

# Community-wide germination strategies in an alpine meadow on the eastern Qinghai-Tibet plateau: phylogenetic and life-history correlates

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**Abstract** In this study, we built up a database of 633 species (48 families, 205 genera) from an alpine meadow on the eastern Qinghai-Tibet plateau. Our objective was to assess the effects of phylogenetic and life-history (life form, perenniality, seed size, dispersal strategy and period) background on the community-wide germination strategies. We found that the seeds of shrubs, perennials, and well-dispersed plants, and the smaller seeds germinated more and comparatively earlier. In one-way ANOVAs, phylogenetic groups explained 12% of the variance in GT (mean germination time for all seeds germinated of

each species); life-history attributes, such as seed size, dispersal strategy, perenniality and life form explained 10%, 7%, 5%, and 1% respectively, and dispersal period had no significant effect on GT. Multifactorial ANOVAs revealed that the three major factors contributing to differences in GT were phylogenetic relatedness, seed size and dispersal strategy (explained 4%, 5% and 4% of the interspecific variation independently, respectively). Thus, seeds germination strategies were significantly correlated with phylogenetic and life-history relatedness. In addition, phylogenetic relatedness had close associations and interactions with seed size and dispersal strategy. Then, we think phylogeny and life-history attributes could not be considered mutual exclusively. Seed germination, like any other trait, is shaped by the natural history of the species and by the evolutionary history of the lineage. And a large percentage of the variance remained unexplained by our model, which suggested important selective factors or parameters may have been left out of this analysis.

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Seed size

## Introduction

The timing of seed germination is a life history trait of early expression in plants' ontogeny, and thus is

expected to affect many other characters of late expression, like fecundity and survivorship (González-Astorga and Núñez-farfán 2000). And because the timing of seed germination of each species in a community is determined by a multitude of biotic and abiotic selective factors acting simultaneously, often in opposing directions (Garwood 1983), it is remarkable that many seed plants exhibit extensive variation in the time of seed germination after fruit ripening.

It has been argued theoretically that the presence of seed dormancy would be associated with life history attributes such as reduced seed size, non-directed seed dispersal, and short perennality (Venable and Lawlor 1980; Venable and Brown 1988; Rees 1993, 1994). In addition, according to recent studies, it is reasonable to expect that, within a family or a genus, reproductive characters, such as seed size and germination, could be affected by phylogenetic constraints and developmental allometries that limit segregation (Kochmer and Handel 1986; Feinsinger 1987; Herrera 1992; Jordano 1995; Smith-Ramírez et al. 1998; Figueroa and Armesto 2001; Zhang et al. 2004).

Seed germination time is a trait of primary significance for the reproductive success of plants and has been the focus of demographic, population, community, and evolutionary studies (Washitani and Nishiyama 1992; Nilsson et al. 1994; Olff et al. 1994; Cheplick 1996). Community-wide studies of the seed germination would be useful to assess the effects of evolutionary and ecological factors. However, there have been few studies on the timing of germination in a whole community. Therefore, we expect to advance our understanding on evolutionary and ecological factors regulating seed germination time in plant communities. In this study, we built up a database of 633 species (involving 10 classes, 48 families, 205 genera) collected from the alpine meadow on the eastern Qinghai-Tibetan plateau. The objectives of this investigation were (1) to characterize seeds germination strategies, with a primary focus on delayed germination, for an assemblage of 633 species in the alpine meadow on the eastern Qinghai-Tibet plateau and (2) to partition interspecific variation in seed germination time among phylogenetic and life-history constraints.

## Materials and Methods

### Study site

The region of this study is located on the eastern Qinghai-Tibet plateau (101°–103° E, 34°–35°70' N). The altitude ranges from 2,800 m to 4,200 m, and the climate is cold Humid-Alpine with mean annual rainfall of 450–780 mm. Mean annual temperature is 1.2°C with −10.7°C on January and 11.7°C in July, and there are on average 270 frost days a year. The grassland type mainly belongs to alpine meadow (59.32%), which is dominated by many monocotyledons, for example, Gramineae, Cyperaceae, and various dicotyledons, such as Ranunculaceae, Polygonaceae, Saxifragaceae, Compositae, Scrophulariaceae, Gentianaceae, and Leguminosae.

