in an Alpine Meadow

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## Abstract

The blue-white gentian, *Gentiana leucomelaena* (Gentianaceae), bears two colors of flowers (blue and white) within populations and on individual plants. We tested the hypothesis that these flower morphs have different mating systems associated with differences in pollinator species and visitation rates. This hypothesis was driven by the fact that colorful flowers are often more likely to be pollinated by diverse animal pollinators, and by the observation that white *G. leucomelaena* flowers always blossom earlier than blue ones in early spring, when few pollinators are available. This hypothesis was investigated by determining the pollination success, daily duration of flower opening, flower life span, the number of pollen grains and ovules per flower, pollinator visitation rates, and seed output for both color morphs during the flowering season of 2009. Hand-pollination and pollinator-exclusion experiments were also conducted to determine whether the two color morphs differed in their ability to self-pollinate. In general, blue flowers were found to have a trait combination that favors a significantly higher degree of pollinator specificity and cross fertilization, whereas white flowers were characterized as pollinator generalists and self-fertilizing. We speculate that the difference in the floral structure and function between blue and white flowers and their coexistence are likely adaptive in unpredictable and often pollinator-limited environments such as those found in the alpine meadows of the Qinghai-Tibet Plateau.

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## Introduction

Flowering plants have evolved a diversity of trait combinations that attract specific kinds of animal pollinators (Galen, 1999; Harder et al., 2001; Asikainen and Mutikainen, 2005; Ghazoul, 2006; Stang, et al., 2006; Morales and Traveset, 2009). Among the traits determining pollinator visitation rates and species groups of pollinators, flower color has been long recognized as one of the most important ones (Darwin, 1859; Menzel and Shmida, 1993; Schemske and Bierzychudek, 2001). Indeed, flower color polymorphism may to some extent determine the possibility of cross-fertilization and eventually plant fitness through mediating pollinator behavior (Waser and Price, 1981; Charlesworth, 2006).

Flower color polymorphism is well known to be associated with pollinator species diversity. For instance, bees preferentially visit purple, blue, and yellow flowers; birds typically visit red and pink flowers (Wilson et al., 2004); and moths nocturnally favor white flowers (Rausher, 2008). It is possible, therefore, that different pollinators visit different color flowers produced by conspecifics in the same plant population. This speculation fully acknowledges that floral color polymorphism may also be associated with different mating systems as well as different pollinators. For example, in *Delphinium nelsonii* populations, white and pale flowers are less visited by natural pollinators than blue ones, even though both colored flowers yield similar seed production when artificially pollinated (Waser and Price, 1981). Similarly, in *Camissonia cheiranthifolia* populations of Southern California, large colorful nectar-producing flowers are strongly

self-incompatible, whereas small plain flowers are usually self-compatible, while simultaneously possessing contrasting pollinator visitation rates (Raven, 1969). These and other studies suggest that colorful flowers are often capable of cross-fertilization in contrast to non-colored, plain flowers.

Besides flower color, flower size (Galen, 1999), odor (Galen and Kevan, 1980; Shuttleworth and Johnson, 2009), nectar volume, pollen production (Harder and Thomson, 1989; Harder et al., 2001; Irwin et al., 2004), and individual life span (Van Doorn, 1997; Abdala-Roberts et al., 2007) are also important traits for successful cross-pollination. Insects usually prefer large flowers because of the enhanced visibility and the possibility of high reward quality or quantity (Galen and Cuba, 2001). For example, bumblebees often visit large flowers first after entering a mixed-species plot (Galen and Stanton, 1989). Moreover, pollinators such as bees favor flowers with more pollen and larger nectar volume (Smith and Cobey, 1994; Nepi et al., 2009). Wasps also use the nectar volume as a guide for visiting flowers (Scora, 1964). In addition, variations in floral longevity are often associated with habitat, pollinator type, taxonomic class, and breeding system (Primack, 1985). The flowers of animal-pollinated species have to remain open for longer times to be pollinated in contrast to self-pollinated species, which typically have much shorter flower life spans (Primack, 1985; Van Doorn, 1997). Animal pollinators mostly depend on a combination of these traits, rather than a single trait. For example, species favored by bees are often characterized by possessing blue or purple flowers, short and wide

corolla tubes, wide limbs, inserted stigmas, and small amounts of concentrated nectar, while the moth-pollinated flowers are usually white and fragrant with long floral tubes that often open at night (Rausher, 2008). Thus, it is reasonable to suppose that flower color is correlated with other attractive/rewarding traits like flower size, pollen productivity, nectar volume and concentration, life span, and diurnal cycles, forming suites of traits that characterize different pollinator types and/or mating systems.

