

Light-dependent associations of germination timing with subsequent life-history traits and maternal habitats for 476 angiosperm species of the eastern Tibetan Plateau grasslands

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Abstract

Germination timing is a key transition of life history. It not only links subsequent life-history traits, such as plant height and flowering time, but also provides a link to the previous generation through the influence of the maternal environment. Environmental factors may mediate these key links, and consequences of this process may influence species regeneration and dispersal. However, little is known about how environmental factors mediate these key links. Here, germination timing under high (natural light) and low light treatments was estimated for 476 angiosperm species of the eastern Tibetan Plateau grasslands. Furthermore, we used standard (std) and phylogenetic (phy) comparative methods to test if germination timing was associated with plant height, flowering time and maternal habitats under both light treatments. Germination timing was positively correlated with plant height only in low light in std-methods. Germination timing was associated with onset of flowering in both light treatments in std-methods, but only in low light when using phy-methods. Germination timing was positively correlated with elevation only in low light when using both comparative methods. Germination timing was correlated with water in maternal habitat only in high light when using both comparative methods. Germination timing was associated with light in maternal habitat in both light treatments in std-methods, but only in high light when using phy-methods. In summary, light-dependent associations of germination timing with subsequent life-history traits and maternal habitats may influence the probability of plant species life-cycle completion and influence distribution and dispersal of plant species in natural plant communities.

Keywords: community, elevation, flowering time, life cycle, phylogenetic comparative methods, plant height

Introduction

Germination timing is a key transition of life history (Chiang *et al.*, 2009). It not only links subsequent life-history traits (Donohue *et al.*, 2010), such as plant height and flowering time, but it also provides a link to the previous generation through the influence of the maternal environment (Donohue, 2009). Environmental factors may mediate these key links, and consequences of this process may influence species regeneration and dispersal. However, little is known about how environmental factors mediate these key links.

Light availability is critical to germination behaviour and subsequent seedling survival, as well as fitness of subsequent life stages (Fenner, 2000; Fenner and Thompson, 2005). The germination response to light availability can also have scaled-up effects on community assembly, diversity and dynamics (Connell and Slatyer, 1977; Nevo, 1997; Fenner and Thompson, 2005; Hautier *et al.*, 2009). In fact, many of the processes of succession and community assembly that are driven by light availability occur at the germination and seedling stage (Van Couwenberghe *et al.*, 2013). Thus, it is important to study how light availability mediates the link between germination timing and subsequent life-history traits and maternal habitats.

Plant height is central to plant ecological strategies, because it is a major determinant of a species' ability to compete for light (Moles *et al.*, 2009). Germinants from seeds of shorter plant species may require a head-start in growth in order to be competitive with germinants from seeds of taller plant species. One might predict,

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therefore, that seeds of shorter plant species should germinate earlier than those of taller plant species. Flowering time is another key trait in plant life history, because it is central to plant reproductive success and determines environmental conditions during fruit maturation, seed maturation timing and dispersal timing (Galloway and Burgess, 2012). Thus, flowering time may correlate with germination timing.

The maternal environment is an important component of the life cycle of plants, since it can link previous and subsequent life cycles (Donohue, 2009). When conditions are suitable, seeds of species from high elevation with a short growing season are predicted to germinate quickly to maximize biomass accumulation and the probability of life-cycle completion. In dry habitats, seeds usually germinate promptly after rain (Fenner and Thompson, 2005). Shade experienced under dense canopies at the stage of seed maturation inhibits seed germination in select taxa (reviewed by Van Couwenberghe *et al.*, 2013). Thus, we predict that seeds from shaded habitats germinate later than those from open habitats.

In this study, under field conditions with high light (natural light) and low light (experimental light manipulation), we monitored germination timing of

476 species from a grassland community of the eastern Tibet Plateau. Specifically, we addressed two questions: (1) is germination timing correlated with subsequent life-history traits (plant height and onset of flowering) and maternal habitats (elevation, water in maternal habitat and light in maternal habitat); and (2) if these associations are significant, is their significance dependent on light treatments?

