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Divergent Norms of Reaction to Temperature in Germination Characteristics among Populations of the Arctic-Alpine Annual, *Koenigia islandica*

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Abstract

The timing of germination is a critical life-history trait for annual plants because it coordinates development from seedling to reproduction with the growing season. Arctic/alpine annuals are subject to the constraints of an especially brief growing season in which temperature both acts as a germination cue and limits growth. The evolution of phenotypic plasticity in response to temperature is thus expected, measured as norms of reaction. Strong selection in combination with differences among habitats in appropriate temperature cues leads to the expectation of population divergence in norms of reaction. Their form may be complex; no *a priori* mathematical distribution can be assumed. Here, we use nonparametric smoothing (Loess) to detail norms of reaction of germination to temperature, and find significant genetic divergence among six widely distributed arctic and alpine populations of the annual *Koenigia islandica* from Norway, the U.S.A., and Canada. Germination plasticity is discussed in relation to temperature regimes recorded under field conditions; however, any adaptive inference based on lab observations must be interpreted cautiously. The present results indicate strong population differentiation in plasticity, and highlight the necessity to consider evolved differences in life-history traits when evaluating the vulnerability of arctic and alpine species to a changing climate.

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Introduction

Temperature is the main limiting factor for plant life in the short and unpredictable growing season that characterizes arctic and alpine tundra habitats (Billings and Mooney, 1968). Temperature is the primary environmental factor regulating seed germination in temperate regions (Baskin and Baskin, 1988), and is expected to be of critical importance for plant establishment in arctic and alpine environments. In fact, the severity and shortness of the growing season means that successful reproduction by seed may be impossible in some years (Billings, 1974; Körner, 1999). Because climate change is predicted to be disproportionately pronounced in these environments (IPCC, 2007), the existence of evolved population divergence provides a particularly pertinent demonstration of the relevance of a species' basic evolutionary ecology in making inferences about vulnerability to change (Franks et al., 2007; Bell and Collins, 2008).

Differences in environmental characteristics among arctic and alpine habitats, created by the gradients of latitude and elevation along the extensive range of tundra in the northern hemisphere, are expected to lead to divergent adaptations within plant species occupying these habitats. This may be particularly true in the case of the few exclusively seed reproducing annual species inhabiting these environments (Bliss, 1956; Wagner and Simons, 2008), environments in which perennality and vegetative reproduction are dominant features of the floras (Billings and Mooney, 1968; Bliss, 1971). Annual plants become increasingly scarce at higher latitudes and elevations, both in number and proportion of species. Only 1–2% of the flora of most arctic and alpine regions is represented by annual species (Billings and Mooney, 1968), presumably because of the challenges of completing a life cycle

in one short growing season (Billings, 1974). In contrast, up to 62% of the plant species in some warm desert habitats are ephemeral annuals (Venable et al., 1993). Because seed germination is a critical life-history trait in arctic and alpine annuals, local adaptation would increase sensitivity to environmental change. The evaluation of germination reaction norms to temperature in these species thus provides a model through which to study the extent of population divergence of critical life-history traits.

Interpopulation differences in germination characteristics, including germination responses to temperature, exist in plant species with wide geographic distributions (Milberg and Andersson, 1998), and adaptations of seed germination responses to different thermal regimes have been the subject of previous studies (Meyer et al., 1989; Meyer and Monsen, 1991; Schütz and Milberg, 1997). Despite the theoretical importance of annuals in arctic and alpine environments, virtually all studies of plastic responses of germination to temperature have been restricted to polycarpic perennials (Mooney and Billings, 1961; Cavieres and Arroyo, 2000). The few existing studies on germination responses to temperature in arctic and alpine annuals were conducted either on a single population (Heide and Gauslaa, 1999), or addressed differences among species, and under a single temperature environment (Reynolds, 1984a). Furthermore, most studies on germination differences among populations have employed seeds obtained directly from the field, and do not allow for separation of genetic and environmental components (Fenner, 1991; Wagner and Simons, 2008) in germination responses.

Although of course it is difficult to infer the particular adaptive significance of germination responses measured under controlled conditions, an experimental approach allows for the

TABLE 1
Environmental characteristics of the sites of origin of the *Koenigia islandica* populations.