This possibility was explored using the alpine blue-white gentian (*Gentiana leucomelaena*; Gentianaceae), which is widely distributed on the Tibetan Plateau. This species has two flower colors (blue and white) within populations as well as within individual plants. Flowers are produced in early spring and therefore typically experience low and fluctuating temperatures. In the region selected for our study, the potential pollinators include honeybees (*Apis cerana cerana*), flies, ants, and moths. Preliminary studies have shown that most white flowers emerge early during the flowering season (in spring), whereas blue flowers dominate in the late season (Mu et al., 2010). Because pollinators are usually less frequent in the early than the late flowering season in alpine areas (Kudo, 1993; as well as this study), we hypothesized that blue flowers are more likely characterized by traits that are associated with the insect pollinators, whereas white flowers should tend to be characterized by traits facilitating self-pollination. To test this hypothesis, we determined floral opening cycles, life span, pollen-ovule ratios, pollinators and their visitation rates, and seed output for both flower color morphs. As noted, these traits are mostly related to pollinator visitation rates. Therefore, we experimentally determined the reproductive success for both color morphs when hand-pollinated and when bagged to exclude animal pollinators to test whether the white flowers are more successful at self-pollination than blue ones, and to test whether blue flowers are more successful at cross-pollination than their white counterparts. As noted, the primary objective of this study was to test whether the two color morphs possess different trait combinations that in theory are predicted to relate to different pollination and mating systems.

## Methods

### STUDY SITES

This study was conducted in permanent plots at the Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences (31°50′–33°22′N and 101°51′–103°23′E), in the eastern section of the Qinghai-Tibetan Plateau (altitude 3500 m). The mean annual precipitation is 749.1 mm, 80% of which is concentrated in the growing season from May to October. The mean annual temperature is 1.4 °C, with maximum and minimum monthly means of 10.9 °C and –10.3 °C in July and January, respectively (Gao et al., 2008).

The meadow community is dominated by *Agrostis trinii*, *A. hugoniana*, *Potentilla anserina*, and *Anemone trullifolia* var. *linearis*. Total vegetation coverage is more than 95% with a plant height of ~30 cm. The soil is characterized by high organic matter content (247 g kg<sup>-1</sup>) and low availability of N (673.75 mg kg<sup>-1</sup>) and P (6.32 mg kg<sup>-1</sup>) (Song et al., 2007).

### SPECIES DESCRIPTION

*Gentiana leucomelaena* is an annual herbaceous species in the *Gentiana* sect. *Chondrophyllae* (He et al., 1988). It grows along streams and in alpine meadows and scrubs in China, with altitudes ranging from 1900 to 5000 m. Plants are 5–10 cm in height and

produce protandrous flowers with corolla diameters and depths of 1.2 and 1.0 cm, respectively; the campanulate-shaped corolla consists of 10 connate petals. Both floral color morphs (blue and white) have dark blue spots within the corolla throat. Flowering occurs between March and May, when few plant species in the study site flower. Fruits mature from April to June.

Each plant typically produced 5 to 15 tillers, each produced one flower; not all the flowers bloomed at the same time. There were three types of individuals within the study population, i.e., individuals with blue or white flowers only, and individuals with both flowers. The proportion of the three types of individuals varies with time during the flowering season (Mu et al., 2010). Even in individuals with both color morphs, the number of blue flowers is usually larger than that of white flowers, although the frequency varies during the flowering season. A previous survey shows that from late March to middle May, the frequency of white flowers decreases from 98.68% to 10.16%, while the blue morphs increase from 1.32% to 89.84% (Mu et al., 2010).

### FLOWER LONGEVITY AND DAILY EXPOSURE TIME

A 50-ha permanent plot was set up in the study site wherein cattle grazing has been prevented since 2007. Within the plot, five 5 m × 5 m subplots were established on 21 March 2009. These subplots were close to a roadside and grass hummock because the species distribution is usually disturbance-dependent (He et al., 1988). The total number of flowers was, on average, ~2000 within each subplot.