### The database

In this study, we built up of a database of 633 species (involving 10 classes, 48 families, 205 genera) collected randomly from the alpine meadow on the eastern Qinghai-Tibet plateau. The sample represented around 65% of the species, 50% of the genera, and 50% of the families reported from the area. Seeds were gathered from July to October in 2004, and species included not only native but also introduced ones. Seeds were collected at the start of natural dispersal. Enveloped seeds were spread on tables at room temperatures (approximately 15°C) until they were dry. Seed weight was defined as the weight of the embryo and endosperm, plus the seed coat. Structures having the function of contributing to dispersal were not included as part of the seed weight. Seeds were pooled per species, and we randomly selected three subsamples of 100 seeds from the pooled samples. The average weight of the three subsamples was used as seed weight variable. The germination experiment was started on the middle 10 days of March (starting season of germination), in 2005. Seeds were placed in covered Petri dishes (9 cm diameter) on a double layer of moistened filter paper, and then placed in temperature chambers (made in Canada) at a diurnal fluctuation of 15°C (20°C day, 12 h; 5°C night, 12 h) with 24-h

darkness (not considering the effect of light) and a relative humidity of about 70%. The temperature of the germination trial resembled natural conditions for April and May: it approximated to the daily maximum and minimum temperature in 5 cm-deep soil. All of the species had three replicates of 50 seeds. Every day, the percentage of seeds germinated was recorded, newly emerged seedlings were removed from the Petri dishes and seeds were regularly watered with distilled water. A seed was considered germinated when the radicle was visible. In view of germination rate, seedling establishment and shorter growth season in the flora, the experiment of seed germination lasted 60 days. In addition, the remaining nongerminated seeds were tested for viability by staining with tetrazolium chloride (Moore 1972).

#### Statistical analyses and comparative method

The following analyses were conceived for the data set generated by laboratory assays. Mean germination time (GT) was estimated as follows:  $\sum(G_i \times i) / \sum(G_i)$  (Figueroa and Armesto 2001), where  $i$  was the days of seed germination counted since the day of sowing (day 0) and  $G_i$  was the number of seeds germinated on day  $i$ . Seeds that did not germinate at the end of the assay were not considered in this calculation.

For multifactorial ANOVAs of mean germination time of species, we grouped these species in various major categories, defined as follows:

(1) Phylogenetic groups. Several phylogenetic classifications of flowering plants based on molecular and morphological attributes are available. We used the Angiosperm Phylogeny Group II (2003) to assign the affiliation of each species to higher levels because it is the most complete and updated molecular phylogeny available. We grouped species by higher taxonomic levels because of the low number of species within many genera or families in the flora on the eastern Qinghai-Tibet plateau. Then the species in the studied flora were grouped into the following ten groups: 1. Monocots (two families, three genera and 16 species); 2. Commelinids (three families, 25 genera and 73 species); 3. Eudicots (3 families, 17

genera and 63 species); 4. Core Eudicots (6 families, 20 genera and 82 species); 5. Rosids (2 families, 6 genera and 9 species); 6. Eurosids I (10 families, 31 genera and 99 species); 7. Eurosids II (3 families, 14 genera and 28 species); 8. Asterids (3 families, 5 genera and 14 species); 9. Euasterids I (10 families, 36 genera and 119 species); 10. Euasterids II (6 families, 48 genera and 130 species).

(2) Life history categories (The life-history information of species for this study was drawn primarily from the Flora of China and based on our field observations yearly): (a) life form—species in the flora were grouped into the following three major growth-form categories: woody plants (48 species, the most were shrubs), graminoid plants (73 species, including all species of Poaceae, Juncaceae and Cyperaceae), and forbs (512 species, including all non-woody species that are not graminoid). (b) perenniality—species in the flora were grouped into two classes: annuals (168 species, including a few biennial) and perennials (465 species). (c) seed size—seeds were sorted by weight into 16 size classes:  $\leq 0.05$  mg (33 species),  $\leq 0.1$  mg (55 species),  $\leq 0.5$  mg (165 species),  $\leq 1$  mg (109 species),  $\leq 1.5$  mg (66 species),  $\leq 2$  mg (44 species),  $\leq 2.5$  mg (36 species),  $\leq 3$  mg (16 species),  $\leq 3.5$  mg (12 species),  $\leq 4$  mg (17 species),  $\leq 4.5$  mg (14 species),  $\leq 5$  mg (7 species),  $\leq 6$  mg (8 species),  $\leq 10$  mg (14 species),  $\leq 20$  mg (26 species),  $>20$  mg (11 species). (d) dispersal related attributes—species in the flora were classified into the following five large groups according to their seed morphological features (Leishman et al. 1995): unassisted (441 species, no obvious morphological structure), ant-adapted (34 species, with an elaiosome), adhesion-adapted (23 species, with hooks, spines or bards), wind-adapted (108 species, with wings, hairs or a pappus), and vertebrate-adapted (27 species, with an aril or flesh). Species were also classified into the following two groups according to ripe period of their fruits in the alpine meadow: species that dispersed their fruits during the summer (July and August), and species that dispersed their fruits during the autumn (September and October).

In addition, GT was log-transformed before statistical analysis to ensure homogeneity of variance.

