

Divergence among arctic and alpine populations of the annual, *Koenigia islandica*: morphology, life-history, and phenology

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Arctic and alpine habitats occur along complex environmental gradients, and over an extensive geographical range. Despite some selective forces common to these habitats, evolutionary divergence among populations of arctic and alpine plants along this gradient is expected. Of particular significance, both in the context of life-history theory and for implications of climate change, are the few annual species that have adapted to the constraints of an unpredictable, short growing season. In this study, morphological, life-history and phenological characters were found to differ significantly among six widely distributed populations of the arctic-alpine annual *Koenigia islandica*. On the basis of morphology and life-history traits, populations from high latitudes, with the exception of Svalbard, performed better in simulated arctic conditions, whereas the low latitude alpine plants from Colorado showed enhanced performance under simulated alpine conditions. On the basis of phenology, the six populations can be clearly grouped into arctic, high latitude alpine and alpine populations: arctic plants were found to develop and flower earliest; alpine plants latest. Because these results were obtained using seeds harvested from plants first grown through a complete generation in growth chambers, they indicate strong genetic differentiation. We discuss possible adaptive explanations for observed differences among the six geographically divergent populations.

Features common to arctic and alpine tundra habitats make them inhospitable to most organisms. However, major differences exist, and complex transitions between arctic and alpine habitats occur over an extensive geographical range. Differences among tundra environments over these gradients may thus be reflected as the evolution of generalized or, alternatively, locally specialized strategies. Current research on “evolutionary tracking” asks whether organisms have the capacity – through standing or mutational genetic variance – to respond to a changing selective regime (Bell and Collins 2008). Thus, the possibility of population differentiation among these harsh environments is of interest not only from evolutionary and biogeographic perspectives, but also because highly specialized arctic/alpine organisms would be especially vulnerable to climate change, which appears to be exaggerated in these habitats (ACIA 2004). Although divergence in morphology, life-history and phenology traits among plant populations as adaptive responses to environmental gradients in general has received attention (Turesson 1922, Primack and Antonovics 1981, 1982, Waser and Price 1985, Potvin 1986, Van Tienderen and Van der Toorn 1991a, b, Li et al. 1998, Santamaria et al. 2003, Macel et al. 2007), little is known about genetically based population differentiation among annual plant species with broad distributions across arctic and alpine habitats.

A common environmental factor along the extensive range of arctic and alpine tundra habitats in the northern hemisphere is the cool and short summer. However, the gradients in both latitude and elevation create important environmental dissimilarities in, among other factors, photoperiod, solar radiation, and mean and magnitude of diurnal fluctuations in temperature (Bliss 1956, Billings and Mooney 1968). There is evidence that perennial plant species inhabiting both arctic and alpine tundra respond to these vast and complex differences through the evolution of genetically differentiated ecological races (Mooney and Billings 1961, Mooney and Johnson 1965, Chapin and Chapin 1981, Galen et al. 1991, Gurevitch 1992, Heide 2005). Annual plants are scarce in arctic and alpine regions, making up <2% of the flora (Billings and Mooney 1968, Molau 1993) whereas annual species may compose up to 50% of the flora in other habitats (Venable et al. 1993). The scarcity of annuals in arctic and alpine habitats must at least in part relate to the constraint of having to complete their entire life cycle in one short and unpredictable growing season, and are therefore expected to show especially marked local adaptation. Studies on arctic-alpine annuals (Löve and Sarkar 1957, Dahl 1963, Reynolds 1984a, b, Wagner and Simons 2008), however, are scarce. A notable exception is a comparative study on a number of characters that, although limited by the number of

populations and individuals used, indicates the potential value of a broader study (Heide and Gauslaa 1999).

In the present study we investigate differentiation in morphology, life-history characters and phenology among six populations of *Koenigia islandica* (Polygonaceae), from a wide distribution of tundra habitats, ranging from high arctic, low elevation environments to low latitude, high elevation alpine ecosystems. Given that annual species make up only 2% of arctic and alpine flora, the number that exist in both habitats are vanishingly few; in many arctic and alpine regions *Koenigia islandica* is the only annual species (Billings 1974). It has a vast circumpolar distribution in the arctic regions of the northern hemisphere, which extends southwards in the alpine regions of temperate mountain ranges of North America and Eurasia (Hultén 1971, Hedberg 1997), and into the southern hemisphere in southernmost South America (Zuloaga and Morrone 1999). Its ability to exist in these harsh environments where perennality is a dominant characteristic of plant life (Bliss 1971), makes *K. islandica* an impressive example of plant adaptation to extreme environments. Because of its low tolerance to high temperatures (Dahl 1963, Reynolds 1984b, Heide and Gauslaa 1999), the distribution of *K. islandica* is directly related to maximum growing season temperatures (Dahl 1951, 1963), and therefore might provide a valuable “early warning” model and contribute to a general understanding of biotic response to environmental change (Sætersdal and Birks 1997).