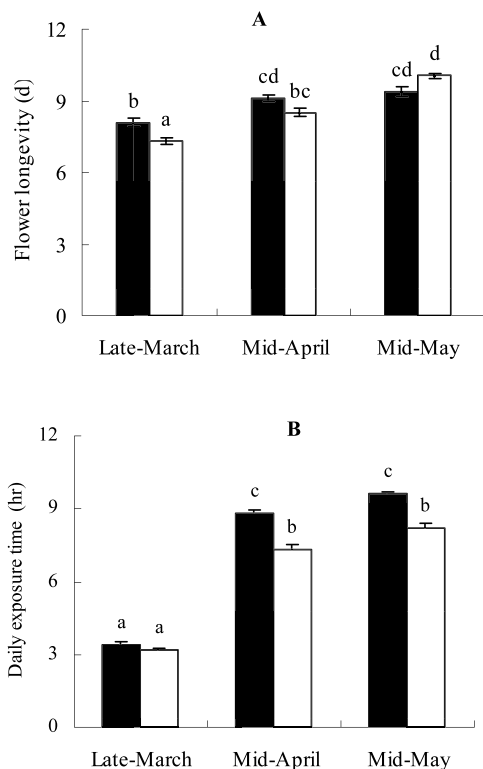
Fifty individuals were randomly tagged and observed daily for each color in late March, middle April, and middle May. There was no obvious damage of tagged individuals during the survey period. “Blue- or white-flowered” individuals were designated based on the first color morph produced during the observation period, even though “blue- or white-flowered” individuals subsequently produced the alternative color morph later in the flowering season. We recorded the dates when flowers first opened and when they wilted; individual flower life span was calculated as the time span between the two dates (following the protocol of Vesprini and Pacini, 2005). We also recorded the time when flowers opened and closed during a minimum of three individual sunny days (in late March, middle April, and middle May). These durations were used to calculate mean daily exposure time for each color morph.

### POLLEN AND OVULE NUMBERS

Thirty flowers of each color morph were collected after flower opening when anthers were not mature. The flowers were randomly chosen from the individuals within subplots and collected from either single- or double-color individuals. Before measurements, anthers were removed and left to dry on paper for 24–48 h at 20 °C. Pollen grains were transferred subsequently into a solution of aniline-blue in lactophenol. Using a dissecting microscope, we recorded the number of pollen grains for each color morph, using the protocols described by Cruden (1977) and Costa and Yang (2009). The number of ovules for each of the collected flowers was also counted. Additionally, we tried to measure the nectar volume, but nectar was produced in undetectable amounts (less than 0.01 μL).

### FLOWER VISITORS AND VISITATION RATES

Within the permanent plot, five 2 m × 2 m subplots were established on 21 March 2009. In each subplot, we observed and



**FIGURE 1.** Means ( $\pm 1$  SE) of (A) flower longevity and (B) daily exposure time of blue and white *Gentiana leucomelaena* flowers. Significant differences ( $P < 0.05$ ) between the two flower morphs as detected by ANOVA (see text) are denoted by different letters above SE bars. Black and white bars denote blue and white flowers, respectively.

recorded pollinators and their visitation frequencies for five sunny days (from mid-April to early May of 2009 when the abundance of flowers and potential pollinators were relatively high during the flowering season) to assess whether differences existed between the two color morphs. Because there are few pollinators in early spring and because pollinator preferences are not likely to change over short time spans, we did not examine visitation rates during other times of the flowering season. At each time, we recorded the total number of flowers for both colors, the pollinator species, and the number of flowers visited per species on an hourly basis from 09:00 to 17:00 (Beijing time). The observation distance was about 3 m for flies, bees, and lepidopterans, and about 1 m for ant species. Visitation rates were calculated for each color morph as the total number of visits divided by the observation time and by the number of flowers observed (e.g. Arroyo et al., 1985; Martén-Rodríguez and Fenster, 2008).

