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Relationships between Flowering Phenology and Functional Traits in Eastern Tibet Alpine Meadow

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Abstract

Flowering phenology is a key life history trait that strongly influences reproductive success. We investigated the relationship between flowering phenology and functional traits of 48 alpine herb species using the Leaf-Height-Seed (LHS) scheme developed by Westoby (1998) to quantify the ecological strategy of the plant species. Phenological data were obtained by weekly observation of fifty $0.5 \times 0.5 \text{ m}^2$ quadrates. Specific leaf area (SLA), height, and seed size of 48 alpine herbs were measured. Comparative generalized estimating equations and generalized linear models suggested that flowering peak date was positively correlated with size of maternal plant (biomass and height) and negatively correlated with seed size. Species with a longer flowering period were smaller and produced bigger seeds than those with a short flowering season. Flowering peak date and duration were negatively correlated, although this correlation was mainly present in annuals, which are relatively rare in the alpine meadow. Relationships between flowering phenology and specific leaf area (SLA) were weak. There were only weak effects of phylogeny on the relationships between flowering phenology and functional traits. Wind-pollinated species flowered earlier than insect-pollinated species. Annuals flowered longer than perennials. Relationships between flowering phenology and functional traits indicate that there is a tradeoff between maternal and reproductive growth, and flowering time may underlie the correlation between plant height and seed size.

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Introduction

Flowering phenology is a key character in plant life history, and it strongly determines plant fitness through reproductive processes (Rathcke and Lacey, 1985). Timing of reproductive events implies a tradeoff between vegetative and reproductive growth (Elzinga et al., 2007; Bolmgren and Cowan, 2008). Plants that flower early may do so before large amounts of photosynthate have been formed, but they would gain more time for seed development and germination. In contrast, late-flowering plants may have much photosynthate for seed growth, but the time for seed development would be decreased due to the ending of the growing season. Given the short and unpredictable growing season (mainly constrained by snowmelt timing) in arctic and alpine areas (Billings, 1974; Kudo and Suzuki, 1999; Inouye and Wielgolaski, 2003; Wielgolaski and Inouye, 2003; Iversen et al., 2009; Vashistha et al., 2009), the allocation of sufficient time for maternal growth and seed production is critical for survival.

Functional traits are a qualitative method for describing plant resource use and life-history strategies. Westoby (1998) proposed the leaf-height-seed (LHS) scheme, which works well in describing species with different life history strategies (Lavergne et al., 2003; Golodets et al., 2009). Those life strategies can be described by the volume resulting from three or more (Westoby, 1998; Westoby et al., 2002) dimensions: (1) Specific leaf area (SLA) represents the resource investments and gains (Reich et al., 1999) and potential relative growth rate (Westoby, 1998; Wright and Westoby, 1999); (2) height represents the ability to compete for light (Falster and Westoby, 2003; Moles et al., 2009); and (3) seed mass is an indicator

of establishment and dispersion (Jakobsson and Eriksson, 2000). However, although flowering phenology is a critical reproductive strategy, it is unclear whether the attributes of flowering phenology (timing and duration) could be influenced by the LHS dimensions. Thus, we measured SLA (leaf dimension), height and biomass (height dimension), seed size (seed dimension), and two phenological traits (peak flowering time and duration) to gain insight on life history tradeoffs between time of flowering and resource allocation.

Relatively little attention has been given to the relationship between flowering phenology and other functional traits at the community level, especially in alpine plants. Previous phenological studies focused on the relationships between flowering phenology and functional traits have been mostly in tropic or temperate ecosystems and focused only on one or a few species (Ollerton and Lack, 1998; Lacey et al., 2003; Dahlgren et al., 2006; Michalski and Durka, 2007; Sola and Ehrlén, 2007; Bolmgren and Cowan, 2008; Ansquer et al., 2009). Moreover, most of these studies did not consider flowering duration (but see Mazer, 1990; Bolmgren et al., 2003) despite its importance in reproduction (Primack, 1985).