We initially used  $\chi^2$ -test to determine the difference in mean percent germination, days to first germination and germination time among the different levels of each factor. We conducted a series of ANOVAs to assess the significance of these factors in determining seed germination time. All ANOVAs were conducted using GLM procedure of SPSS. The type III sum of squares was used to establish the significance level of each effect because the data were unbalanced. This type of analysis calculates the sum of squares for each variable when the variable is added last into the ANOVA model. First, one-way ANOVAs estimated the main effect of each factor on the variance of GT. In addition, two-way ANOVAs were performed on the same data to assess the interactions among each pair of factors. Finally, multifactorial ANOVAs provided the following two types of information (Figueroa and Armesto 2001): (1) variation accounted for by all factors combined and by each class variable independent of the others. A way to measure the effect of each class variable on seed germination time was to conduct a series of ANOVA which included all variables but one (incomplete model). When each of these ANOVA was compared to the ANOVA including all variables (complete model), the difference between the proportion of the total sum of squares (ss) explained by the complete model (its  $R^2$ ) and the  $R^2$  of the incomplete model represented the proportion of the total ss explained by the deleted class variable. This method provided a rough estimate of the amount of variance in seed germination time explained by each of the class variables independent of the others, and provided information that was not available from the one-way or two-way ANOVAs. (2) Associations or correlations between class variables. This method provided another means of detecting associations between class variables. If in the complete ANOVA, a given class variable had a lower  $R^2$  value than in the incomplete ANOVAs from which a different variable had been deleted, the increase in the  $R^2$  value of the first variable would be due to an association (or correlation) or strong interaction with the second variable. In other words, when two variables were strongly associated, the ss of each variable would account for a higher proportion of the total ss of the model when the other was not included in the model. All analyses were performed with the SPSS 12.0 procedure.

## Results

### Seed dispersal in time

The germination data for the 633 species used in the analyses were listed in the appendix (see Supplementary Material), including 66 species that did not germinate. There was a great difference in mean GT among species. GT varied within and among species. For the entire flora, the mean percent germination ranged from 0 (for example, *Gentiana szechenyii*) to 100% (for example, *Elymus excelsus*); the number of days of first germination varied between 1 day (for example, *Kochia scoparia*) and 49 days (for example, *Carex crebra*) after sowing; mean GT varied between 2 days (for example, *Kochia scoparia*) and 52 days (for example, *Carex crebra*).

Tetrazolium tests were carried out and revealed that most nongerminating seeds were still alive at the end of the experiment. That is either the seeds required more time to germinate (germination was poorly synchronized), or they kept up dormancy. We considered that the variation in the percentage among the studied species could be a consequence of differences in the degree of dormancy.

### Phylogenetic correlates

Among the 633 species in the experiment, most species not germinated belonged to Eurosids I (19 species, about 29%), Eudicots (16 species, about 24%), Euasterids I (11 species, about 17%), and Euasterids II (8 species, about 12%).

Percent germination of seeds differed among phylogenetic groups ( $\chi^2 = 6833.29$ , d.f. = 9,  $P < 0.001$ ), species in group 2 (Commelinids) presented the highest germination percentage (26%), and species in group 5 (Rosids) presented the lowest germination percentage (18%) (Fig. 1); the days of first germination was significantly different among phylogenetic groups ( $\chi^2 = 2646.91$ , d.f. = 9,  $P < 0.001$ ), species in group 3 (Eudicots) presented the slowest onset to germination (15 days) and species in group 7 (Eurosids II) presented the earliest onset to germination (6 days) (Fig. 1); mean GT had a significant difference among phylogenetic groups ( $\chi^2 = 3599.11$ , d.f. = 9,  $P < 0.001$ ), species in group 5 (Rosids) presented the longest mean GT (22 days),

species in group 7 (Eurosids II) presented the earliest mean GT (9 days) (Fig. 1); and Tukey's b post-hoc tests indicated significant differences between these two groups ( $P < 0.01$ ).

Phylogenetic groups had a significant effect on interspecific variation in mean GT (Table 1). Phylogenetic groups explained 12% of the variance of GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau (Table 1). However, the percentage of variance in GT explained by phylogeny independently decreased to 4% when removing the effects of the other factors (see methods) (Table 2). A decrease in the proportion of variance explained by phylogeny (with respect to the complete model) was observed when seed size and dispersal strategy were removed from the multifactorial analysis, which suggested associations between phylogenetic relatedness and these two factors for species in the alpine meadow on the eastern Qinghai-Tibet plateau (Table 2).

Two-way ANOVAs yielded significant interaction terms ( $P < 0.01$ ) for phylogenetic relatedness when analyzed in combination with seed size and dispersal strategy (Table 3).

## Life-history correlates

### *Life form*

Among the 633 species in the experiment, 512 species (about 81%) were forbs, 73 species (about 11%) were graminoid plants and 48 species (about 8%) were woody plants. However, species not germinated included 32 forbs (about 6% the total forbs), five graminoid plants (about 7% the total graminoid plants), and 29 woody plants (about 60% the total woody plants).