To allow inferences about the genetic basis of observed differences among the six *K. islandica* populations, experiments used plants resulting from seeds produced under laboratory conditions, and were performed in growth chambers. We used a reciprocal transplant design in which all populations were grown in both simulated arctic and alpine conditions. Reciprocal transplant studies predict that the relative performance of populations will be enhanced in their environment of origin which, when found, is

interpreted as evidence for local adaptation (Waser and Price 1985, Potvin 1986, Van Tienderen and Van der Toorn 1991a, Joshi et al. 2001, Macel et al. 2007). We aimed to test this prediction, as well as the predictions that more northern populations have smaller stature and decreased fecundity, and populations from northern latitudes develop and flower earlier (Chapin and Chapin 1981, Potvin 1986, Winn and Gross 1993, Li et al. 1998). We discuss the possible adaptive nature of observed population differentiation, but stress that adaptive explanations require further test.

Materials and methods

Plant material

Morphology, life-history traits and phenology were investigated in six arctic and alpine *Koenigia islandica* populations originating from a variety of tundra environments (Table 1). Achenes (seeds) of the Svalbard population were collected late in the season in 2004 in Spitsbergen, Svalbard from two sites: one just outside Longyearbyen, the other near the airport, at Hotelneset. Because most of the seeds were already shed, entire plants were collected with moss and soil, and the seeds were sorted from the plant material and soil in the laboratory. Seeds of the Iqaluit population originate from a site near Iqaluit, Nunavut, Canada, from plants growing in moist tundra with mosses. In July 2004 entire portions of tundra turf were collected and transported to Carleton Univ., where the *K. islandica* plants with still unripe seeds were allowed to mature in growth chambers. After maturation the seeds were harvested from the plants and sorted from the substrate. The plant material of the Dovre population was provided by O. Heide and originates from Kongsvoll in the Dovre Mountains (Oppdal, southern Norway). This population has been investigated in an earlier study on *K. islandica* (Heide and Gauslaa 1999) and the field collected seeds were stored for 23 yr at -20°C

Table 1. Environmental characteristics of the sites of the *Koenigia islandica* populations.

Population name	Svalbard	Iqaluit	Dovre	Yukon	Jasper	Colorado
Country	Norway	Canada	Norway	Canada	Canada	USA
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
Population category	(high) arctic	arctic	high latitude alpine	high latitude alpine	alpine	alpine
Elevation (m a.s.l.)	10	15	900	783	2300	~3650
Max. photop. (h)	24	20.83	19.91	19.35	16.9	15
Mean annual temperature (°C)	−6.7 ^a	−9.8 ^b	−0.1 ^c	−3.8 ^d	− ^e	−3.71 ^f
Mean summer temp. (Jun–Aug, °C)	4.2 ^a	6.03 ^b	9.1 ^c	11.4 ^d	7.53 ^e	6.63 ^f
Extreme maximum summer temp. (°C)	21.3 ^a	25.8 ^b	26.8 ^c	31.7 ^d	29 ^e	19 ^g
Mean January temp.	−15.3 ^a	−26.6 ^b	−8.8 ^c	−22 ^d	—	−13.15 ^f
Extreme minimum winter temp. (°C)	−46.3 ^a	−45.6 ^b	−32.4 ^c	−55 ^d	—	−37 ^g
Mean annual precipitation (mm)	190 ^a	412.1 ^b	435 ^c	279.7 ^d	—	930 ^f
Days without snow cover	127 ^a	117.7 ^b	161.6 ^c	188.2 ^d	—	—

^a1961–1990 Longyearbyen Airport, 28 m a.s.l., Norwegian Meteorological Inst.

^b1971–2000 Iqaluit Airport, 33.5 m a.s.l., Environment Canada.

^c1961–1990 Fokstugu Station, 972 m a.s.l., Norwegian Meteorological Inst.