Besides plant traits, biotic and abiotic interactions also play important roles in the reproduction of plants. Phenology may be affected by other organisms (Stiles, 1977; Stone et al., 1998; Oberrath and Bohning-Gaese, 2002; Bolmgren et al., 2003). That is, flowering or fruiting may occur coincidentally with mutualists (i.e. pollinators and seed dispersers), as it may be timed to avoid antagonists (i.e. herbivores or seed predators), thereby increasing the chances of successful reproduction. With respect to abiotic factors, there is huge interannual variation in environmental conditions in alpine areas. Species differing in life form may differ

in reproductive strategy. Perennial herbs have underground root or rhizomystems storing carbohydrates through winter, so their reproductive strategy might be different from “brand new” annuals. Thus, effects of pollination vectors, seed dispersal modes, and life forms on flowering phenology also are considered in our analyses.

It has been suggested that both morphological traits and flowering phenology are phylogenetically conserved within evolutionary lineages, thus related species may share similar characters through common descent (Kochmer and Handel, 1986; Johnson, 1992; Ollerton and Lack, 1992; Westoby et al., 1995; Wright and Calderon, 1995; Oberrath and Bohning-Gaese, 2002). Because the phylogenetic conservation of phenological and functional traits may not meet the assumption that species are independent observations (Felsenstein, 1985), we used a comparative generalized estimating equations (GEE) approach (Paradis and Claude, 2002) for analysis of the comparative data. In the GEE approach, the dependence among species is taken into account with a correlation matrix. Models without considering phylogenetic relations also were conducted (generalized linear models, GLM), and results of different models (GLM and GEE) were compared to see whether phylogeny affected the relationship between flowering phenology and other functional traits.

In this study, our aim was to address the following questions: (1) Is there any correlation between flowering phenology and quantitative functional traits (biomass, height, SLA, and seed size)? That is, can flowering phenology be described by the LHS plant strategy scheme? Also, what is the relationship between flowering time and duration in various species? (2) Does flowering phenology (including time and duration) of species differ between qualitative traits (life forms, pollination modes, and dispersal modes)? (3) Is the relationship between flowering phenology and functional traits constrained by phylogeny?

Materials and Methods

STUDY SITE

The study was conducted in an alpine meadow at The Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University (Azi Branch Station), in Maqu (33°40'N, 101°52'E, altitude 3550 m a.s.l.), Gansu, China, on the eastern Tibetan Plateau. The average temperature is 1.2 °C, ranging from −10 °C in January to 11.7 °C in July. Annual frost days are 270. Annual precipitation, measured over the last 30 years, is 620 mm; mainly in the short summer. The vegetation is typical alpine meadow dominated by *Kobresia graminifolia* (Cyperaceae), *Elymus nutans* (Poaceae), *Anemone rivularis* (Ranunculaceae), *Poa poophagorum* (Poaceae), *Festuca ovina* (Poaceae), and *Carex kansuensis* (Cyperaceae). The average aboveground biomass is 360–560 g m^{−2}. Our research was conducted in an alpine meadow that had been fenced since 2007 and was grazed only in winter.

In 2008, fifty 0.5 × 0.5 m² permanent plots were uniformly established at 3.5 m intervals in the fenced meadow. The flowering phenology of every species in each plot was recorded approximately weekly for 22 times from early May to late September (1, 7, 14, 21, 30 May; 5, 12, 20, 26 June; 4, 11, 17, 24 July; 2, 8, 16, 22, 27 August; 3, 10, 16 September) except the last one (on 7 October, the site was covered by snow). Monitoring of phenology was performed without reference to previous data.