Percent germination of seeds differed among life forms ( $\chi^2 = 21333.08$ , d.f. = 2,  $P < 0.001$ ), species in the woody plants (most of them were shrubs) presented the highest germination percentage (66%), and species in the forbs presented the lowest germination percentage (37%) (Fig. 1); the days to first germination was significant different among life forms ( $\chi^2 = 7167.45$ , d.f. = 2,  $P < 0.001$ ), species in the woody plants presented the earliest germination time (8 days), and species in the forbs presented the latest germination time (11 days) (Fig. 1); mean GT had a significant difference among life forms

( $\chi^2 = 11068.12$ , d.f. = 2,  $P < 0.001$ ), species in the woody and graminoid plants presented the shorter mean GT (13 days), and species in the forbs presented the longer mean GT (16 days) (Fig. 1).

One-way ANOVAs indicated that life form had a significant effect on the interspecific variation in mean GT (Table 1). But life form could explain only 1% of the variance of GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau when we considered only one categorical variable. The effects of life form assessed by a multifactorial ANOVA accounted for an identical percentage of the variance in GT (Table 2).

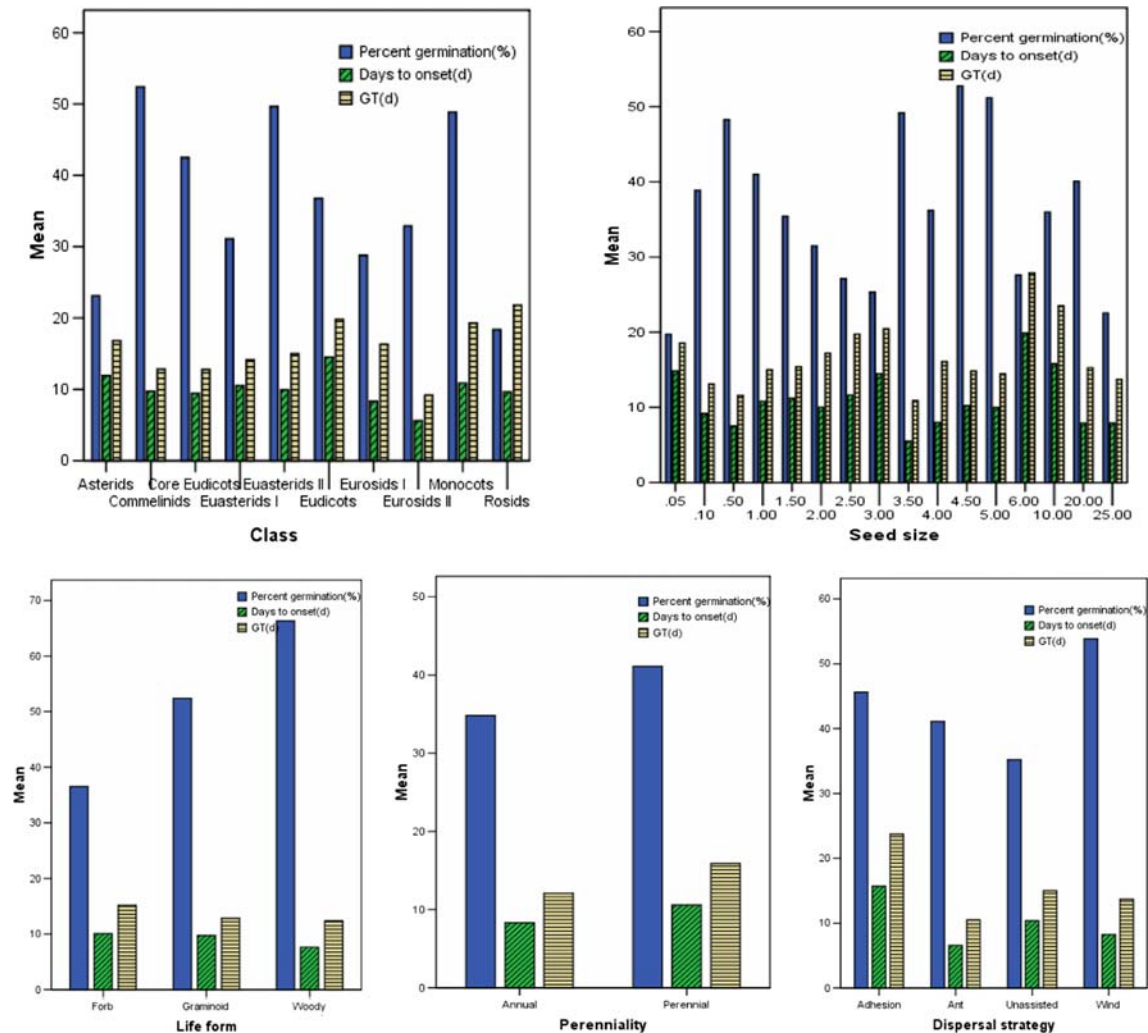
Two-way ANOVAs yielded significant interaction terms ( $P < 0.01$ ) for life form when analyzed in combination with seed size (Table 3).