^d1971–2000 Burwash Airport, 807 m a.s.l., Environment Canada.

^e1963–2003 Adams Creek, 2210 m a.s.l., Environment Canada. Dash indicates data not available.

^f1951–1985 Niwot Ridge D1 Station, 3749 m a.s.l. (Greenland 1989). Dash indicates data not available.

^g1952–1970 Niwot Ridge D1 Station, 3749 m a.s.l. (Barry 1973).

before being propagated through an entire generation in the laboratory to produce fresh seed for the present study. The seeds of the Yukon population were collected at Rat Lake, on the eastern shore of Kluane Lake in Yukon, Canada, in August 2003. The seeds of the Jasper population were collected in August 2004 south-west of Jasper, Alberta, Canada, from two sites on the slopes of the ridge connecting Whistler Mountain and Indian Ridge, as well as one site at Indian Pass. The seeds of the Colorado population were collected in August 2003 and originate from four locations in the Front Ranges: Guanella Pass Campground and Summit Lake in the Mount Evans Wilderness, as well as Toll Memorial and Sundance Mountain in the Rocky Mountain National Park (for more information, see Wagner and Simons 2008). The seeds were dried after collection at room temperature and stored at -18°C for further use.

By collecting seeds from several different locations (subpopulations) for some populations, and by sampling hundreds of seeds from as many individuals as possible in all cases, a representative sample from each population was insured, even though it is sometimes difficult to tell individual *K. islandica* plants apart in the moss embedded, dense patches in which this species typically grows.

Laboratory seed propagation

For the purpose of both multiplying the number of available seeds and reducing maternal effects (Quinn and Colosi 1977, Roach and Wulff 1987, Simons and Johnston 2006), the investigated populations were grown through one (Svalbard, Iqaluit and Jasper) or two generations (Dovre, Yukon and Colorado) in the laboratory. Seeds were germinated on wet filter paper in Petri dishes in germination chambers (Enconair SG-30, Biochambers, Winnipeg, MB, Canada). The seed germination conditions for the Dovre, Yukon and Colorado populations were of long day (LD) photoperiod (15:9) at 24°C day and 17°C night temperatures, while for the seed germination of the Svalbard, Iqaluit and Colorado populations a continuous 24 h photoperiod and constant 27°C were used. The resulting seedlings in each case were moved to growth chambers (Enconair GC-40) in pots with a 1:8 mixture of sand and peat-based standard growing medium. The first generation of laboratory-reared plants of the Dovre, Yukon and Colorado populations were grown under LD photoperiod at 15°C day and 10°C night temperatures, while the second generation of these populations, as well as those from Svalbard, Iqaluit and Jasper were grown in both a continuous photoperiod at 14°C day and 5°C night temperatures and a LD photoperiod at 18°C day and 6°C night temperatures. In every case the resulting seeds were harvested, dried at room temperature and stored at -18°C until used.

Experimental procedures

Experiments testing for differences in morphology and life-history traits, as well as in phenology among populations, were performed in two growth chambers (Enconair GC-40). One chamber was used to simulate a high arctic environment above the Arctic Circle (hereafter referred to as the arctic

chamber). The day temperature was set to 14°C for 18 h and the night temperature to 5°C for 6 h. The chamber, constantly lighted, was illuminated for 18 h d^{-1} by both fluorescent and incandescent bulbs ($550 \mu\text{mol m}^{-2} \text{s}^{-1}$). Daylight was extended for the remaining 6 h only by incandescent bulbs ($\sim 40 \mu\text{mol m}^{-2} \text{s}^{-1}$). A second growth chamber was used to simulate growing conditions in an alpine environment at approximately 40°N latitude (alpine chamber). Day and night temperatures (12 h; 18°C and 6°C) and a LD photoperiod were maintained, with 12 h of $550 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density lighting, prolonged to 15 h by incandescent bulbs. Plants were grown in plastic trays in individual cells in a 1:8 mixture of sand and peat-based standard growing medium. The experiments were reciprocal transplants, in which both arctic and alpine populations were grown in both growth chambers.