Materials and methods

Study region

The study area was located on the north-eastern edge of the Tibetan Plateau in China ($101^{\circ}05' - 104^{\circ}40'E$, $32^{\circ}60' - 35^{\circ}30'N$, about 40,000 km², see Fig. 1). The altitude ranges from 1200 to 4800 m, and the climate is typically continental plateau climate with a mean annual precipitation of 450–780 mm (mainly in summer and autumn) and a mean annual temperature of $-4 - 9^{\circ}C$. The growing season generally ranges from late April–late May to late October–early November. The grassland types are mainly alpine meadow and temperate/subalpine steppe, which are

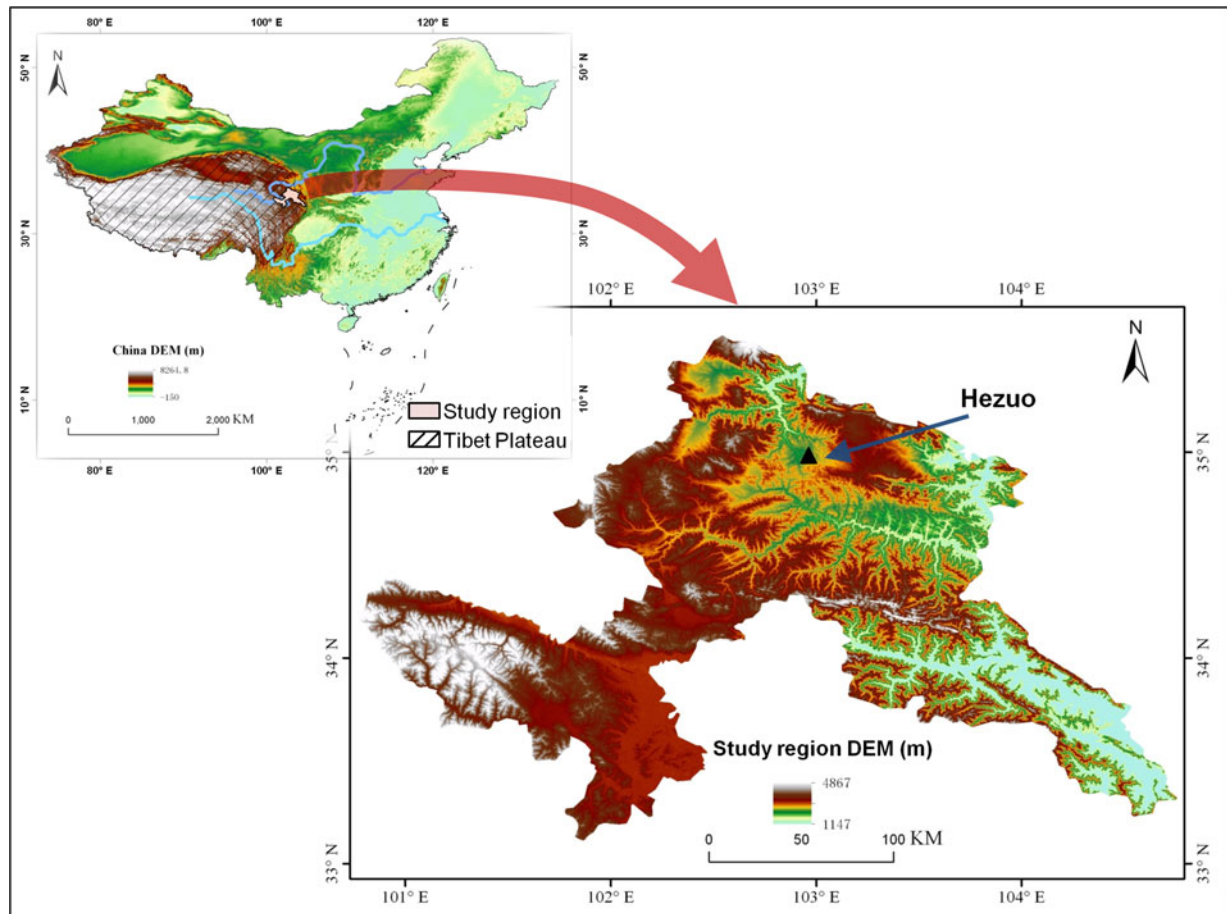


Figure 1. (Colour online) Digital elevation map (DEM) of the study region and China.

dominated by native monocotyledons (predominantly species in the Poaceae and Cyperaceae families) and by native dicotyledons (predominantly species in Ranunculaceae, Polygonaceae, Saxifragaceae, Asteraceae, Scrophulariaceae, Gentianaceae and Fabaceae). Sheep and yak have grazed these grasslands for centuries.