Population name	Svalbard	Iqaluit	Dovre	Yukon	Jasper	Colorado
Country	Norway	Canada	Norway	Canada	Canada	U.S.A.
Latitude	78°14'N	63°44'N	62°18'N	61°10'N	52°49'N	~40°N
Longitude	15°35'E	68°34'W	9°36'E	138°25'W	118°08'W	~105°42'W
Elevation (m a.s.l.)	10	15	900	783	~2300	~3650
Maximum photoperiod (h)	24	20.8	19.9	19.3	16.9	15
Summer photoperiod (Jun–Aug, h)	24–19.1	19.9–14.6	19.1–14.5	18.7–14.3	16.5–13.7	14.8–13.1
Mean annual temperature (°C)	–6.7 ^a	–9.8 ^b	–0.1 ^c	–3.8 ^d	— ^e	–3.71 ^f
Mean summer temperature (Jun–Aug, °C)	4.2 ^a	6.0 ^b	9.1 ^c	11.4 ^d	7.5 ^e	6.6 ^f
Extreme maximum summer temperature (°C)	21.3 ^a	25.8 ^b	26.8 ^c	31.7 ^d	29.0 ^e	19.0 ^g
Extreme minimum summer temperature (°C)	–8.4 ^a	–10.2 ^b	–6.0 ^c	–8.5 ^d	–12.2 ^e	–12.0 ^g
Days/year with maximum temperatures >20°C	0.06 ^a	1.2 ^b	9.9 ^c	28.1 ^d	5.6 ^e	0 ^g
Mean annual precipitation (mm)	190 ^a	412.1 ^b	435 ^c	279.7 ^d	— ^e	930 ^f
Mean summer precipitation (Jun–Aug, mm)	51 ^a	160 ^b	180 ^c	158 ^d	256 ^e	172 ^f
Mean growing season length (days/year with minimum temperatures >0°C)	99 ^a	93 ^b	140 ^c	106.8 ^d	— ^e	47 ^g

^a 1961–1990, Longyearbyen Airport, 28 m a.s.l., Norwegian Meteorological Institute.

^b 1971–2000, Iqaluit Airport, 33.5 m a.s.l., Environment Canada.

^c 1961–1990, Fokstugu Station, 972 m a.s.l., Norwegian Meteorological Institute.

^d 1971–2000, Burwash Airport, 807 m a.s.l., Environment Canada.

^e 1963–2003, Adams Creek, 2210 m a.s.l., Environment Canada. Dash indicates data not available.

^f 1951–1985, Niwot Ridge D1 Station, 3749 m a.s.l. (Greenland, 1989).

^g 1952–1970, Niwot Ridge D1 Station, 3749 m a.s.l. (Barry, 1973).

characterization of population differentiation in norms of reaction. The characterization of complex reaction norms is desirable both because it measures the potential for phenotypic response to environmental conditions, and because divergent patterns in reaction norms may reflect local adaptation.

In this study we analyze variation in norms of reaction of germination traits among natural populations of an arctic-alpine annual, using seeds produced in the laboratory under uniform conditions to eliminate environmental effects (Quinn and Colosi, 1977; Simons and Johnston, 2006). Loess smoothing techniques (Shipley and Hunt, 1996), were recently introduced for the characterization of complex norms of reaction (Simons and Wagner, 2007) and are used here for this purpose. This technique avoids the limitations associated with assumptions of linear or other simple norms of reaction (Izem and Kingsolver, 2005; Simons and Wagner, 2007). In addition, to obtain information on field conditions, soil surface temperatures at selected sites were monitored continuously throughout one year.