#### THE EFFECT OF CROSS- AND SELF-POLLINATION ON SEED PRODUCTION

For each color morph, we bagged 50 flowers before anther dehiscence and stigma receptivity (as indicated by the opening of bilobed stigma bearing feather-like trichomes) to determine the potential for autonomous self-pollination. Additionally, another 50 flowers were randomly selected, emasculated, hand-pollinated using pollen from other individuals, and then bagged to examine seed productivity arising from cross-pollination. Fruit ripening was determined when the pod extended from the corolla; this occurred one or two days before fruit opening. After fruit ripening, the bags were removed and seeds were individually

**TABLE 1**

Summary of ANOVA for the effects of flower color (white vs. blue) and flowering time (early, middle, and late flowering season) on flower longevity and daily exposure of blue and white *Gentiana leucomelaena* flowers. The 'subplot' was assigned as a random factor in the analysis.

		df	F	P	
Flower longevity	Flower color (C)	1	27.31	0.01	
	Flower Time (T)	2	66.93	<0.001	
	Subplot (S)	4	0.559	0.693	
	C $\times$ T	2	6.304	0.002	
	C $\times$ S	4	2.87	0.081	
	T $\times$ S	8	2.391	0.017	
	C $\times$ T $\times$ S	8	2.67	0.008	
	Error	207			
Daily exposure time	Flower color (C)	1	13.328	<0.001	
	Flower Time (T)	2	130.019	<0.001	
	C $\times$ T	2	2.116	0.126	
		Error	94		

counted and weighed (to 0.1 mg) for each flower. Empty seeds, which are easily recognized (see Larrinaga et al., 2009), were recorded as aborted.

#### DATA ANALYSIS

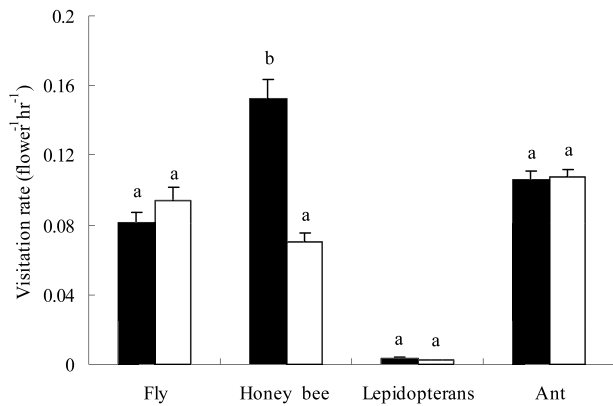
All the data on the study traits were tested for normality before analysis. The differences in pollen and ovule numbers and pollinator visitation rates were assessed using one-way ANOVA. Three-way ANOVAs were employed to examine the effects of flower color and flowering time (late March, middle April, and middle May) on flower longevity with subplot as a random factor. Two-way ANOVAs were used to detect the effects of these variables on daily exposure time. In addition, we calculated seed set ratios, i.e., the ratio of the number of mature seeds to the total number of seeds (including the number of aborted seeds), to characterize the success of pollination. The data for seed set ratios and seed output were log-transformed ( $\log[1 + X]$ ) to achieve normality. Two-way ANOVAs were used to determine the effect of fertilization type and flower color on seed set ratios, seed size, seed number, and seed output per flower. All statistical analyses were performed using SPSS Version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A.).

## Results

#### FLOWER LONGEVITY AND DAILY EXPOSURE TIME

Flower longevity ranged between 7 and 10 days. However, it differed between the two color morphs and varied over the flowering season (Fig. 1, A; Table 1). In late March, blue flowers had a significantly longer life span than white ones ( $N = 50$  for each color,  $F = 7.80$ ,  $P < 0.05$ ), whereas in mid-May, life span was slightly but statistically insignificantly longer for white flowers than for blue ones. Flower longevity increased significantly over the course of the flowering season for both color morphs, i.e. from 8.1 d to 9.4 d among blue flowers ( $N = 50$ ,  $F = 10.5$ ,  $P < 0.05$ ) and from 7.3 d to 10.0 d among white flowers ( $N = 50$ ,  $F = 59.21$ ,  $P < 0.05$ ).