Seed collection

The 476 species used in this study (see species list in supplementary Table S1, available online) included most of the common and dominant species found in the region. Seeds were collected from the field at the start of natural dispersal, in 2008. Seeds were collected from 20+ individual plants for the majority of species, and from all available individual plants for the remaining rare species. The collected seeds were allowed to air-dry to a constant mass at room temperature (approximately 15°C) and were pooled across individuals of a species before being weighed and planted. The seeds were stored dry at room temperature (approximately 15°C) before they were used in the experiment.

Field germination experiment

The germination experiment was carried out at the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University on the eastern Tibetan plateau, in Hezuo (34°55'N, 102°53'E, see Fig. 1), Gansu, China, on a broad, flat site at 2980 m above sea level. At the site, the mean annual temperature is 2.0°C, ranging from -10°C in January to 11.7°C in July; the maximum growing season temperature is 23.6–28.9°C (see annual temperature for 2009 in supplementary Fig. S1, available online). Mean annual precipitation over the previous 35 years has been 532 mm, characterized by a short, cool summer. The area has 2294 h of sunshine and more than 270 days of frost per year. The vegetation is dominated by *Elymus* sp., *Roegneria* sp., *Festuca* sp. and *Anemone* sp.

We tested germination under two light treatments: high light (100% unfiltered light, i.e. natural light) and low light (manipulated with a plastic shade net). Based on the photosynthetically active radiation (PAR) at 1 cm above the soil surface under and outside the shade net, light availability under low light treatment (mean PAR = 40 675.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 3997.9) was only about 2.9% of that of high light (mean PAR = 112946.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 6419.7). PAR was recorded with a Decagon Sunfleck Ceptometer (Decagon, Pullman, Washington, DC, USA).

The temperature under the different light treatments differed by an average of 1.3°C (paired *t*-test: *t* = 11.00, *df* = 110, *P* < 0.0001; noonday means with

standard error for 111 days in June–September: high light, 17.9 ± 0.46°C; low light 16.6 ± 0.37°C). Furthermore, it is known that temperature can affect germination time (Baskin and Baskin, 2001). Thus, we recognize that temperature is a possible confounding factor on our estimates of germination timing. Most studies, however, have found that germination is earlier under increased temperature (e.g. Bierhuizen and Wagenvoort, 1974; Garcia-Huidobro *et al.*, 1982). In our study, we found that germination was relatively late under the high light (higher temperature) treatment (see Zhang *et al.*, 2014), suggesting that the effects of temperature on germination timing were minimal with respect to the effects of light availability in our experiment.

For each treatment, we germinated 300 seeds of each species, distributed over three replicate pots per species (i.e. for each species there were: 100 seeds × 3 pots × 2 treatments). Seeds were placed on grey cotton fabric on top of local soil (see soil information in supplementary Table S2, available online) in plastic pots. The pots were kept in plastic pools (see photographs of experimental facilities in supplementary Fig. S2, available online). The germination trials began on 9 June 2009, because germination at high elevation is mainly restricted to short periods in early summer (Bliss, 1971), and ended on 30 September 2009 (114 d). Every day, the number of germinated seeds was recorded and newly emerged seedlings were removed from the pots. The pools were regularly watered and always full of water to keep the soil and grey cotton fabric wet. A seed was considered germinated when the radicle was visible. In addition, to avoid seeds being washed out by rain, we covered the plots with tarpaulins at night and on rainy days. Seed viability was assessed with the tetrazolium test before the germination experiment on 3 × 50 additional seeds (Hendry and Grime, 1993) and for all the seeds we could find in the pots at the end of germination experiment.

Characterization of life-history traits and maternal habitats

Germination timing

We calculated germination timing [GT, days (d) post planting] as the mean time to germination (MTG) for each species under both light availability treatments.

Plant height

Adult plant height (m) of each species was from the information in Flora China Editing Group (2004). For some climbing species (*n* = 5), plant height was assessed from the average value of all species used in the study.