We investigate differentiation in norms of reaction of germination to temperature among six populations of *Koenigia islandica* L. (Polygonaceae). This species is the most widespread arctic-alpine annual plant. It has an extensive circumpolar distribution in the northern hemisphere, from high arctic regions beyond the 80th parallel, to alpine habitats as far south as the 30th parallel in the temperate mountain ranges of Eurasia and North America (Hultén, 1971; Hedberg, 1997). *K. islandica* also has disjunct populations in southernmost South America (Zuloaga and Morrone, 1999). The six populations investigated originate from a range of habitats spanning 38° of latitude, from high arctic ecosystems to low latitude, high elevation alpine environments. In these habitats, the studied populations are subjected to vastly different environmental factors. The most important of these factors include photoperiod, temperature, precipitation, and length of growing season. The present experiment isolates population differentiation in norms of reaction to temperature for germination of both unstratified and stratified seeds. Although this study can validly detect population differentiation, particular

adaptive explanations for differences in norms of reaction are speculative.

Materials and Methods

PLANT MATERIAL

Achenes—hereafter referred to as seeds—were obtained from six populations that experience vastly different environmental conditions (Table 1). Because *K. islandica* usually grows intertwined and in dense patches, often embedded in moss mats, it is difficult to ascertain how many individuals were sampled at each location. For some of the populations the seeds were collected from several sites (subpopulations), and care was taken to collect seeds from as many individuals as possible, even from the populations represented by more than one subpopulation. The danger of obtaining an unrepresentative sample from a single subpopulation is minimal, however, because hundreds of seeds were collected, and only 3–15 seeds are produced per individual. More details on the sites and plant material are provided elsewhere (Wagner and Simons, 2008, 2009a).

Because previous experiments showed no significant differences in seed germination among subpopulations (Wagner and Simons, 2008), subpopulations (i.e. Svalbard, Jasper, and Colorado; see Wagner and Simons, 2008, 2009a) were pooled for the present work. After collection, all seeds were dried at room temperature and stored for 2–3 months at –18°C until used, with the exception of the Dovre population seeds, which had been stored for 23 years at –20°C. For all populations, fresh seeds were propagated through an entire generation for use in the present study (see following).

LABORATORY SEED PROPAGATION

For the purpose of minimizing possible maternal effects (Quinn and Colosi, 1977; Roach and Wulff, 1987), and the effects of different lengths of storage time of the field-collected seeds (see

above), the studied populations were grown through at least one complete (seed to seed) generation in the laboratory. For the Yukon and Colorado populations, second generation seeds were used. To produce progeny seeds for the study of norms of reaction, the seeds originally collected from the field were germinated in germination chambers (Enconair SG-30, BioChambers Inc., Winnipeg, Manitoba) under long day (LD) photoperiod (15:9) at 24°C day and 17°C night temperatures on wet filter paper in petri dishes. The resulting seedlings were transplanted to pots in a 1:8 mixture of sand and peat-based standard growing medium and grown in growth chambers (Enconair GC-40). *Koenigia islandica* is self compatible and has mostly cleistogamous, self-pollinating flowers (Hedberg, 1997); therefore, no cross-pollination was performed by hand in this study. Seeds of each population were produced, in equal proportion, from plants grown under “arctic” (continuous photoperiod, 14°C day, 5°C night), and under “alpine” (LD photoperiod, 18°C day, 6°C night) conditions. The seeds were harvested, dried at room temperature, and stored for up to two years at –18°C until further use.

EXPERIMENTAL CHARACTERIZATION OF NORMS OF REACTION

Norms of reaction for seed germination for the six *K. islandica* populations were tested using a thermogradient plate. Preliminary experiments show that the Loess method can distinguish among norms of reaction of conspecifics (Simons and Wagner, 2007). The plate was lined with filter paper moistened with distilled water, and placed in continuous fluorescent light (ca. 50 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density). Twenty to twenty-five of both unstratified and stratified (45 days in complete darkness, 4°C) seeds of each of the six populations were placed on the plate at 12 discrete temperature positions; 10 positions with 3°C spacing from 6°C to 33°C along the temperature gradient, with two additional positions at 28°C and 31°C.

Within each temperature position groups of 5–7 seeds for each population and stratification treatment were placed in randomly assigned positions, and a new randomization was used for each temperature. Germinated seeds were counted and removed every day over a 60-day period and germination, as well as time from imbibition to germination (time to germination) was recorded for individual seeds. Viability tests were performed on nongerminating seeds with seed coats slit, using a 1% Triphenyl tetrazolium chloride (TTC) solution. After 24 hours in a growth chamber, a seed was deemed viable if the embryo or the radicle appeared red.