### *Seed size*

Among the 66 species not germinated in the experiment, species of smaller seed size (<10 mg) was 44 (about 67%). The mean seed size of species not germinated was  $8.2240 \pm 1.2696$  mg, and the mean seed size of species germinated was  $0.1607 \pm 0.1557$  mg. And when mean seed size was  $\leq 3$  mg (excluding the smallest size class), percent germination decreased with increasing seed size ( $r = -0.893$ ,  $P = 0.007$ ), but days to onset ( $r = 0.857$ ,  $P = 0.014$ ) and mean GT ( $r = 0.964$ ,  $P < 0.001$ ) increased. However, there was no significant correlations between seed size and germination indexes when mean seed size was  $\geq 3.5$  mg, yet a negative tendency between seed size and percent germination could be observed (Fig. 1).

Percent germination of seeds differed among size classes ( $\chi^2 = 20928.72$ , d.f. = 15,  $P < 0.001$ ), species in the smallest and the biggest size classes presented the lowest germination percentage (20% and 22%, respectively), and species in size classes of 4–4.5 mg presented the highest germination percentage (52%) (Fig. 1); the days to first germination was significant different among size classes ( $\chi^2 = 5723.48$ , d.f. = 15,  $P < 0.001$ ), species in size classes of 3–3.5 mg presented the earliest germination time (6 days), and species in size classes of 5–6 mg presented the latest germination time (20 days) (Fig. 1); Mean GT had a significant difference among size classes ( $\chi^2 = 8065.52$ , d.f. = 15,  $P < 0.001$ ), species in size





**Fig. 1** Mean percent germination, days to onset and mean GT of seeds in different taxonomic groups. Numbers of species is 567 (excluding 66 species not germinated)

**Table 1** One-way ANOVAs that partition the variance of germination time in the experiment

Source of variation	d.f.	F	P	R <sup>2</sup>
Phylogenetic group	9	8.38	**	0.12
Seed size	15	4.26	**	0.10
Dispersal strategy	3	14.87	**	0.07
Perenniality	1	30.56	**	0.05
Life form	2	3.01	*	0.01
Dispersal period	1	0.001	ns	0.00

\*= $P < 0.05$ , \*\*= $P < 0.01$ , ns =  $P > 0.05$ . Numbers of species is 567

classes of 3–3.5 mg presented the shortest GT (11 days), and species in size classes of 5–6 mg presented the longest GT (28 days) (Fig. 1).

One-way ANOVAs indicated that seed size had a significant effect on the interspecific variation in mean GT (Table 1). Seed size could explain 10% of the variance of GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau when we considered only one categorical variable. However, seed size explained only 5% of interspecific variance in mean GT when the effects of other factors were removed. An increase in the proportion of variance explained by seed size was observed when phylogeny

and perennality were removed from the multifactorial analysis, which suggested associations between seed size and these two factors (Table 2).

Two-way ANOVAs yielded significant interaction terms ( $P < 0.01$ ) for seed size when analyzed in combination with phylogeny, life form, and perennality (Table 3).

### Perennality

Species not germinated included 14 annuals (about 8% the total annuals) and 52 perennials (about 11% the total perennials).

Percent germination of seeds differed among perennality groups ( $\chi^2 = 5568.75$ , d.f. = 1,

**Table 2** Multifactorial ANOVAs for the independent effects of each factor and their associations

Source of variation	d.f.	<i>F</i>	<i>P</i>	<i>R</i> <sup>2</sup>	d.f.	<i>F</i>	<i>P</i>	<i>R</i> <sup>2</sup>
Complete model					Phylogenetic group removed			
Phylogenetic group	9	9.57	**	0.14				
Life form	2	7.37	**	0.01	2	3.67	*	0.01
Perennality	1	20.13	**	0.04	1	44.04	**	0.08
Seed size	15	2.76	**	0.07	15	3.91	**	0.10
Dispersal strategy	3	10.34	**	0.06	3	13.41	**	0.07
Dispersal period	1	0.43	ns	0.001	1	0.29	ns	0.00
Model	31	6.22	**	0.26	22	6.84	**	0.22
Total	567							
Life form removed					Perennality removed			
Phylogenetic group	9	9.43	**	0.14	9	9.33	**	0.14
Life form					2	7.19	**	0.01
Perennality	1	17.00	**	0.03				
Seed size	15	2.67	**	0.07	15	3.08	**	0.08
Dispersal strategy	3	10.84	**	0.06	3	10.01	**	0.05
Dispersal period	1	0.42	ns	0.001	1	0.06	ns	0.000
Model	29	6.03	**	0.25	30	5.77	**	0.24
Seed size removed					Dispersal strategy removed			
Phylogenetic group	9	9.22	**	0.13	9	9.10	**	0.13
Life form	2	7.10	**	0.01	2	7.01	**	0.01
Perennality	1	19.40	**	0.03	1	19.13	**	0.03
Seed size					15	2.62	**	0.07
Dispersal strategy	3	11.82	**	0.06				
Dispersal period	1	0.14	ns	.000	1	0.08	ns	0.000
Model	16	9.67	**	0.21	28	5.46	**	0.22
Dispersal period removed								
Phylogenetic group	9	9.58	**	0.14				
Life form	2	7.38	**	0.01				
Perennality	1	20.16	**	0.04				
Seed size	15	2.76	**	0.07				
Dispersal strategy	3	10.35	**	0.06				
Dispersal period								
Model	30	6.42	**	0.26				