Trial 1

This initial experiment – performed prior to the availability of seeds of all populations – comprises only the Dovre and Yukon populations, as well as four Colorado subpopulations and is included mainly to contrast differentiation among subpopulations from Colorado with differentiation among distant populations. Because two growth chambers were not available at the same time, an arctic chamber experiment was performed starting in July 2004 and an alpine chamber experiment starting January 2005. Because of the reciprocal nature of the design, any differences in function of the chamber over time would result in differences in performance among arctic and alpine treatments, but not in the relative performance of arctic and alpine populations within these treatments. The seeds for this trial were germinated in an SG-30 germination chamber under a LD photoperiod (ca $70 \mu\text{mol m}^{-2} \text{s}^{-1}$) and 24°C day and 17°C night temperatures. Untreated seeds, scarified seeds, seeds stratified for 45 d at 4°C in the dark and seeds that received both treatments were used (Wagner and Simons 2008). Germinated seeds were transferred to arctic and alpine chambers to complete their life cycle (see above).

Upon seed maturation, the plants were harvested and the following morphological and life-history traits were measured and recorded: height, number of seeds, seeds per infructescence, infructescences per individual, total seed mass as well as above-ground dry plant mass (after drying to constant weight at 50°C). From these measurements the total aboveground plant mass (as the sum of seed mass and aboveground plant mass), the average mass of one seed, and reproductive allocation of each plant (total mass of the seeds/total plant weight) were also inferred. The very high shoot/root ratio of 5–7 for *K. islandica* plants (Reynolds 1984b) makes the above-ground reproductive allocation a good approximation of the actual reproductive allocation that would include the root mass as well (Heide and Gauslaa 1999).

Trial 2

This focal experiment was performed with all six populations concomitantly in both arctic and alpine chambers. Seeds were germinated in a continuously lighted SG-30 germination chamber (photosynthetic photon flux density

as above) at constant 27°C prior to transfer to the arctic and alpine chambers. Sixteen trays were placed in each chamber, with each tray containing all six populations in blocks randomized within trays. A new randomization was used for each tray, and the randomization was identical in the two chambers. The individual plants were monitored every second day throughout their life cycle for the presence of 11 vegetative and 11 generative phenological stages (Table 2). After most plants reached their last phenological stage, they were harvested, and the same morphological and life-history traits as in trial 1 were measured and recorded.

Statistical analysis

Fixed effects analyses of variance (ANOVA) were used to test for the effects of chamber, population and their interaction on the measured morphological and life-history traits. The chamber $\times$ population interaction is of particular importance in a reciprocal environment design, and tests the relative performance of populations across the environments after controlling for environmental response common to all populations. ANOVAs were performed independently on several traits. We use a recent method, known as the false discovery rate (FDR) to minimize the frequency of significant results that are type-1 errors (Benjamini and Hochberg 1995, Garcia 2003); a method that does not suffer from loss of power as do methods such as Bonferroni correction. Because of the differences in goals and experimental design, the results of the two experimental trials were analysed separately. Only the most important results for trial 1 are reported, whereas complete ANOVA results are reported for the second trial, which uses all six populations. When the analysis of variance indicated significant effects ($p > 0.05$), post-hoc Tukey HSD-tests were performed to further examine differences detected. Additional ANOVA and Tukey HSD analyses were performed on the subpopulations of the Colorado population in the first trial.

The phenological data (second trial only) were expressed as the number of days from germination until the occurrence of the observed phenological stage. Because multiple measures are taken within subjects over time, we performed a multivariate analysis of variance (MANOVA) for repeated measures, testing for the effect of chamber, population, the within- subjects effect of stage, and interaction terms. The null hypothesis of no difference among populations in time taken to reach different stages is

tested with the stage $\times$ population interaction term. F tests and Wilk's Lambda (λ) tests were used. Because of the large number of stages measured, insufficient degrees of freedom preclude analysis of the whole set of phenological stages. Therefore, the nine most relevant vegetative, and the eight most relevant generative stages of the total of eleven stages were included in the analysis. Stage 0 for both the vegetative and generative phenology – attained right after germination – is identical for all populations and was therefore not included. Vegetative stage 6 – appearance of a 2nd branch – was reached only by a relatively small percentage of individuals, and has been excluded as well. Similarly, the generative stages 2 and 5, with low percentages of occurrence, were not included in the analysis.

Results

Trial 1

This reduced trial is included mainly because it allows a comparison of differentiation among three distant populations with differentiation among Colorado subpopulations (see Methods). Morphological and life-history traits differ significantly and markedly among the three *K. islandica* populations, whereas among the four subpopulations from Colorado the few differences are also much less marked. After correcting for FDR, no chamber $\times$ subpopulation interaction effects, and only 2 of 9 traits, both morphological – plant mass and height – are significantly different among the Colorado subpopulations. In contrast, 8 out of 9 traits are significantly different among the three main populations included in this trial.