Onset of flowering

Each species was assigned to one of the following three groups (Mazer, 1989), based on information in the Flora China Editing Group (2004): early ($n = 88$), flowering begins in May; middle ($n = 338$), flowering begins in June; and late ($n = 50$), flowering begins in July and August.

Elevation

The elevation information of each species was from our collection records. We measured the elevation of the sampling site of each species.

Maternal habitats

Based upon our collection records, we recorded information on two aspects of the maternal habitat for each species: water and light. For water, species were assigned to one of five groups: severe drought ($n = 23$), mild drought ($n = 117$), moderate moisture ($n = 227$), wet ($n = 50$) and aquatic ($n = 9$, e.g. swamp). For light, species were assigned to one of three groups: shade (about 0–30% natural light, $n = 42$, e.g. under closed canopy), moderate shade (about 30–90% natural light, $n = 89$, e.g. forest/scrub margin) and open (about 90–100% natural light, $n = 345$). Two or three experienced observers made collection records on water and light conditions of the habitat.

Prior to analyses, germination timing (d), plant height (m) and elevation (m) were log-transformed to optimize normality of frequency distributions.

Phylogeny construction

A composite phylogeny of all species was constructed with Phylomatic version 3 (Webb and Donoghue, 2005) based on the angiosperm megatree (R20091120.new). This tree was further resolved based on the Angiosperm Phylogeny Website version 12 (Stevens, 2001 onwards). Branch lengths were made proportional to time using the 'bladj' function in the program Phylocom 4.0 (Webb *et al.*, 2008) and divergence time estimates based on fossil data (Bell *et al.*, 2010; Smith *et al.*, 2010).

Correlations of germination timing with life-history traits and maternal habitats

We used both standard linear regression and phylogenetic generalized linear models (PGLM) to test if germination timing was correlated with continuous variables (plant height and elevation) in both light treatments. Standard linear regression was conducted using the 'lm' functions of the R package 'stats'. Phylogenetic generalized linear models control for the effects and degree of phylogenetic signal in the dependent variable (Revell and Harrison, 2008). Phylogenetic generalized linear models were conducted using the 'plgs' functions of the R package 'caper' version 0.5 (Orme *et al.*, 2011). For the PGLM, phylogenetic signal (Pagel's λ) was estimated for the dependent variable using a maximum likelihood framework. Pagel's λ can vary from 0 (no phylogenetic signal) to 1 (strong phylogenetic signal) (Pagel, 1999; Freckleton *et al.*, 2002).

Germination timing also was compared among onset of flowering, water in maternal habitat or light in maternal habitat in both light treatments, using both standard ANOVAs and phylogenetically corrected ANOVAs (phyANOVAs). Standard ANOVAs were conducted using the 'aov' functions of the R package 'stats'. The phylogenetically corrected ANOVAs were performed similarly to the phylogenetic generalized linear model (PGLM), but using the 'gls' function and 'corPagel' function in the R package 'nlme' and R package 'ape' version 3.0-8 (Paradis *et al.*, 2004).

Results

Germination timing was significantly positively correlated with plant height, but only in low light in standard linear regression ($P = 0.0141$, Table 1 and Fig. 2). Germination timing was significantly associated with the onset of flowering in both light treatments in standard ANOVAs (high light: $F_{2,473} = 8.8$, $P = 0.0002$; low light: $F_{2,473} = 13.0$, $P < 0.0001$, Table 2 and Fig. 3) but only in low light when using phylogenetically corrected ANOVAs

Table 1. Results of standard linear regression and phylogenetic generalized linear models (PGLM) testing correlations of log germination timing with log plant height and log elevation. Significant P values ($P < 0.05$) are indicated in bold

| Light treatments | Continuous variables | Standard linear regression | | | | PGLM | | | |
|------------------|----------------------|----------------------------|-----|----------------|---------------|--------|------|----------------|---------------|
| | | Slope | F | R ² | P (Slope) | Slope | F | R ² | P (Slope) |
| High light | Log-height | 0.031 | 1.3 | 0.003 | 0.2580 | 0.0025 | 0.01 | 0.000 | 0.9173 |
| | Log-elevation | -0.13 | 2.5 | 0.005 | 0.1130 | -0.045 | 0.4 | 0.001 | 0.5144 |
| Low light | Log-height | 0.065 | 6.1 | 0.013 | 0.0141 | 0.043 | 3.4 | 0.007 | 0.0647 |
| | Log-elevation | -0.24 | 8.6 | 0.018 | 0.0036 | -0.18 | 7.1 | 0.015 | 0.0078 |