FLOWERING PHENOLOGY

At each monitoring time, plants with petals, anthers, filaments, or calyces were recognized as “flowering.” We did not

use the flowering onset date and “absolute duration” (last date flower appears—first date flower appears + 1) because they are easily biased by a few outliers, especially for data only collected for 1 year. Flowering time is calculated as the dates weighted by flowering proportion of each species:

$$peak\ date = \frac{\sum_{t=1}^T n_t \cdot d_t}{\sum_{t=1}^T n_t}, \quad (1)$$

where d_t is the day of the year of observing time t ; T is the total observing times; and n_t is the number of plots in which focal species is flowering.

If we consider time as a resource, the duration of flowering is the breadth of a temporal niche a species occupied; thus, flowering durations were quantified by Pianka's index of niche breadth (Pianka, 1973):

$$Duration = \frac{1}{\sum_{t=1}^T P_t^2}, \quad (2)$$

where

$$P_t = \frac{n_t}{\sum_{t=1}^T n_t}. \quad (3)$$

TRAITS SAMPLING AND MEASUREMENT

The 48 most common species were chosen for traits measurement. These species accounted for 90% of the aboveground biomass in the community. Samples of whole adult plant were collected when the focal species were in flowering or had already flowered in more than half of the plots in which it appeared (i.e. if a species appeared in 42 plots and 21 of them were in flowering or had flowered, samples of this species were collected). Similarly, we collected seeds for each species when they were mature in more than half of the plots in which it occurred. We did not collect the belowground parts due to the high density of roots in the alpine community. For clonal species, a ramet is regarded as an individual (Niu et al., 2008).

Biomass, height, SLA, and seed size were measured following Cornelissen et al.'s (2003) procedures for all 48 species and for 20–25 individuals of each species sampled randomly in the field (but outside the monitoring plots). Plant heights were measured when collected. Two leaves (one leaf for some species) from each single plant were used for determining leaf area. Leaves were scanned as a computer image and the area were measured by ImageJ (Rueden and Eliceiri, 2007), and then they were dried and weighed. After leaves were scanned, the whole plant were dried and weighed. Seeds were collected for each species, and three samples of 100 randomly chosen seeds were dried and weighed. Seed size is the average mass of the three samples. All samples were dried at 80 °C for 48 h and weighed with a Sartorius balance (10^{−4} g accuracy).

POLLINATION MODE, SEED DISPERSAL MODE AND LIFE FORM

Species with showy conspicuous flowers were categorized as insect-pollinated, while those without obvious floral attractants

TABLE 1

(A) Bivariate correlation (Pearson) analysis between biomass and other traits, based on 48 species and 27 PICs.

Correlation of biomass	Flowering Peak Date	Flowering Duration	Height	SLA	Seed size
Cross-species ($N = 48$)	0.489**	-0.060	0.518**	-0.368*	0.269a
PIC ($N = 27$)	0.510**	-0.163	0.570**	-0.600**	0.381*

(B) Bivariate correlation (Pearson) analysis between height and other traits, based on 48 species and 27 PICs.

Correlation of height	Flowering Peak Date	Flowering Duration	Biomass	SLA	Seed size
Cross species ($N = 48$)	0.657**	-0.277	0.518**	0.155	-0.149
PIC ($N = 27$)	0.676**	0.091	0.570**	0.064	0.189

(C) Bivariate correlation among function traits based on 48 species and 27 PICs. Correlations on cross-species analyses are given on the upper triangle ($n = 48$), and analyses on PICs are given on the lower triangle ($n = 27$).

	Seed size	Height	SLA
Seed size		-0.149	-0.335*
Height	0.184		0.114
SLA	-0.112	0.064	

** . Significant at 0.01 level; * . Significant at 0.05 level; a. Significant at 0.1 level.

were categorized as wind-pollinated (Rabinowitz et al., 1981). None of these species appeared to have characteristics of water-, bird-, or animal-pollinated flowers. Seed dispersal mode was coded as wind-dispersed (anemochorous) and nonassisted dispersed (autochorous). Since there is only one species (*Anemone rivularis*) whose seed are dispersed by rodents and cattle (zoochorous and ectozoochorous), data for this species were removed from the consideration of seed dispersal mode. Life form was coded as perennials or annuals. Data for *Halenia elliptica* were removed from consideration of life form because it is the only biennial.