STATISTICAL ANALYSIS

Plastic responses in seed germination along the temperature gradient for the six *K. islandica* populations were analyzed using the Loess nonparametric smoothing function (Cleveland, 1979). This procedure allows the assessment of the shape of complex norms of reaction and permits testing for differences among populations (Simons and Wagner, 2007). Each population and each treatment was analyzed in two steps; in the first the smoothing parameter based on the unbiased selection criterion AIC_{C1} (SAS Institute Inc., 2003) was independently obtained (Hurvich et al., 1998); in the second a SAS PROC LOESS analysis was performed using the single smoothing parameter selected in the first step (SAS Institute Inc., 2003). The final output of the analysis contained the predicted smoothed surface for the

population and treatment, as well as its 95% confidence limits. Although a conservative test, non-overlap of the 95% confidence limits allows for statistical inferences on differences between norms of reaction of populations and treatments across temperatures (Simons and Wagner, 2007).

SOIL SURFACE TEMPERATURE MEASUREMENTS

To obtain information about the thermal regimes experienced by *K. islandica* plants in the field, monitoring of soil surface temperatures in three of the *K. islandica* sites was performed in 2005–2006. A total of 13 miniature temperature data loggers (iButtons, Maxim Integrated Products Inc., Sunnyvale, California) were placed at the soil surface, a few tens of centimeters apart at one location, among *K. islandica* plants in Colorado, Yukon, and Svalbard sites. We provide the field-measured temperature data for two reasons: first, as qualitative evidence that, within a year, temperatures differ among sites; second, to provide an overall impression of the range of temperatures experienced within sites. The duration of the soil surface temperature measurement experiment was only one year; therefore, we cannot estimate the variability of snowmelt among sites.

For accessibility reasons, in Svalbard the temperature loggers were placed in a *K. islandica* population in Adventdalen, approximately 10 km from the Svalbard populations described above. The Adventdalen site is very similar to the site where the seeds of the Svalbard population were collected. The iButtons recorded soil surface temperatures with a precision of 0.5°C every 4 hours for approximately 11 months, from midsummer 2005 until midsummer 2006. Most of the data loggers—10 out of the 13 and at least one from each region—were successfully retrieved after the experiment, and the downloaded temperature records were used to infer the time of snowmelt and the temperatures to which *K. islandica* seeds are subjected at the time of germination.

Results

The shapes of reaction norms to temperature indicate a distinct peak in germination for most populations, below and above which germination is markedly inhibited (Figs. 1 and 2). The Loess methods reveal dissimilarities in temperature reaction norms among populations for both germination fraction (Fig. 1) and time to germination (Fig. 2). Shapes of the reaction norms for germination fraction are significantly different for most populations (Fig. 1). Moreover, cold stratification of the seeds induced significantly different germination responses to temperature in four of the six populations (Fig. 1). With the exception of the Dovre population, the untreated *K. islandica* seeds germinated with high percentages in a relatively narrow range of high temperatures, from 27 to 30°C. Temperatures below 21°C seemed to inhibit the germination of unstratified seeds for all populations except Dovre, and at 33°C all six populations exhibited poor germination (Fig. 1).

A small percentage of the seeds germinated during the stratification treatment at 4°C in the dark (Svalbard, 0.74%; Colorado, 3.33%; Dovre, 5.92%; Iqaluit, 6.66%; Jasper, 12.96%). Cold stratification significantly increased the seed germinability of the Yukon population, and greatly reduced the temperature requirement for germination (Fig. 1); stratified seeds of this population germinated at temperatures as low as 6°C, whereas the unstratified seeds did not germinate at all below 18°C. Cold stratification lowered the minimum germination temperature also in the Iqaluit and Jasper populations, but had little influence on