Trial 2

Trends in morphological and life-history characters are similar to those of trial 1; the chamber $\times$ population interactions, after controlling for false discovery rate, were significant for all traits, indicating significant ecotypic differentiation among populations (Table 3). Post-hoc Tukey HSD-tests (analyses not presented) show that the high-latitude populations from Iqaluit, Dovre and Yukon accumulated significantly more biomass in the simulated arctic conditions, a tendency which was found, unexpectedly, also in the Jasper population. However, the significant chamber $\times$ population interaction for aboveground biomass is primarily a result of comparatively high positive slopes across the alpine to arctic chambers for Dovre and Yukon (Fig. 1b). Enhanced growth of the plants from Colorado relative to other populations under alpine conditions is suggested by the consistently negative slopes across the alpine to arctic chamber environments for this population (Fig. 1). Very similar growth was, surprisingly, exhibited by the high arctic Svalbard plants (Fig. 1); the post-hoc Tukey HSD-tests also showed that only one trait – seeds per infructescence – was significantly larger in the arctic chamber for this population.

Populations can be grouped into three categories: arctic, high latitude alpine and alpine (Table 1). Analyses performed on population category data yield similar results to analyses performed on individual populations, with only

Table 2. Phenological stages of *Koenigia islandica* individuals.

Vegetative stages	Generative stages
0 – cotyledons only	0 – no buds/flowers
1 – 1st leaf	1 – 1st buds visible
2 – 1st pair of leaves	2 – buds just before opening
3 – 2nd pair of leaves	3 – 1–30% of flowers open
4 – 3rd pair of leaves	4 – 31–60% of flowers open
5 – 1st branch	5 – full flowering
6 – 2nd branch	6 – flowers wilted
7 – 1–30% of leaves dry	7 – 1st achenes visible
8 – 31–60% of leaves dry	8 – achenes mostly green
9 – 61–99% of leaves dry	9 – achenes mostly brown
10 – plants dead	10 – achenes fully ripe and dispersing

Table 3. Analysis of variance for morphological and life-history traits of six populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions (trial 2). No false rejections were found through calculation of false discovery rate (at $\alpha = 0.05$) for multiple comparisons (see Methods).

Source	DF	MS	F	p
Morphological traits				
Plant mass:				
chamber	1	189.74	115.07	<0.0001
population	5	52.06	31.57	<0.0001
chamber $\times$ population	5	4.78	2.89	0.01
Total aboveground mass:				
chamber	1	1813.92	153.85	<0.0001
population	5	405.59	34.40	<0.0001
chamber $\times$ population	5	62.50	5.30	<0.0001
Height:				
chamber	1	69.31	7.03	0.008
population	5	864.81	87.76	<0.0001
chamber $\times$ population	5	52.72	5.34	<0.0001
Life-history traits				
Total seed mass:				
chamber	1	830.33	157.06	<0.0001
population	5	174.46	33.00	<0.0001
chamber $\times$ population	5	43.90	8.30	<0.0001
Number of seeds:				
chamber	1	9289.34	203.06	<0.0001
population	5	1664.77	36.39	<0.0001
chamber $\times$ population	5	786.28	17.18	<0.0001
Seeds/infructescence:				
chamber	1	490.34	75.09	<0.0001
population	5	372.57	57.06	<0.0001
chamber $\times$ population	5	26.52	4.06	0.001
Infructescences/individ.:				
chamber	1	117.05	126.35	<0.0001
population	5	29.22	31.54	<0.0001
chamber $\times$ population	5	8.57	9.25	<0.0001
Average mass of a seed:				
chamber	1	0.01	0.51	0.47
population	5	1.63	83.82	<0.0001
chamber $\times$ population	5	0.05	2.47	0.03
Reproductive allocation:				
chamber	1	1.15	30.60	<0.0001
population	5	0.74	19.87	<0.0001
chamber $\times$ population	5	0.43	11.47	<0.0001

two discrepancies in significance ($\alpha = 0.05$, after correcting for multiple comparisons using FDR) over all traits and effects: the chamber $\times$ population category effect is no longer significant for plant mass nor for number of seeds per infructescence.