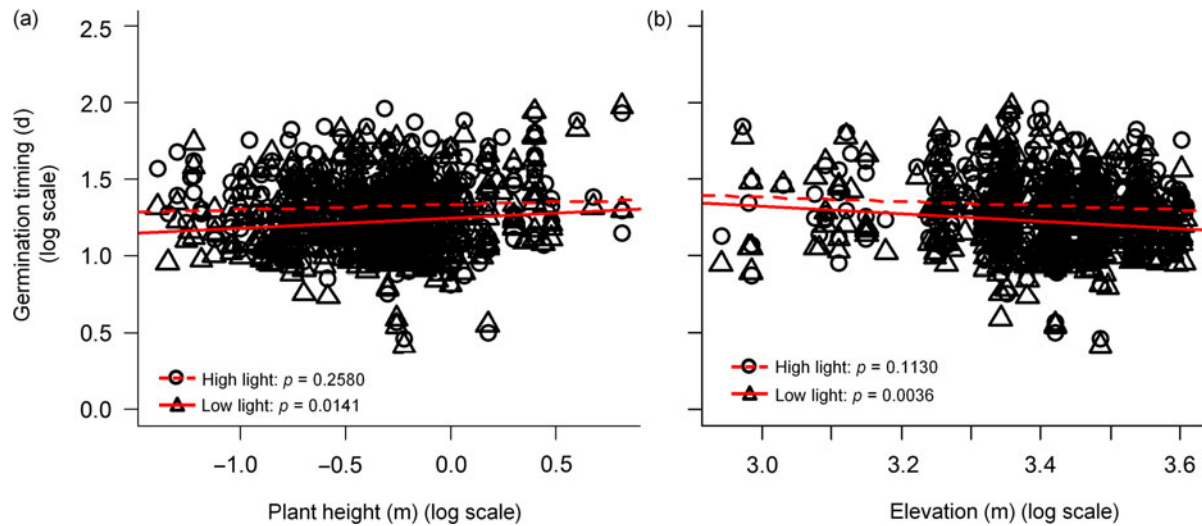


Figure 2. (Colour online) Bivariate regressions across species using standard linear regression: (a) log germination timing and log plant height; (b) log germination timing and log elevation. Germination timing: d post planting.

($F_{2,473} = 3.2$, $P = 0.0403$, Table 2 and Fig. 4). Germination timing was negatively correlated with elevation only in low light when using both comparative methods (standard linear regression: $P = 0.0036$; PGLM: $P = 0.0078$, Table 1 and Fig. 2). Germination timing was significantly correlated with water in maternal habitat only in high light when using both comparative methods (standard ANOVAs: $F_{4,471} = 2.5$, $P = 0.0439$; phyANOVAs: $F_{4,471} = 3.7$, $P = 0.0060$, Table 2 and Figs 3 and 4). Germination timing was significantly associated with light in maternal habitat in both light treatments in standard ANOVAs (high light: $F_{2,473} = 7.9$, $P = 0.0005$; low light: $F_{2,473} = 6.8$, $P < 0.0012$, Table 2 and Fig. 3) but only in high light when using phylogenetically corrected ANOVAs ($F_{2,473} = 4.1$, $P = 0.0167$, Table 2 and Fig. 4).

In general, there were significant correlations of germination timing with water and light in maternal habitats in high light but not in low light, while there were significant correlations of germination timing with plant height, onset of flowering and elevation in low

light but not in high light, i.e. there are light-dependent associations of germination timing with subsequent life-history traits and maternal habitats.

The conflict between standard comparative methods and phylogenetic comparative methods may only result from the species in our data having strong phylogenetic dependence [phylogenetic signal (Pagel's λ) of germination timing = 0.83, see Zhang *et al.*, 2014] and uneven distribution of sampled species numbers in different taxa (Qi *et al.*, 2014).