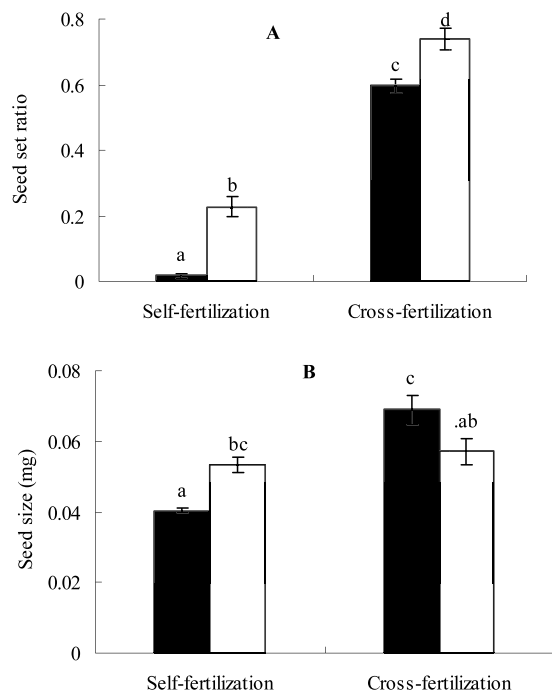
The daily exposure time also changed during the flowering season. From 22 to 31 March, the daily flowering time was, on average, only 3 h for both colors (Fig. 1, B; Table 1), but increased until the end of flowering season (Fig. 1, B). Blue flowers had



**FIGURE 2.** Means ( $\pm 1$  SE) of diurnal variation in daily mean pollinator visitation rates (including honeybees, flies, ants, and lepidopterans) for blue and white *Gentiana leucomelaena* flowers. Significant differences ( $P < 0.05$ ) between the two flower morphs as detected by ANOVA (see text) are denoted by different letters above SE bars. Black and white bars denote blue and white flowers, respectively.

significantly longer daily flowering times than white ones in mid-April ( $N = 50$ ,  $F = 26.29$ ,  $P < 0.05$ ) and mid-May ( $N = 50$ ,  $F = 48.83$ ,  $P < 0.05$ ). The opening time for both color morphs varied throughout the season ( $N = 50$ ,  $F = 8.53$ ,  $P < 0.05$ ). In late March, blue and white flowers did not open until 12:00. In middle April, blue flowers opened, on average, at 08:35, while white flowers, on average, did not open until 09:13.

Based on the individual flower life spans and mean daily opening times, we calculated the total opening time for individual flowers and found that it was significantly longer for blue flowers than for white ones ( $N = 50$ ,  $F = 57.1$ ,  $P < 0.05$ ). The time ranged



**FIGURE 3.** Means ( $\pm 1$  SE) of reproductive success characterized by (A) seed set ratios and (B) seed size for blue and white *Gentiana leucomelaena* flowers. Significant differences ( $P < 0.05$ ) between the two flower morphs as detected by ANOVA (see text) are denoted by different letters above SE bars. Black and white bars denote blue and white flowers, respectively.

**TABLE 2**

Results of two-way ANOVA for the effects of flower color (white vs. blue) and fertilization type (cross vs. self-pollinated) on seed set ratio, seed size, and seed output per flower for blue and white *Gentiana leucomelaena* flowers.

		df	F	P
Seed set ratio	Fertilization type (F)	1	37.196	<0.001
	Petal color (C)	1	369.600	<0.001
	F $\times$ C	1	1.053	0.306
	Error	155		
Seed size	Fertilization type (F)	1	10.668	<0.05
	Petal color (C)	1	30.415	<0.001
	F $\times$ C	1	5.066	<0.05
	Error	133		
Seed output	Fertilization type (F)	1	13.597	<0.05
	Petal color (C)	1	34.088	<0.001
	F $\times$ C	1	17.109	<0.001
	Error	133		

from 23.36 h to 82.00 h for white flowers and from 27.54 h to 90.24 h for blue flowers in late March and middle May, respectively.

#### POLLEN AND OVULE NUMBERS

The number of pollen grains was more than 2000 and the number of ovules was more than 100 for both color morphs, and varied little among flowers within each color morph. However, blue flowers produced significantly more pollen grains than white flowers ( $13,027.2 \pm 970.87$  versus  $10,480.1 \pm 512.84$ , respectively;  $N = 30$ ,  $F = 5.38$ ,  $P < 0.01$ ). In contrast, white flowers produced a greater number of ovules compared to blue flowers ( $152.9 \pm 6.65$  versus  $194.9 \pm 8.05$ , respectively;  $N = 30$ ,  $F = 16.2$ ,  $P < 0.01$ ). Consequently, pollen to ovule ratio was significantly higher for blue compared to white flowers ( $88.27 \pm 7.51$  versus  $56.31 \pm 3.35$ , respectively;  $N = 30$ ,  $F = 15.4$ ,  $P < 0.01$ ).