Dependent variable is germination time in laboratory assays. For each main factor,  $R^2$  is the proportion of the sum of squares attributed to the main effect. To calculate the proportion of the variance explained by only one of the main factors, we subtracted the  $R^2$  of the incomplete ANOVA with that factor removed, from the  $R^2$  of the complete model. \*= $P < 0.05$ , \*\*= $P < 0.01$ , ns =  $P > 0.05$ . Numbers of species is 567

$P < 0.001$ ); the days to first germination was significant different among perennality groups ( $\chi^2 = 1579.42$ , d.f. = 1,  $P < 0.001$ ); mean GT had a significant difference among perennality groups ( $\chi^2 = 2454.99$ , d.f. = 1,  $P < 0.001$ ). Perennials had a higher percent germination (41%), later days to onset (11 days) and longer mean GT (16 days) (Fig. 1).

One-way ANOVAs indicated that perennality had a significant effect on the interspecific variation in mean GT (Table 1). Perennality could explain 5% of the variance of GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau when we considered only one categorical variable. However, perennality explained only 2% of interspecific variance in mean GT when the effects of other factors were removed. An increase in the proportion of variance explained by perennality was observed when phylogeny was removed from the multifactorial analysis, which suggested associations between perennality and phylogeny (Table 2).

Two-way ANOVAs yielded significant interaction terms ( $P < 0.01$ ) for perennality when analyzed in combination with seed size and dispersal strategy (Table 3).

### Dispersal-related correlates

Among the 66 species not germinated in the experiment, the dispersal strategy of 34 species was unassisted (about 5% the total unassisted species), that of 27 was vertebrate-adapted (about 100% the total vertebrate-adapted species), that of three was wind-adapted (3% the total wind-adapted and that of

two was adhesion-adapted (9% the total adhesion-adapted species).

Percent germination of seeds differed among dispersal strategies ( $\chi^2 = 20503.47$ , d.f. = 3,  $P < 0.001$ ), wind-adapted species presented the highest germination percentage (53%), and unassisted species presented the lower germination percentage (35%) (Fig. 1); the days to first germination was significant different among dispersal strategies ( $\chi^2 = 7620.30$ , d.f. = 3,  $P < 0.001$ ), ant-adapted species presented the earliest days to onset (7 days), and adhesion-adapted species presented the slower days to first germination (16 days) (Fig. 1); mean GT had a significant difference among dispersal strategies ( $\chi^2 = 10483.80$ , d.f. = 3,  $P < 0.001$ ), ant-adapted species presented the shortest GT (11 days), and adhesion-adapted species presented the longer GT (24 days) (Fig. 1). And because all the seeds of vertebrate-adapted species did not germinate, they presented the lowest percent germination, the slowest days to onset and the longest mean GT (not including in Fig. 1).

One-way ANOVAs indicated that dispersal period had no significant effect and dispersal strategy had a significant effect on the interspecific variation in mean GT (Table 1). Dispersal strategy could explain 7% of the variance of GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau when we considered only one categorical variable. However, dispersal strategy explained only 4% of interspecific variance in mean GT when the effects of other factors were removed. An increase in the proportion of variance explained by dispersal strategy was observed when phylogeny was removed from the multifactorial analysis, which suggested

**Table 3** Two-way ANOVAs that partition the variance of germination time in laboratory assays (except nonsignificant interaction)

Source of variation(A/B)	Effect of A				Effect of B				Effect of A × B			
	d.f.	F	P	R <sup>2</sup>	d.f.	F	P	R <sup>2</sup>	d.f.	F	P	R <sup>2</sup>
Phylogenetic group/seed size	9	9.95	**	0.16	15	3.19	**	0.09	135	1.95	**	0.24
Phylogenetic group/dispersal strategy	9	9.16	**	0.13	3	12.38	**	0.06	27	2.97	**	0.05
Life form/seed size	2	3.50	*	0.01	15	4.71	**	0.12	30	2.82	**	0.09
Perennality/seed size	1	33.60	**	0.06	15	3.67	**	0.09	15	2.25	**	0.05
Perennality/dispersal strategy	1	33.38	**	0.06	3	14.38	**	0.07	3	4.94	**	0.03

Variance attributed to phylogenetic group, life history and dispersal-related traits of the plants (except dispersal period because of its nonsignificant main effect). For each main factor, R<sup>2</sup> is the proportion of the total sum squares contributed by the sum of squares of the main effect. \*= $P < 0.05$ , \*\*= $P < 0.01$ , ns =  $P > 0.05$ . Numbers of species is 567



associations between dispersal strategy and phylogeny (Table 2).