Differences in developmental timing, or phenology, exist among the six *K. islandica* populations. The between-subjects “population” effect reveals significant differences in both the time taken for vegetative development (Table 4) and in the overall generative development (Table 5). Some of the populations tended to develop and flower earlier in the arctic chamber (results not presented), and several within-subjects interactions with chamber effect were significant for both vegetative and generative phenology (Table 4 and 5). For vegetative phenology, the within-subjects stage $\times$ population effect is marginally non-significant; the time taken for *K. islandica* to reach different vegetative phenological stages shows little difference among populations (Table 4). Vegetative traits of the arctic populations from Svalbard and Iqaluit developed earlier than these traits in alpine populations from Jasper and Colorado.

Generative phenology, however, shows more marked differentiation among populations, as indicated by the highly significant stage $\times$ population effect (Table 5). Again, the plants of the arctic populations from Svalbard and Iqaluit form buds and flowers several days earlier than the plants of the alpine populations from Jasper and Colorado. The plants from Dovre and Yukon have again a somewhat intermediate position: at the beginning of their life cycle the Yukon plants develop more like the arctic populations and the Dovre plants like the alpine populations, but at the end of their life cycle they form a category on their own.

These trends are more evident when the populations are collapsed into three categories: arctic, high latitude alpine and alpine (Table 1). The MANOVA indicates in this case that the stage $\times$ population category interaction is now significant for both vegetative and generative phenologies (results not presented) and the differentiation among these categories is more marked (Fig. 2). The three-way within-subjects interaction term provides further evidence for greater differentiation among populations in generative compared to vegetative phenology: differences in stage timing expressed between the two chamber environments are strongly dependent on population for generative (Table 5), but not for vegetative (Table 4) phenology.

Discussion

Annual plant species in arctic and alpine environments are subjected to particularly strong selective pressures from abiotic factors (Billings 1974), and are therefore expected to differentiate into marked ecological races. In this study we test for genetically based differentiation in morphology, life-history traits and phenology in six arctic and alpine populations of *Koenigia islandica*, originating from sites spread over a wide range of latitudes and elevations, with a correspondingly great variety of climatic conditions.

The results of the experiments performed in the simulated arctic and alpine environments suggest significant differentiation among populations. The use of material obtained in the laboratory, in which population positions were randomized during development, suggests that the differences are evolved and ecotypic rather than due to phenotypic plasticity generated by maternal or other environmental effects. The ecotypic nature of these dissimilarities is reinforced also by the comparatively minor differences observed among subpopulations of the Colorado population. This strong among-population divergence seems to reflect the reproductive strategies of *K. islandica*; in tundra environments as well, genetic variation in inbreeding species exists mainly among populations (Molau 1993).

Important differences observed among populations, regardless of experimental environment, confirm the general finding that ecotypes originating from higher latitudes or from harsher environments show smaller stature and decreased fecundity relative to more southern populations, or those from milder climates (Mooney and Billings 1961, Hickman 1975, Chapin and Chapin 1981, Porvin 1986, Gurevitch 1992, Winn and Gross 1993, Li et al. 1998, Heide 2005). For example, the larger average mass per seed