STATISTICAL ANALYSIS

All the values of functional traits were log-transformed to meet the assumptions of statistical tests. The relationship between biomass, height, and other traits was quantified using Pearson correlation and P values, and both cross-species values and phylogenetically independent contrasts (PICs) were analyzed.

We used two models to analyze the relationship between flowering phenology and LHS schemes: comparative generalized estimating equations (GEE) and generalized linear models (GLM). In the comparative GEE model, independence among species was taken into account with a correlation matrix. It has been suggested that phenology is the result of selective pressure (Fenner, 1998) and is constrained by phylogenetic relations. Thus, for non-independence from phylogeny (Felsenstein, 1985), we conducted GEE analysis for predicting flowering peak date and duration. Models without consideration of phylogenetic relations were also conducted with GLM to see if the relationships between flowering phenology and other functional traits were phylogenetically constrained.

In pre-analysis, bivariate correlation analysis indicated that biomass was highly correlated with other traits (Table 1, A), while height, SLA, and seed size were orthogonal with each other (Table 1, B and C). Thus, biomass was excluded from GLM and GEE analyses.

Phylogenetic relations were generated by Phylomatic (Webb and Donoghue, 2005) (Appendix 1) and assumed to be polytomous. All lengths of branches are set to 1 arbitrarily (Ackerly, 2000). The comparative GEE analysis were conducted with the “APE” package (Paradis et al., 2004). All statistical analyses were performed in R 2.9.0 (R Development Core Team, 2009). PICs were generated by Phylocom (Webb et al., 2008).

Results

COMPARATIVE GENERALIZED ESTIMATING EQUATION AND GENERALIZED LINEAR MODELS

Results of GEE and GLM were similar (Table 2). Plant height ($p < 0.001$ in GLM; $p < 0.001$ in GEE) and seed size ($p = 0.041$ in GLM; $p = 0.003$ in GEE) was significant in predicting variation of flowering peak date, but the effect of SLA was not significant. Wind-pollinated species flowered earlier than insect-pollinated ones in both the GLM ($p = 0.014$) and GEE ($p = 0.002$) analyses. The difference of flowering peak dates between annuals and perennials was significant in GLM only. Seed dispersal modes (wind- or unassisted-dispersal) had no effect on flowering peak date ($p = 0.875$ in GLM; $p = 0.264$ in GEE). In the GLM analysis, the only variable significant in predicting variation of flowering duration was seed size ($p = 0.012$). But in the analysis of GEE, height ($p = 0.017$) and seed size ($p < 0.001$) were also significant in predicting variation, while signs of their coefficients were opposite to the model of flowering peak date. SLA was marginally significant ($p = 0.050$). With regard to life form, annuals flowered longer than perennials ($p = 0.029$).

BIVARIATE CORRELATIONS BETWEEN BIOMASS AND OTHER TRAITS

Biomass was highly correlated with flowering peak date ($r = 0.489$, $p < 0.001$ for cross-species; $r = 0.510$, $p < 0.001$ for PICs) (Table 1, A), height ($r = 0.518$, $p < 0.001$ for cross-species; $r = 0.570$, $p < 0.001$ for PICs) (Table 1, A), and SLA ($r = -0.368$, $p < 0.05$ for cross-species; $r = -0.600$, $p < 0.010$ for PICs) (Table 1, A). Height was orthogonal with seed size and SLA ($r = 0.155$, $p > 0.1$ for cross-species; $r = 0.064$, $p > 0.1$ for PICs) (Table 1, B). No correlation was found between biomass and flowering duration ($r = -0.060$, $p > 0.1$ for cross-species; $r = -0.163$, $p > 0.1$ for PICs) (Table 1, A). The relationship between biomass and seed size was marginally significant in the species-based analysis ($r = 0.269$, $p < 0.1$), but when phylogenetic relations were considered, they were significantly correlated ($r = 0.381$, $p < 0.05$).