Two-way ANOVAs yielded significant interaction terms ( $P < 0.001$ ) for dispersal strategy when analyzed in combination with phylogeny and perenniality (Table 3).

## Discussion

### Phylogenetic correlates

A growing number of studies have documented that phylogenetic correlates of plant species within a community could account for a significant proportion of interspecific variation in reproductive attributes. For example, phylogenetic closeness explains much interspecific variation in breeding systems, seed mass, flowering phenologies, fleshy-fruit attributes, and in general, reproductive syndromes in Mediterranean, tropical, and temperate floras (see Figueroa and Armesto 2001). In this study, phylogenetic groups significantly influenced GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau and together with seed size and dispersal strategy, accounted for the greatest fraction of the variance explained by the complete model.

The biological and evolutionary interpretation of this close association between germination time and phylogenetic affinity in the alpine meadow on the eastern Qinghai-Tibet plateau is complex. One possible interpretation is that phylogeny imposes limits to variability in reproductive attributes within a clade, because of similar developmental and design constraints in related species (Mckittrick 1993; Miles and Dunham 1993; Ackerly and Donoghue 1995; Figueroa and Armesto 2001). For example, species in the genus *Poa* exhibited similar patterns of dispersal in time, as well as species in *Adenophora*, *Saussurea* and so on. The result in this study was in accord with some other studies. For example, Seven species of *Alstroemeria* that grew in different mountains and lowland habitats in South America exhibited similar germination strategies, regardless of their habitats of origin (see Figueroa and Armesto 2001); Baskin et al. (1993) reported analogous results when dormancy types were compared among species in the family Asteraceae; species belonging to the Arcto-Tertiary flora and presently inhabiting deciduous North

American forest, had germination strategies that matched those of congeneric species presently occurring in East Asia (Baskin and Baskin 1998). These examples lend support to the hypothesis that germination strategies can be stable evolutionary attributes, thus constrain interspecific variation in germination behavior.

### Life-history correlates

#### *Life form*

No clear relation between life form and seed germination strategies has been reported in the literature. For example, Garwood (1983) did not find a consistent relation between life form and germination parameters in a neotropical forest flora; in contrast, Grime et al. (1981) found that seeds of annuals and perennial herbs germinated faster than seeds of shrubs and trees in a temperate flora; and Figueroa and Armesto (2001) found in Chiloé that seed germination of trees was significantly delayed relative to shrubs, vines and herbs. In this study, we found life form had a significant effect on mean GT, but could explain only 1% of the variance of mean GT for species in the alpine meadow on the eastern Qinghai-Tibet plateau. And to our interesting, the seeds of woody plants germinated more and earlier than those of graminoid plants and forbs (Fig. 1). However, most of the germinated woody plants were shrubs. In addition, in the 60-day experiment, 7% graminoid plants, 6% forbs and 60% woody plants did not germinate and not be considered in the analysis. If we took it into account, the woody plants might be the most delayed germination group. We think faster seed germination of shrubs might be the results of their slower growth velocity relative to the herbs, because faster germination would help them obtain competition advantages in time and space.

#### *Seed size*

Seed size may affect germination and seedling characteristics and, then, population recruitment. Hence, large numbers of researches focus on the relationship of seed size and seed germinability. Seed size has been found to affect germination rate and

percent germination (Mckersie et al. 1981; Piper 1986; Wulff 1986; Winn 1988; Counts and Lee 1991; Vera 1997; Eriksson 1999). But no accordant relationship between seed size and germination strategies has been reported in the literature, and very few studies have been addressed to test seed size and germination within a whole plant community. Seed size has also been studied in connection with many other aspects of plant biology (Baker 1972; Foster 1986; Mazer 1989; Westoby et al. 1992; Bond et al. 1999; Leishman et al. 2000; Jurado and Flores 2005). Many authors have predicted that species with lighter seeds will be more likely to have some form of dormancy (Thompson and Grime 1979; Grime et al. 1981; Venable and Brown 1988; Rees 1993), and empirical data support this evidence in most (Grime et al. 1981; Carter and Ungar 2003), but not all environments (Jurado and Westoby 1992; Leishman and Westoby 1994). In this study, we found that percent germination along seed size classes presented bimodal distribution (Fig. 1). Except the smallest seed size classes, seed germinability presented significant negative correlation with seed size ( $\leq 3$  mg), and approximate negative correlation with seed size ( $\geq 3.5$  mg). One possible interpretation was faster germination of smaller seeds could obtain competitive advantage in time and space (Hendrix 1984), and larger seeds germinated slower due to their usual opacity of thick or hard seed coats (Pearson et al. 2002).