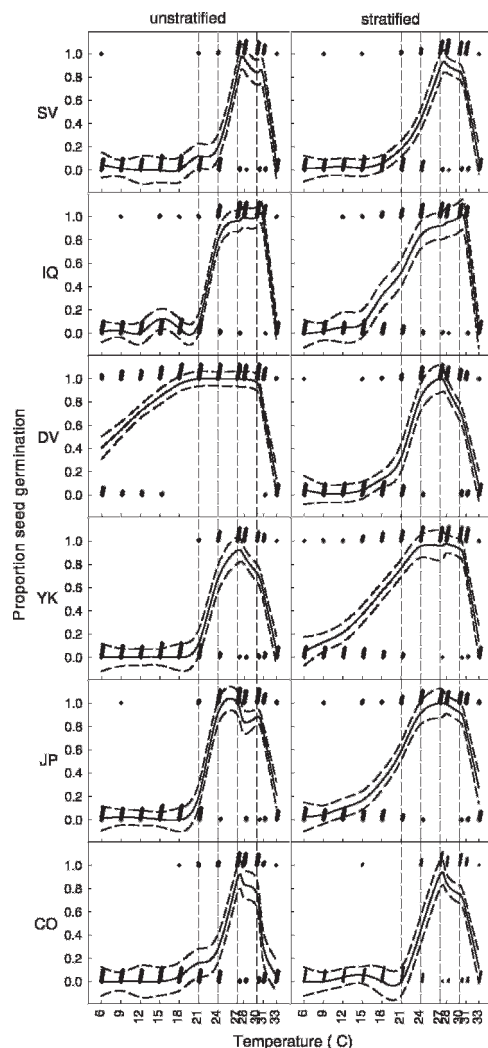


FIGURE 1. Norms of reaction for germination fraction for six populations of *Koenigia islandica* in response to temperature as predicted by the Loess smoothing procedure. The populations are from Svalbard (SV), Iqaluit (IQ), Dovre (DV), Yukon (YK), Jasper (JP), and Colorado (CO). Germination data (diamonds) for unstratified (left) and stratified (right) seeds are offset so as to reveal all individual germination and nongermination events at each of the 12 temperatures. The predicted Loess function (solid line) is fitted using an objectively selected smoothing parameter. Broken lines are the 95% confidence limits. Vertical lines are added to aid in comparison of norms of reaction.

seed germination of the Svalbard population, and virtually no effect in the case of the Colorado population (Fig. 1). Unexpectedly, cold stratification had a negative effect on seed germination of the Dovre population, producing a significant decrease in the seed germinability at lower temperatures (Fig. 1), and increasing the time to germination at any temperature (Fig. 2). The germination responses to temperature observed in the present experiment are corroborated by independent results of Simons and Wagner (2007), in which seed material from a previous generation, and from only three of the six *K. islandica* populations along with another monocarpic plant species were tested under fewer temperature environments.

In the case of unstratified seeds, the 95% confidence limits of Loess predictors for the six populations overlap at and above the optimal temperatures of 27°C, and also at temperatures below 21°C, with the notable exception of the Dovre population. At

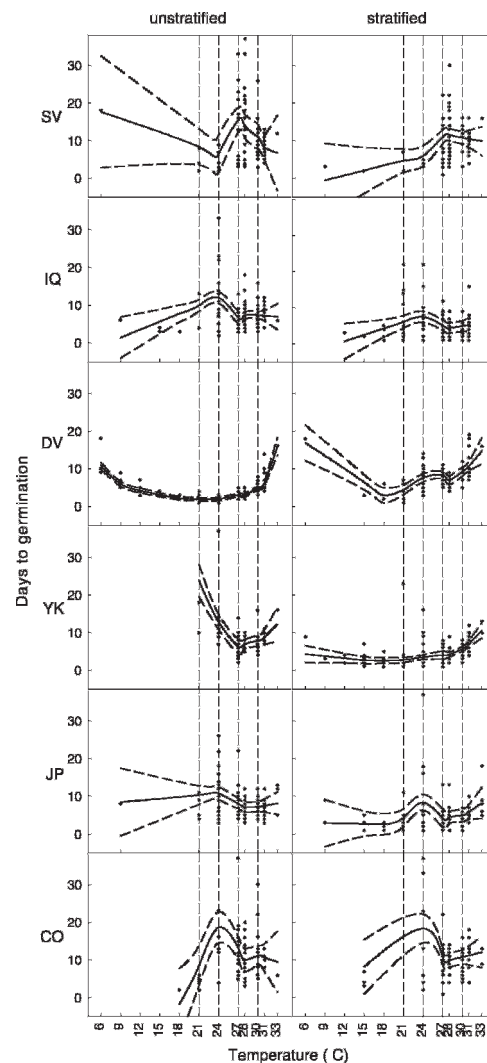


FIGURE 2. Norms of reaction for time to germination for six populations of *Koenigia islandica* in response to temperature as predicted by the Loess smoothing procedure. Diamonds are for individual germination events of unstratified (left) and stratified (right) seeds at each of the 12 temperatures. For further details, see Figure 1.