TABLE 2

Estimates of generalized linear models (GLM) and comparative generalized estimating equations (GEE) analyses on flowering peak date and duration. For GEE model, the degrees of freedom (dfP) were recalculated as part of the analysis.

(A) Model: GLM; Independent variable: Flowering Peak Date; Proportion of Variance: 0.641. $F_{6,41} = 12.230$, $p < 0.001$.

Coefficients	Estimate	Std.Error	<i>t</i>	<i>p</i>
(Intercept)	175.692	42.127	4.171	<0.001 ***
Height	49.067	7.730	6.347	<0.001 ***
SLA	-7.835	18.954	-0.413	0.681
Seed size	-8.679	4.119	-2.107	0.041 *
Pollination: Wind	-14.056	5.448	-2.580	0.014 *
Seed dispersal: Wind	1.040	6.576	0.158	0.875
Life form: Perennial	-15.943	6.449	-2.472	0.018 *

(B) Model: GEE; Independent variable: Flowering Peak Date; Proportion of Variance: 0.613. Estimated Scale Parameter: 275.070, dfP = 12.731

Coefficients:	Estimate	Std.Error	<i>t</i>	<i>p</i>
(Intercept)	145.966	22.319	6.540	0.001 ***
Height	46.146	3.925	11.756	<0.001 ***
SLA	1.042	9.181	0.114	0.914
Seed size	-10.954	2.277	-4.811	0.003 **
Pollination: Wind	-17.727	3.251	-5.453	0.002 **
Seed dispersal: Wind	-5.045	4.072	-1.239	0.264
Life form: Perennial	-2.469	3.450	-0.716	0.502

(C) Model: GLM; Independent variable: Flowering Duration; Proportion of Variance: 0.243. $F_{6,41} = 2.193$, $p = 0.063$

Coefficients:	Estimate	Std.Error	<i>t</i>	<i>p</i>
(Intercept)	6.065	4.698	1.291	0.204
Height	-1.352	0.862	-1.568	0.125
SLA	1.850	2.114	0.875	0.387
Seed size	1.214	0.459	2.642	0.012 *
Pollination: Wind	-0.679	0.608	-1.117	0.271
Seed dispersal: Wind	-0.150	0.733	-0.204	0.839
Life form: Perennial	-0.584	0.719	-0.812	0.422

(D) Model: GEE; Independent variable: Flowering Duration; Proportion of Variance: 0.230. Estimated Scale Parameter: 3.256, dfP = 2.731

Coefficients:	Estimate	Std.Error	<i>t</i>	<i>p</i>
(Intercept)	5.708	2.428	2.351	0.059 a
Height	-1.420	0.427	-3.325	0.017 *
SLA	2.475	0.999	2.478	0.050 a
Seed size	1.692	0.248	6.832	0.001 **
Pollination: Wind	-0.008	0.354	-0.024	0.982
Seed dispersal: Wind	0.575	0.443	1.299	0.244
Life form: Perennial	-1.087	0.375	-2.896	0.029 *

** . Significant at 0.01 level; *. Significant at 0.05 level; a. Significant at 0.1 level.

FLOWERING PEAK DATE AND DURATION

Flowering peak date and duration were negatively correlated ($r = -0.335$, $p = 0.020$ for cross-species; $r = -0.097$, $p = 0.632$ for PICs) (Table 3 and Fig. 1). This negative correlation agreed with results from the GLM and the GEE analyses, that sign of estimation on height and seed size was opposite between flowering peak date and duration. However, this correlation was mainly due to the annuals, although the sample size was small. For perennials, this relationship is not significant (Table 3 and Fig. 1).