### Perenniality

In this study, perenniality had significant effect on delayed GT and could explain 2% variation of GT independently. Seeds of perennials germinated more, but later. Our result was in accord with from Rees (1993), who found that seed dormancy was negatively associated with plant longevity (Rees's data base also did not contain trees). There was a possible explanation for that. Plants producing seeds more than once in their lifetime (i.e., most perennial plants) will be less dependent on one particular set of seeds than monocarpic plants that produce seeds only once (i.e., annuals, biennials and some perennials such as bamboos and agaves). Annual and biennial species would thus be more dependent on their seeds to persist in a given environment, and therefore might

have seeds with more selective germination requirements (Jurado and Westoby 1992; Rees 1996; Jurado and Flores 2005). Hence short-lived plants might have a tendency to include more dormancy species than long-lived ones.

On the other hand, annual species have developed two extreme seed dispersal strategies: 'escape' or 'protection' and 'opportunistic' or 'cautious' strategies (Guterman 2000). In suitable environment, an annual plant genotype with no seed dormancy would maximize its arithmetic average population growth rate but become extinct the first year reproduction failed. A genotype that never germinated would also become extinct as a result of seed mortality (Rees 1994). Hence, in a variable environment, seed dormancy of annuals spreads the risk of germination over time, which becomes more advantageous especially when the frequency of bad years was higher.

### Dispersal-related effects

Seed dispersal is alleged to provide plants with many advantages, including (1) escape from specialist predators and pathogens attracted or supported by the parent; (2) spreading the risks encountered by seeds in a spatially variable environment; (3) prevention or reduction of competition between parent and offspring, and (4) location of 'safe sites' where seeds can successfully germinate and establish, such safe sites are often, but not always, vegetation gaps or larger areas of disturbance (Fenner and Thompson 2005).

In this study, seeds dispersal period had no significant effect on the interspecific variation of GT, but dispersal strategy had significant effect on variation of GT and could explain independently 4% of the total variance in GT in the alpine meadow (Table 2). There was a trend that seeds of well-dispersed species could germinate more (Fig. 1). Wind-adapted species had earlier germination time and shorter mean GT, and subsequent adhesion-adapted, ant-adapted and unassisted species, all the seeds of vertebrate-adapted species had no germination in the 60-day experiments (not included in Fig. 1). One impossible interpretation was special dispersal could be regarded as a mechanism to avoid or minimize sibling competition. In most seeds germinated, restricted dispersal (e.g. unassisted species)

can lead to competition among the siblings produced by a fecund maternal plant (Cheplick 1992, 1993, 1996). It has been suggested that in some species seed dormancy may have evolved to reduce the risk of sibling competition by spreading germination out in time (Venable and Brown 1988; Nilsson et al. 1994). For example, seeds of poor-dispersed species were most likely to experience sibling competition (i.e., those in the lower tiller nodes) and showed low percent germination and slow germination rate (Nilsson et al. 1994); and Venable and Lawlor (1980) noted that there was a strong tendency for the poorly dispersed morph to have delayed germination and the well-dispersed morphs to have rapid germination.

In addition, ant-adapted species had earliest germination time and shortest germination time, which may be the result of escaping collection by ants (ants do not collect seedlings). That ensured higher survival of the seedlings by fast germination (Guterman 2000). Furthermore, seeds dispersed by frugivores must survive the passage through the digestive tract of the animal, they should delay germination longer than their abiotically dispersed relatives.

## Conclusion

In summary, germination strategies in the alpine meadow on the eastern Qinghai-Tibet plateau were affected by phylogenetic and life-history factors. The phylogenetic groups could explain the biggest proportion in variance of mean GT, which could argue the strong phylogenetic constraints. But the seeds of shrubs, perennials and well-dispersed species, and the smaller seeds presented more and earlier germination, which could be regarded as an adaptation to different lifestyles formed in the long-term ecological time. In addition, phylogenetic relatedness had close associations and interactions with seed size and dispersal strategy. Thus, we think phylogeny and life-history attributes could not be considered mutually exclusively. Seed germination, like any other trait, is shaped by the natural history of the species and by the evolutionary history of the lineage.

In this study, phylogenetic relatedness, seed size, and dispersal strategy were the three factors making the greatest contribution to the multifactorial model (13% of the variance in GT). However, the complete

statistical model explained only 26% of the variation in GT among the species in the alpine meadow. The factors perenniality and life form combined explained 3% of the variation in GT, and dispersal period had no effect on GT. Thus, a large percentage of the variance remained unexplained by our model suggests that at the geographic scale investigated in this study, important selective factors or parameters may have been left out of this analysis. For example, a greater portion of the variance in GT could be accounted for by family membership ( $R^2 = 0.40$ ,  $F = 9.16$ ,  $P < 0.001$ ) in the dataset. However, the veracity accounted for by family was in doubt because many families were collected only one or two species. And we suggested that other possible correlates may exist between dispersal in time and additional phylogenetic related morphological and physiological seed attributes that were not evaluated in this study (e.g. endosperm mass).

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