intermediate temperatures, however, significant differences can be observed in the seed germination of some of the populations, with the populations from the two extremes—the high arctic population from Svalbard and the low latitude alpine population from Colorado—exhibiting similar germination responses to temperature (Fig. 1). Cold stratification of the seeds increased the differences among populations (Fig. 1).

The results of the TTC viability test indicated that over 97% of the ungerminated seeds of both unstratified and stratified treatments of all six populations were viable, with the exception of all seeds subjected to 33°C during the two month experiment, which appears therefore to be lethal.

The temperature records of the miniature temperature loggers (iButtons) allowed assessments of the temperature regimes experienced by *K. islandica* plants and seeds, from summer 2005 until summer 2006 (Fig. 3) and permitted inferences on the date of snowmelt as well as the germination conditions of *K. islandica* in the field (Table 2). At the Colorado site the temperatures registered the greatest diurnal oscillations during the snow-free period, with maxima exceeding 20°C and minima at or a few

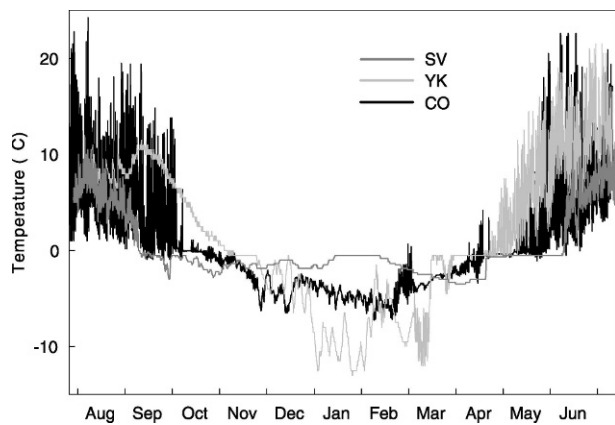


FIGURE 3. Soil surface temperatures between July 2005 and July 2006 at the three *Koenigia islandica* sites, as recorded by miniature temperature loggers (iButtons). SV (dark gray) = Svalbard; YK (light gray) = Yukon; CO (black) = Colorado.

degrees above 0°C for most of the growing season (Fig. 3). In the northern sites of Yukon and Svalbard the daily temperature fluctuations during the snow-free period were less pronounced, and a decrease in amplitude with increasing latitude is apparent across the three locations. In all three sites the soil surface temperatures were constant, and remained near 0°C under the snow cover (Fig. 3). Immediately following snowmelt, the temperatures again registered strong oscillations, with daily maxima exceeding 20°C within a few days at the Colorado site (Table 2).

Discussion

Vegetative reproduction and distribution of flower and seed formation over more than one year are dominant reproductive strategies (Heide, 1992) in arctic and alpine habitats. For annuals, however, reproductive success depends on the completion of a life cycle from seed to seed in one short, cool, and unpredictable growing season (Billings, 1974). A key component of success thus depends on seed germination, which must be timed to maximize the use of the growing season yet minimize the risk of germinating too early or too late, when seedling survival is low. Temperature is among the most important cues available for adaptive germination response (Baskin and Baskin, 1988). The existence of population differentiation indicative of local adaptation would suggest the vulnerability of arctic-alpine annuals to rapid environmental change. In this study we find genetic population differentiation in norms of reaction of germination responses to a gradient of temperature environments among six populations of the arctic-alpine annual *Koenigia islandica*, originating from a geographically wide range of arctic and alpine habitats.