Discussion

THE LHS STRATEGY SCHEMES AND FLOWERING PHENOLOGY

Our results show that a species' reproductive (flowering) timing can be described by its height and seed size in LHS schemes. The flowering phenology implies a tradeoff between vegetative and reproductive growth. Larger species flowered later due to the requirement for a longer period for vegetative growth.

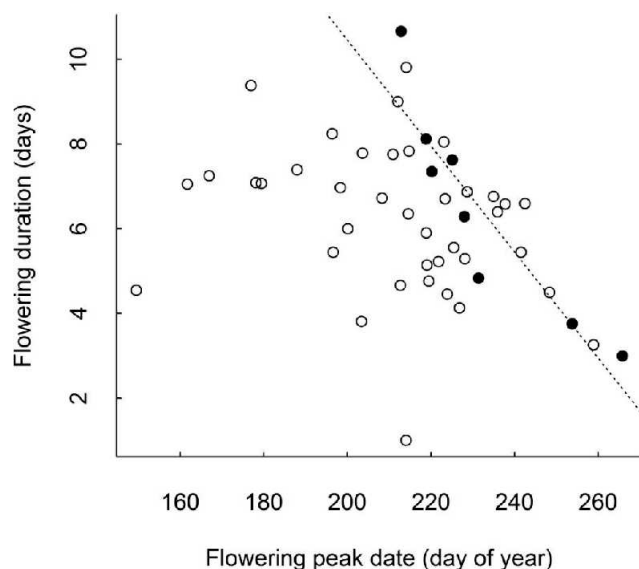


FIGURE 1. Relationship between flowering peak date and duration. Regression line of annuals is shown. $R^2 = 0.109$ for full sample, 0.829 for annuals, and 0.072 for perennials. Correlations are shown in Table 3.

Previous work focused on single species showed a different pattern. That is, larger individuals often flowered earlier (Ollerton and Lack, 1998; Lacey et al., 2003; Bustamante and Burquez, 2008) because they were older and had the advantage of more stored resources than smaller ones. However, this relationship varied among species, years, and populations, indicating that plant size was not the only determinant of optimal flowering time. In many studies on single species (woody, shrub, or Cactaceae herbs), the aboveground parts (stems) are maintained after seed production, and thus few resources and little time are required to produce the plant body. However, for alpine herbs no stems remain alive over winter, therefore, plant size is constrained by the short period of time for vegetative growth before flowering. Similarly, perennials flowered earlier than annuals because perennials “save” time on roots and rhizomes development (Table 2 and Fig. 1), which is probably accomplished by preformation in most cases.

Species with larger seeds tend to flower earlier than those with small seeds, which is coincident with previous inter-species reports (Mazer, 1990; Bolmgren and Cowan, 2008) in both GLM and comparative GEE models. A possible reason for flowering earlier is that larger seeds require not only the resource accumulated from parents’ vegetative growth, but also a longer developmental period before dispersal (Mazer, 1990). Relationships among flowering time, duration, and seed size in our study (Table 2D and Fig. 1) also confirmed that larger-seeded species flower earlier and longer than smaller-seeded ones.

As a basic assumption of the LHS strategy scheme, Westoby (1998) stated that each dimension varies widely between species at any given level of the other two. Thus, a degree of independence is assumed to exist among the three traits. Plant height and seed size are crucial components of a plant species’ ecological strategy. Height represents competitive ability and fecundity (Rösch et al., 1997; Aarssen and Jordan, 2001), and seed mass is related to dispersal and establishment ability (Jakobsson and Eriksson, 2000; Leishman et al., 2000). Although many researchers have found a strong positive correlation between plant height and seed mass (Diaz et al., 2004; Wright et al., 2007), this correlation was not found in our study.

TABLE 3

Bivariate correlation analysis on flowering peak date and duration. Pearson correlations were shown.