Discussion

Correlation of germination timing with subsequent life-history traits

As predicted, germination timing was significantly positively associated with plant height in the low light treatment. This is consistent with the hypothesis that early germination has evolved in part to reduce

Table 2. Results of standard ANOVAs and phylogenetically corrected ANOVAs (phyANOVAs) testing correlations of log germination timing with life-history traits or maternal habitat. Significant P values are indicated in bold

| Light treatments | Discrete variables | Standard ANOVAs | | | phyANOVAs | |
|------------------|--------------------|-----------------|------|--------------------|-----------|---------------|
| | | df | F | P | F | P |
| High light | Onset of flowering | 2, 473 | 8.8 | 0.0002 | 0.6 | 0.5432 |
| | Water habitat | 4, 471 | 2.5 | 0.0439 | 3.7 | 0.0060 |
| | Light habitat | 2, 473 | 7.9 | 0.0005 | 4.1 | 0.0167 |
| Low light | Onset of flowering | 2, 473 | 13.0 | < 0.0001 | 3.2 | 0.0403 |
| | Water habitat | 4, 471 | 0.9 | 0.4860 | 1.5 | 0.1930 |
| | Light habitat | 2, 473 | 6.8 | 0.0012 | 2.6 | 0.0747 |

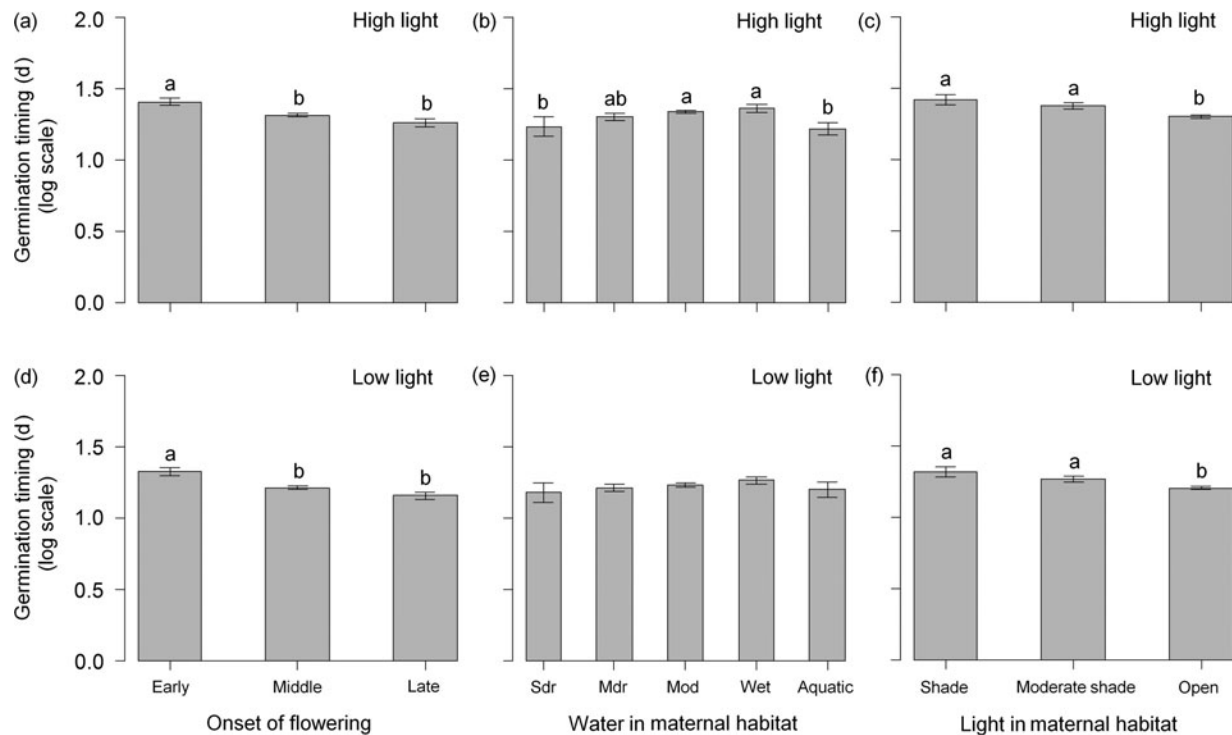


Figure 3. Variation of log germination timing with onset of flowering, water in maternal habitat and light in maternal habitat, under high and low light treatments. Error bars correspond to standard errors. Differences in letters indicate significant differences ($P < 0.05$) among the different groups. We compared means of different levels using least significant difference (LSD).

differences in competitiveness for light between short and tall plant species, and that seeds from short plant species can acquire a competitive head-start by germinating early. This positive association may contribute to coexistence of short and tall plant species. However, this association was only found in the reduced light treatment, i.e. this association depended on light availability.