Seed germination without a prior period of cold stratification can be considered equivalent to the germination of freshly matured seeds late in the autumn of the same growing season. With the exception of seeds from the Dovre population, which seem to germinate rapidly and with high percentages across a broad temperature range, seeds not subjected to cold stratification require high temperatures for germination (Fig. 1), a characteristic commonly encountered in arctic and alpine species (Mooney and Billings, 1961; Sayers and Ward, 1966; Chabot and Billings, 1972; Olson and Richards, 1979; Acharya, 1989; Kibe and Masuzawa, 1994; Nishitani and Masuzawa, 1996; Heide and Gauslaa, 1999; Giménez-Benavides et al., 2005). The temperature at which greatest germination occurs for *K. islandica* is among the highest observed in plant species from arctic and alpine environments, and is in sharp contrast to the low temperatures optimal for growth in this species (Reynolds, 1984a; Heide and Gauslaa, 1999). This discrepancy (Amen, 1966), together with the almost complete lack of germination at temperatures below 21°C, indicate that all populations except Dovre have a conditional, or temperature-dependent dormancy (Meyer et al., 1989; Shimono and Kudo, 2005). This is also supported by the high viability (>97%) for ungerminated seeds, and may be an important mechanism to avoid mortality caused by late spring frost (Amen, 1966; Billings and Mooney, 1968; Chabot and Billings, 1972).

Differences among population reaction norms for unstratified seeds (Fig. 1) apparent at intermediate temperatures are suggestive of adaptive germination responses. In particular, temperature requirements for germination were least restrictive for the population originating from the mildest climate (Dovre), whereas an elevated and sharp minimum temperature threshold is observed for seeds from populations of intermediate climate severity (Iqaluit, Yukon, Jasper). The greatest resemblance in norms of reaction exists between the two populations from the “opposite” habitats; the high arctic population from Svalbard and the low latitude, alpine population from Colorado. Although we had not predicted this result, the comparatively high temperature needed to attain maximum germination fraction and the protracted germination process might be explained by the fact that these two populations originate from the most severe climates; the Svalbard site has a high arctic climate (moderated in part by the warming effect of the Gulf Stream), and the Colorado site, despite being at relatively low latitude, is at high elevation and experiences relatively low summer temperatures (Wagner and Simons, 2009a). Furthermore, these observed germination patterns are consistent with previous findings that seeds from warmer sites germinate rapidly over a broader temperature range, while seeds from higher elevation and colder climates require higher germination temperatures and germinate more slowly (Meyer et al., 1989; Cavieres and Arroyo, 2000). The failure of the Svalbard and Colorado populations to achieve 100% germination is consistent with previous observations that seed germination fractions

TABLE 2

Soil surface temperature regimes after snowmelt in three *Koenigia islandica* sites for May–July 2006 as recorded by miniature temperature loggers (iButtons).

Site	Date of snowmelt	Days to 15°C*	Days to 20°C*	Days to 25°C*	Max [†] (°C)
Svalbard	16 June	26	—	—	16.5
Yukon	05 May	27	60	—	21.5
Colorado	27 May	7	11	11	25.5

* Time elapsed from date of final snowmelt until temperature reaches or exceeds this value. Dash indicates temperature never reached value.

† Maximum temperature observed during the entire growing season.

generally decrease with increasing latitude or elevation of origin (Amen, 1966; Dorne, 1981).

Assessment of norms of reaction for germination in seeds subjected to cold stratification simulates spring or early summer germination of seeds that have spent the winter under snow cover. More rapid, and increased germinability following stratification observed in three of the six *K. islandica* populations was also manifested through a decrease in the minimum temperature required to elicit germination, especially in the Yukon population and, to a lesser extent, in the Iqaluit and Jasper populations. This is consistent with the results of previous germination studies on arctic and alpine plants (Bell and Bliss, 1980; Densmore, 1997; Shimono and Kudo, 2005). An increased germination percentage and a decrease in light requirement for germination when stratified (Wagner and Simons, 2009b) are typical of summer annuals, which germinate in the spring after spending the winter as seeds under the snow cover and set seed in late summer or autumn (Baskin and Baskin, 1988).