#### POLLINATORS AND VISITATION RATES

Both color morphs were visited by flies, ants, and honeybees (*Apis cerana cerana*), and (occasionally) by lepidopterans, albeit at different times. Ants visited flowers significantly earlier than flies ( $2 \pm 0.5$  d) or honeybees ( $7 \pm 1.5$  d;  $N = 5$ ,  $F = 11.04$ ,  $P < 0.05$ ) and started visiting flowers on 10 April. Furthermore, flies tended to visit flowers between 09:00 and 11:00 and 15:00 and 17:00 and honeybees mostly visited at 13:00–15:50, whereas ants showed no preference in their visitation times. The mean daily visitation rate was significantly higher for blue as opposed to white flowers ( $N = 25$ ,  $F = 41.93$ ,  $P < 0.01$ ), but not for ants, flies, and lepidopterans (Fig. 2). The visitation rates of bees was  $0.152$  flower<sup>-1</sup> h<sup>-1</sup> for blue flowers and  $0.071$  flower<sup>-1</sup> h<sup>-1</sup> for white flowers.

#### THE EFFECT OF POLLINATION TYPE ON SEED PRODUCTIVITY

Pollination type and flower color both significantly influenced reproductive successes (Fig. 3; Table 2). As expected, pollinator exclusion significantly reduced the seed set ratio, individual seed mass, and seed number per flower for both morphs (all  $P < 0.01$ ). However, the reduction in magnitude differed between the two color morphs. Blue flowers had significantly lower seed set ratios, seed size, and seed output per flower than white flowers for flowers

that were treated as being capable of self-pollination. With the exception of seed set ratios, this trend was reversed when flowers were hand-pollinated (Fig. 3).

## Discussion

Our data show that the blue flowers of *G. leucomelaena* remain open on a daily basis for longer times, have longer total exposure times, and larger pollen-to-ovule number ratios compared to white flowers. Theoretically, these traits should make blue flowers more attractive to animal pollinators compared to white ones. Consistent with this speculation, honeybee visitation rates are observed to be significantly higher for blue as opposed to white flowers, although other arguably less specialized pollinators show no detectable preference for either flower color morph. Additionally, compared to white flowers, blue flowers have a significantly higher reproductive output when cross-pollinated and a lower reproductive output when self-pollinated. These results collectively suggest that blue flowers are better adapted for cross-fertilization than white ones.

It has long been known that flower color is an important trait that can enhance pollinator specificity (Darwin, 1859; Asikainen and Mutikainen, 2005). In the context of this study, it is important to note that bees have good color vision (particularly in the ultraviolet, blue, and yellow spectrum ranges), whereas flies prefer white or yellow flowers (Briscoe and Chittka, 2001) and therefore preferentially visit blue compared to white flowers (Ackermann and Weigend, 2006; Thomson and Wilson, 2008). Consistent with this trend, honeybees (*Apis cerana cerana*) were observed to visit blue *G. leucomelaena* flowers much more frequently than white flowers, particularly during the middle flowering season. Because honeybee visitation rates are higher for blue compared to white flowers and because honeybees usually have higher pollination efficiencies compared to flies, blue flowers have a greater reproductive success compared to white flowers. A similar phenomenology is reported for white-flowered individuals of *Delphinium nelsonii*, which have lower seed production than blue ones pollinated naturally, presumably because bumblebees favor blue flowers over white ones (Waser and Price, 1981). In contrast, some pollinators do not discriminate between white and blue flowers (Schemske and Bierzychudek, 2001). In our study, ants, flies, and lepidopterans showed little or no preference between the two gentian color morphs, and typically visit flowers visitors less frequently than honeybees (especially in early spring; J. P. Mu, personal observation). These observations detract from the hypothesis that the two floral color morphs reflect divergent natural selection based on pollinator preferences (e.g. flies vs. bees), although this hypothesis cannot be excluded. Clearly, a number of traits other than flower color contribute to pollinator specificity or reproductive success. In the case of one of the examples previously mentioned, hummingbirds and bumblebees may favor blue *D. nelsonii* flowers not because of the color of the corolla but because blue flowers produce more or higher quality nectar than white ones (Waser and Price, 1981), just as some bees or flies discriminate among flowers differing in pollen or nectar quality or quantity (Smith and Cobey, 1994; Nepi et al., 2009). In our case, we could not measure nectar volume with sufficient accuracy because of low nectar production. However, we did find that the blue gentian flowers most often visited by bees had greater amounts of pollen. We also observed that the extent to which the interior floral structure is exposed affected the success of being pollinated. Although flowers often close and desiccate after fertilization, depending on pollen removal and deposition (Sato,