Germination and flowering are two key fundamental developmental transitions which combine to determine the overall life-cycle and generation time of many plants (reviewed in Chiang *et al.*, 2009). Previous studies showed that significant components of the same genetic pathway are shared between flowering and germination regulation in *Arabidopsis thaliana* (Chiang *et al.*, 2009). Using interspecies comparative analysis, we found an association between germination timing and flowering time. However, this association depended on light availability, i.e. the association was only found in the reduced light treatment. Based on life-history theory, there is a complex network of links among life-history transitions/traits. Environmental changes can mediate this network of links at the community level. Our results suggest that harsh environmental conditions (e.g. low light condition) at the germination stage strengthened the links between germination timing and subsequent life-history traits – plant height and flowering time.

Correlation of germination timing with maternal habitats

Climatic correlations across elevation gradients influence the distribution of plant species (Wang *et al.*, 2009). As elevation increases, the growing season becomes shorter. As predicted, germination timing and elevation were significantly negatively correlated under low light, such that species from high elevation germinated earlier. When conditions are suitable, the seeds of alpine plants may germinate quickly to maximize biomass accumulation and the probability of life-cycle completion. However, germination timing and elevation were not significantly negatively correlated under high light treatment. Thus, effects of maternal elevation were apparent only under harsh conditions (i.e. low light condition).

Germination timing was significantly associated with water in the maternal habitat under high light treatment, and species from both extremely dry and aquatic habitats (extreme habitats) germinated earlier compared with those from other habitats (Figs 3b and 4b). In dry habitats, seeds usually germinate promptly after rain, resulting in the maximum period of time with moist soil for seedling establishment (Fenner and Thompson, 2005). Seedlings in aquatic habitats may be at risk of being submerged, and thus seeds may germinate in a short time when conditions are suitable.