The apparent lack of response to cold stratification in the populations from the most extreme environments and longest winter period (Svalbard and Colorado) suggests that the seeds of these two populations require longer stratification periods to break their conditional dormancy (Meyer et al., 1989; Meyer and Monsen, 1991; Cavieres and Arroyo, 2000). However, previous independent experiments, specifically looking at effects of stratification and scarification indicated that, at least in the case of the Colorado population, a longer stratification period (20 weeks) failed to improve the germination fractions (Wagner and Simons, 2008). Regardless, the requirement of high temperatures and light for germination (Wagner and Simons, 2009b), together with the platykurtic distribution of time to germination—seeds of these two populations were still germinating after over a month from imbibition (Fig. 2)—indicate that the populations from Svalbard and Colorado seem to have evolved a “cautious” (Schütz, 2002) or diversified bet-hedging germination strategy (Simons and Johnston, 2006). It also confirms the observations of Amen (1966) and Dorne (1981) that germination generally decreases with increasing severity of the climate.

The anomalous germination traits of the Dovre population (Figs. 1 and 2) are consistent with the observation that seeds from milder sites germinate rapidly and over a broader temperature range (Meyer et al., 1989). The decrease in germinability (Fig. 1) and increase in time to germination (Fig. 2) at low temperatures, together with the strong light requirement induced by stratification (Wagner and Simons, 2009b) is consistent with germination characteristics of some winter annuals—plants which germinate in autumn, pass the winter in vegetative state under the snow cover, and flower, set seed and die the following spring or summer (Milberg and Andersson, 1998)—but is a most surprising trait for an arctic-alpine annual.

That population differentiation in reaction norms overlapped only in the high temperature range for stratified seeds (Fig. 1) does not support the observation of Schütz and Milberg (1997) that cold stratification decreases germination variability among geographically different populations. Rather, it supports the finding that in some cases stratification increases the differences in germination responses of different populations (Milberg and Andersson, 1998).

The high temperatures corresponding with peak germination in most observed *K. islandica* populations beg the question of how germination occurs in the field, where even the extreme maximum summer air temperatures (Table 1) never, or only seldom, reach the temperatures required under laboratory conditions. It has been suggested that the distribution and biology of *K. islandica* is

related to the thermal balance and microclimatic conditions of its growing sites as much as it is related to the macroclimate. Soil surface temperatures, under the effect of solar radiation on clear days in early summer, can greatly exceed the air temperatures, especially in more southern, alpine locations (Dahl, 1963). This is substantiated by the soil surface temperature monitoring experiment. The iButtons placed at the soil surface among the *K. islandica* plants indicated that temperatures at the soil level reached temperatures sufficient for seed germination in only a few days after snowmelt in the Colorado site (Table 2) and at the Yukon site, to temperatures within the first few weeks at which about half of the overwintered (stratified) seeds would germinate. In the Svalbard site, however, the maximum recorded soil temperature during the entire growing season was only 16.5°C (Table 2), a temperature at which even the cold-stratified seeds germinate at less than 20% under laboratory conditions. Although it is believed that pronounced fluctuations in temperature as observed at all sites following snowmelt might represent a signal for germination, temperature fluctuations in the laboratory failed to promote germination (Wagner, 2007). Another possibility is that an opportunistic germination behavior of the Svalbard population causes germination only on the rare occasions when the relatively high temperatures are reached. However, this is contradicted by the observation that *K. islandica* is plentiful in this location on a yearly basis. A third explanation is that the 45-day stratification period was insufficient to break dormancy. Fourth, the iButton data were gathered during a single season, and may be atypical. Finally, the possibility exists that large soil seed banks persist over many years, from which only a small fraction germinates each season. Reynolds (1984b) found only a small residual bank of viable seeds in a *K. islandica* population from Montana, but populations in more severe climates might have larger and more persistent seed banks (Cavieres and Arroyo, 2001). Further investigation is needed to shed light on this aspect.

The Loess procedure used in this study has provided evidence of strong population differentiation in norms of reaction to temperature in germinating seeds of arctic-alpine annual species. Although we can conclude that these differences are genetically based, our adaptive explanations of this differentiation remain speculative until specifically tested. Local adaptation of a critical life-history trait suggests important implications of environmental change for arctic-alpine plants and is an area that merits more intensive study.

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