	Life Form	<i>N</i>	Cor	<i>p</i>
cross_species	Annuals	8	−0.910	0.002
	Perennials	39	−0.269	0.098
	Full Sample	48	−0.335	0.020
PICs	Annuals	6	−0.919	0.010
	Perennials	23	−0.190	0.932
	Full Sample	27	−0.097	0.632

The lack of correlation between height and seed size may be due to the following reason: taller species generally have larger seeds than shorter species (Leishman et al., 1995; Leishman et al., 2000; Diaz et al., 2004; Moles et al., 2004; Wright et al., 2007), but this correlation was not consistent. Relationship between plant height and seed size was “wedge-shaped” rather than simply linear in log-log plots (Grubb et al., 2005): small species produce small seeds, while large species produce either many small seeds or few large seeds. In the community we studied, large and late-flowering species are mainly wind-dispersed Asteraceae species, which tend to generate small seeds.

Our analysis did not find any relationship between vegetative trait (SLA) and flowering phenology. Although leaf traits are key components in the studies of plants (Reich et al., 1999; Wright et al., 2004), SLA had only a weak relationship with flowering phenology (duration only) (Table 2). It has been reported that SLA was correlated with vegetative growth (Leishman et al., 2007), which can constrain reproduction (Sola and Ehrlén, 2007). However, in our study, SLA was only marginally significant in variance of duration. Species with higher SLA tend to flower longer, and this may indicate a trade-off between vegetative and reproductive growth, since SLA was positively correlated with potential relative growth rate (Westoby, 1998). With the consideration of their important role in resource gain and accumulation in plants (Leishman et al., 2007) and the negative correlation between SLA and leaf life span (Reich et al., 1999), further studies on vegetative phenology may find the answers to the relationships between phenology and leaf traits.

FLOWERING PHENOLOGY AND LIFE FORM, POLLINATION, AND DISPERSAL MODE

In addition to quantitative functional traits, life form, pollination, and dispersal mode have also been reported to be key characters in flowering phenology (Rathcke and Lacey, 1985; Johnson, 1992; Iversen et al., 2009). It is expected that the annuals would flower earlier than perennials because annuals experience more risk than perennials due to the great yearly variation in environment and short unpredictable growing season in alpine ecosystem (Iversen et al., 2009). In our study, however, perennials flower earlier than annuals in GLM analysis (Table 2). This may be due to perennial herbs taking advantage of resources storage from previous years, and thereby reducing the time to reach flowering size each year. After consideration of phylogenetic relations, however, the significance of life form disappeared (Table 1, A and B). This result demonstrates that the difference between annuals and perennials is phylogenetically constrained, because most of the annuals are closely related and from a small number of families.

The effect of pollination mode on flowering peak date was congruent with previous research on temperate trees (Rathcke and

Lacey, 1985; Bolmgren et al., 2003) and herbs (Martinkova et al., 2002), with wind-pollinated species flowering significantly earlier than insect-pollinated ones in our study. In wind-pollinated species, the pattern of flowering peak dates are highly family-specific. Sedges (Cyperaceae) flowered very early in the growing season, when leaves and stems of other species are not yet present to interfere with pollen movement. Grasses (Poaceae) reached their peak flowering in the middle of growing season, along with other insect-pollinated species. Though flowering relatively late, species of Poaceae may overcome pollination interference by their height advantage.

Although previous research suggested that flowering phenology is constrained by seed dispersal (Johnson, 1992; Bolmgren and Cowan, 2008), we found no significant differences in flowering time between wind- and unassisted-dispersed species. Of all the species we studied, only *Anemone rivularis* is ectozoochorous, and it would be advantageous if its seeds ripen and are dispersed at the time of animal dispersers' activity. However, animals of our study site (mainly marmots) are active through the summer and autumn. Species of autochory and anemochory seem to have no advantage to ripen and disperse seeds at any particular time. Although wind-dispersed species will send their seeds for a longer distance before their neighbors grow higher, disadvantages of late-ripening fruits can be compensated for by their own increased height. The lack of a relationship between phenology and dispersal may also be due to the clonality of alpine herbs. In alpine species, vegetative reproduction is more common (Bliss, 1971; Billings, 1974). Because sexual reproduction (flowering and seed explosion) is more risky at high altitudes and latitudes, clonal propagation would be more advantageous in natural selection. As fruiting phenology and clone propagation were not considered in this paper, a further study will be required to investigate this.