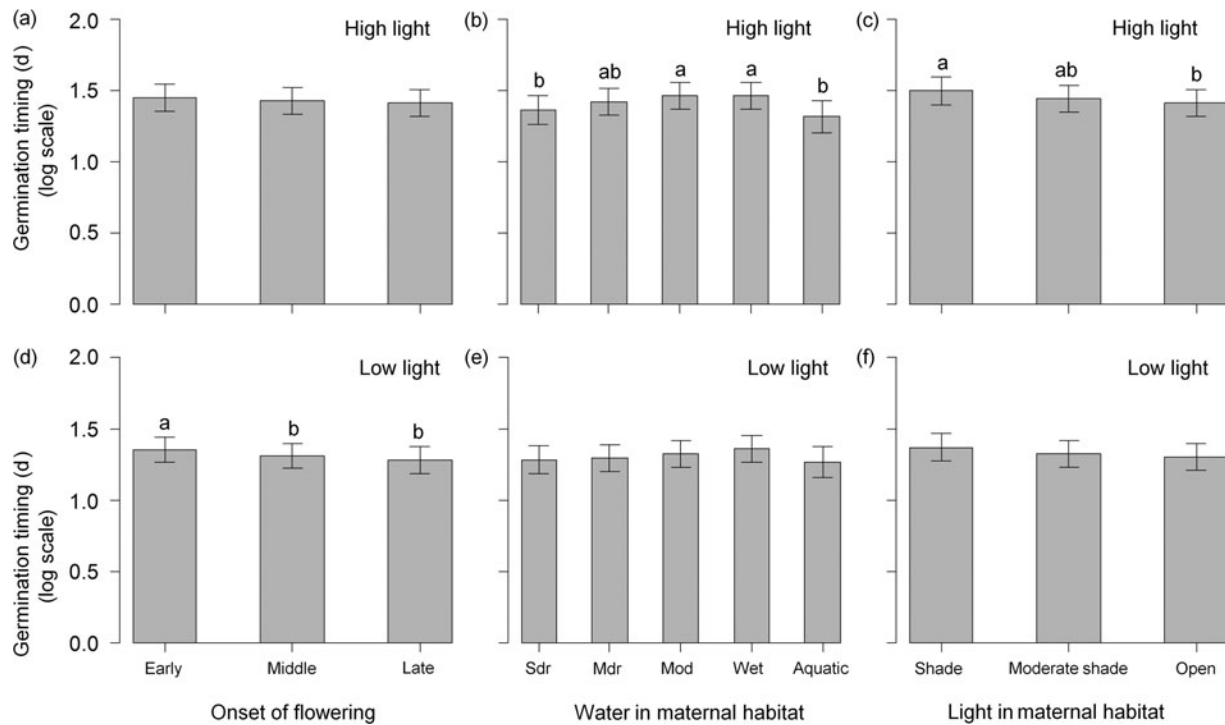


Figure 4. Variation of log germination timing with life-history traits and maternal habitat, under high and low light treatments. Means were calculated based on phylogenetically corrected mean values of species. Error bars correspond to standard errors. Differences in letters indicate significant differences ($P < 0.05$) among the different groups. We compared means of different levels, using phylogenetically corrected ANOVAs.

As predicted, germination timing was also significantly associated with light in the maternal habitat under the high light treatment, and species from shaded habitats germinated later compared with those from open habitats (Figs 3c, 3f and 4c). Low ratios of red to far-red light (R/FR ratio) experienced under dense canopies at the stage of seed maturation inhibit seed germination in select taxa (reviewed by Van Couwenberghe *et al.*, 2013). However, significant associations of germination timing with water and light in maternal habitats were not found in low light treatment, i.e. these associations depended on light availability (see below).

Different maternal environmental factors (elevation, water or light) favoured species with differing germination timing, suggesting local adaptation of species, habitat specialization through germination (ten Brink *et al.*, 2013), or maternal effects on field-collected seeds (Galloway, 2005). Locally adaptive maternal effects result in fitness advantages for species in home habitats and enhance their persistence (Galloway, 2005). The effect of maternal habitat on germination timing depended on light availability at the germination stage. Maternal environment effects can link previous and subsequent life cycles (Donohue, 2009). Harsh environmental conditions (e.g. low light) at the germination stage can strengthen (for elevation) or weaken (for water and light) these links between previous and subsequent life cycles. The first step of colonization of a

new habitat by a species is germination. If maternal environmental effects, in fact, drive the elevation and habitat (light and water) differences in germination timing, maternal effects on germination timing may be important for the distribution and abundance of plant species in natural plant communities. Furthermore, the environmental plasticity of maternal effects on germination may also play a vital role in the regeneration and dispersal of plant species in natural plant communities.

No dormancy-breaking treatments (except dry storage at room temperature) were applied before the experiments in our study, since it is difficult to assess when these pre-treatments are necessary and whether they are effective (Baskin and Baskin, 2003; Norden *et al.*, 2009), especially for a large number of species. However, this may result in overestimation of germination timing of some species. To minimize this problem, species with high levels of dormancy [e.g. *Rosa* sp.; according to Liu *et al.* (2013) and our unpublished data] were not included in the study (see final germination percentage of each species under both light treatments in supplementary Table S1). In addition, Körner (2003) documented that the several months, quiescence (not necessarily low temperature) is responsible for the much greater germination success in spring compared with autumn in the majority of species from subalpine and alpine zones, i.e. storage at winter temperature is not essential.

Conclusions

Associations between germination timing and subsequent life-history traits and maternal habitats under different environments of light availability were analysed using both standard comparative methods and phylogenetic comparative methods at the community level. Germination timing was associated with plant height, onset of flowering, elevation, water in the maternal habitat and light in the maternal habitat. Nevertheless, associations of germination timing with subsequent life-history traits and maternal habitats depended on light availability at the germination stage. Thus, environmental factors can mediate key links in the life cycle of plant species, and this may be decisive for the distribution, abundance and dispersal of plant species in natural plant communities.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0960258514000208>

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Conflicts of interest

None.

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