2002; Parra-Tabla et al., 2009), they must keep open sufficiently long to be visited by pollinators. Indeed, the flowers of some species remain open for several months before being pollinated (Fitting, 1910). In the case of our gentian, white flowers emerge more frequently during the early flowering season, while blue flowers are more frequent in late season (Mu et al., 2010). Because daily exposure time is much longer and flower life span is only slightly shorter in blue compared to white flowers, the total flower exposure time is greater for blue than for white flowers. This difference in total exposure time arguably increases the probability that blue flowers will be visited and pollinated on a daily basis. Another important consideration is that flower mass and size is also related to flower color. In our study, the mass and corolla diameter of individual flowers are significantly larger in the blue morphs compared to white ones (both  $P < 0.001$ ;  $0.067 \pm 0.003$  g and  $1.28 \pm 0.023$  cm in blue flowers, respectively;  $0.058 \pm 0.004$  g and  $1.01 \pm 0.025$  cm in white flowers, respectively). These differences may account for the large amounts of pollen grains produced by blue flowers. Additionally, previous studies have demonstrated that flower size and plant size are often positively correlated to nectar volume and pollen amount (Galen, 1999; Shuttleworth and Johnson, 2009).

To our knowledge, our study is one of the few cases that explicitly supports the hypothesis that flower color polymorphism is associated with differences in mating system type. Clearly, many studies have implied that the type of mating system could be flexible depending on environmental condition and pollinator abundance. For example, in the populations of *Pedicularis hirsute* and *P. dasyantha*, plants that normally cross-fertilize could manifest autogamy when pollinators are limited or rare (Odasz and Savolainen, 1996; Macior et al., 2001). The white flowers of *D. nelsonii* are less frequently visited by insects and produce fewer seeds compared to blue flowers (Waser and Price, 1981), in part due to limited pollen transfer and pollinator visitation. Similarly, in *Linanthus parryae* populations, individuals that produce blue flowers often produce more seeds than white flowers when experiencing drought stress (Schemske and Bierzychudek, 2001). However, these studies do not clearly link flower color with any specific mating system type possibly, in large part, because they did not incorporate experiments conducted to assess the effects of cross- and self-fertilization. By the same token, it was not possible for us to follow in detail the visitation rates and pollination efficiencies of different insect species throughout the entire flowering season. Therefore, our study is limited in that we cannot totally exclude the possibility that white flowers are preferentially visited and pollinated by some as-of-yet unobserved pollinator specialists (even for flies). That is to say, although we are confident with the hypothesis that flower color polymorphism is associated with mating system type, we are still uncertain as to whether flower color polymorphism is related to pollinator species diversity/specificity. The pollination efficiency during the entire flowering season will be addressed in future research.

The differentiation of mating system types between blue and white flowers may be of important ecological significance for our gentian species. The production of white flowers could be advantageous when pollinator density is extremely low. Plants flowering in early spring often suffer extremes in temperature and low pollinator activity at alpine areas (Kudo, 1993), which can favor self-pollinating species (Shamurin, 1958). Likewise, the formation of white flowers is a reproductively viable strategy that simultaneously conserves energy and reduces nutrient costs (e.g. in flower pigments) particularly in cold early spring when pollinator density is low (Charlesworth, 2006). In contrast, cross-fertilizing blue flowers likely help to maintain a comparatively high genetic

diversity for the gentian species under the conditions of high or even comparatively low pollinator densities. Thus, flower color polymorphism can be regarded as a bet-hedging strategy for the survival of some plant species.

In summary, individual *G. leucomelaena* plants that produce white and blue flowers differ significantly in their pollination-related traits including pollen and ovule number, flower daily open time and life span, and biomass allocation. Blue flowered individuals are also more likely to be cross-fertilized than are white flowered plants, as demonstrated by pollinator exclusion experiments, in which blue flowers are shown to have a higher reproductive success when cross-pollinated and lower success when self-pollinated, and by the observation that certain pollinators generally visit blue flowers more frequently than white ones. The coexistence of these two color morphs may benefit *G. leucomelaena* populations in alpine habitats such as those in the Qinghai-Tibet Plateau.

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