Duration of flowering did not differ between pollination modes, although Rabinowitz et al. (1981) reported that for animal-pollinated species flowering duration is longer than that for abiotically pollinated species. Longer flowering duration is advantageous for visitation by pollinators, while wind-pollinated species can benefit from short duration but intense pollen release (Whitehead, 1969). However, this difference of flowering duration was not shown in our study, perhaps because of the short and unpredictable environment of the alpine ecosystem, where abiotically pollinated species can take advantage of bet-hedging flowering to extend flowering duration. Differences in flowering duration were not found in dispersal modes, which may also be due to the short and unpredictable environment. With regard to life form, flowering duration of perennials is shorter than annuals, thus, species in different life forms partition temporal resources in different ways. For perennials, their photosynthate could be saved and used for the next year. Thus they tend to allocate more resources to maternal growth and flower shorter, while for annuals, they need the photosynthate from continuous photosynthesis in order to continue to produce flowers and seeds. Thus annuals tend to flower longer, no matter how risky it could be.

FLOWERING PEAK DATE AND DURATION

A negative correlation between flowering peak date and duration was indicated by the GEE model (Table 2). Species that flower for longer periods are smaller in maternal size and larger-seeded than those that flower for shorter periods. In bivariate analysis, flowering peak date and duration were negatively correlated, but this correlation was highly life form dependent (Table 3). Although there were only eight species and six PICs,

annuals showed strong correlation between flowering peak date and flowering duration (Table 3). We suggest that negative correlations were due to the late flowering time of annuals (Fig. 1; Table 2, A and B). None of the annuals reached their peak of flowering before the middle of the growing season. The late reproduction made the remaining length of the growing season a limiting resource, as all annuals tend to flower as long as they can. Thus, length of growing season was limited, and a negative correlation became logical. Perennials also showed a negative correlation between flowering peak date and duration, but it was not significant. Late-flowering perennials have a narrower range of flowering duration than early and mid-flowering species. These data suggest that late flowering species are constrained by reproductive growth, while early flowering species are constrained by vegetative growth.

TAXONOMIC BIAS

The relationship between flowering phenology and ecological traits may be the adaptive outcome of natural selection, which favors certain combinations of reproductive strategies. Although both phenology and functional traits showed a phylogenetically conserved pattern in our study (data not shown), the similar result of analysis with or without phylogenetic consideration indicated that taxonomic biases do not substantially contribute to the relationship between them. We inferred that evolutionary divergence in flowering phenology is correlated with divergences in ecological traits of alpine herbs. However, lengths of branches in the phylogenetic tree are arbitrary. Thus the result should be interpreted with caution.

Conclusion

Using comparative methods, we found that early flowering species flower longer and produce larger seeds, but their maternal size is smaller than late flowering species. On the other hand, late flowering species, which are larger at maturity, have a relatively short flowering period and produce small seeds. No effect of SLA on flowering phenology was found. Wind-pollinated species flower earlier than unassisted ones. Perennials flowered longer than annuals. Reproductive timing implies a tradeoff between vegetative and reproductive growth. However, to understand more about the annual life history cycle of a species, further studies on vegetative and fruiting phenology are required. SLA and seed dispersal mode had no effect on flowering phenology in this study, but since they are linked with vegetative and fruiting phenology, further studies on them are needed.

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Appendix 1.

Forty-eight species and their phylogenetic relations in calculating phylogenetically independent contrasts (PICs) and conducting comparative generalized estimating equations (GEE).