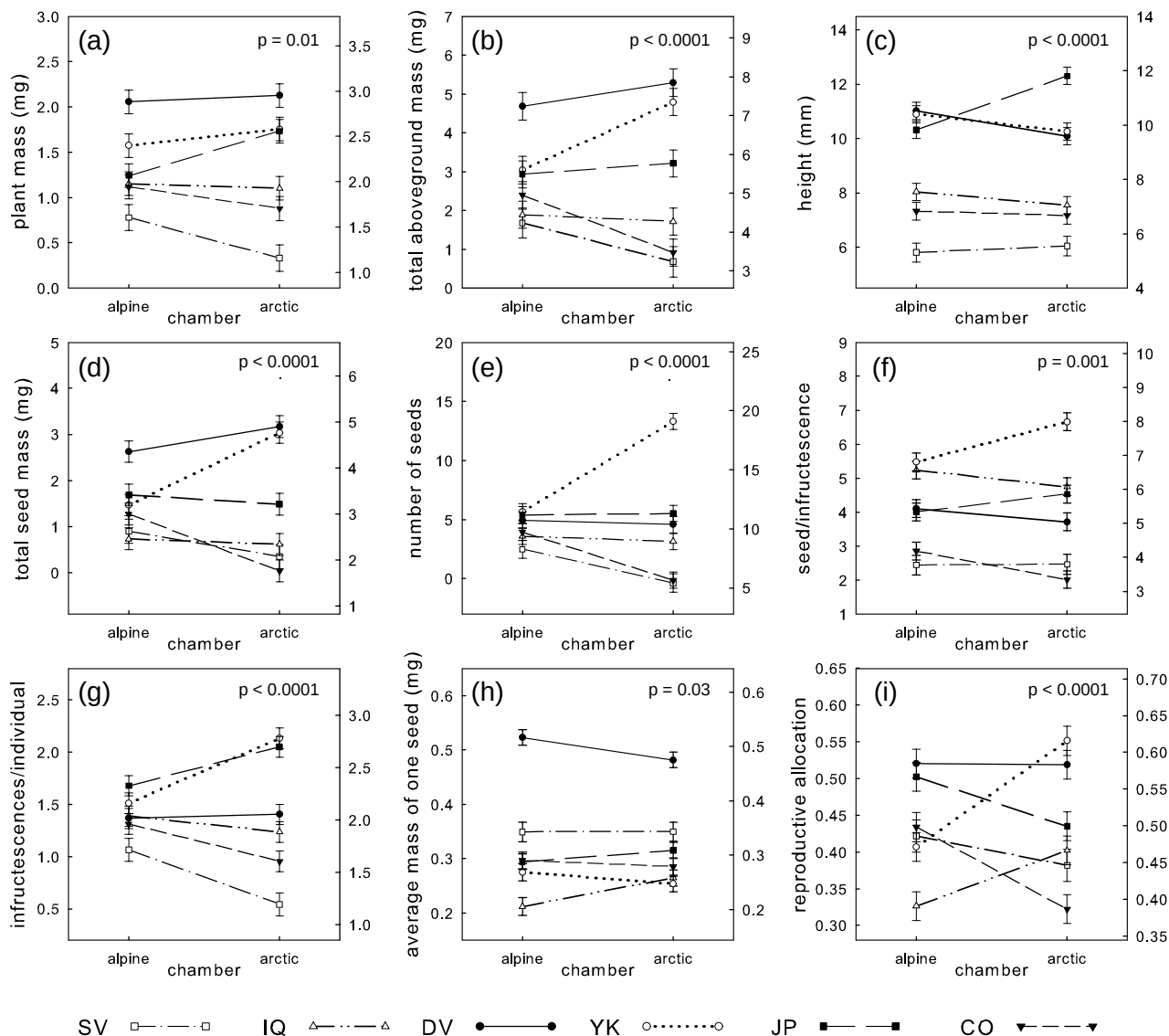


Figure 1. Morphological (a–c) and life-history traits (d–i) of six populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions. Trial 2. SV (dash-dotted line) – Svalbard; IQ (dash-dot-dotted line) – Iqaluit; DV (solid line) – Dovre; YK (dotted line) – Yukon; JP (long dashed line) – Jasper; CO (short dashed line) – Colorado. To represent chamber × population interactions rather than simple differences in trait means across environments, means for each trait are shifted to coincide along the two y-axes (see Methods). Left y-axis is for alpine chamber values; right y-axis for arctic chamber values. P-values are for chamber × population interaction. No false rejections (type-I errors) at $\alpha = 0.05$ were detected after controlling for false discovery rate. Error bars indicate ± 1 SD.

of the Dovre population appears to be a population-specific trait, and smaller overall sizes and lower seed production seem to be characteristics of the plants from Svalbard and Iqaluit, the northernmost among those studied. The small stature of plants from Colorado appears contradictory; however, whereas this population is the southernmost in this study and among the most southern populations in the global distribution of *K. islandica*, it also originates from the environment with one of the highest elevations and harshest climate, with some of the lowest growing season temperatures.

It is the relative performance of populations growing in the contrasting chamber environments, given by the chamber × population interaction, that provides information on local adaptation. In both trials, three populations from northern latitudes – Iqaluit, Dovre and Yukon – grew

better in the environment more closely simulating their environment of origin, i.e. the arctic chamber, while the performance of the low latitude alpine population from Colorado was enhanced relative to other populations when grown under alpine conditions. This result is consistent with local adaptation and the general observation that populations in reciprocal transplants do better in conditions closer to their environment of origin (Waser and Price 1985, Potvin 1986, Van Tienderen and Van der Toorn 1991a, Galen et al. 1991, Joshi et al. 2001, Macel et al. 2007). However, important exceptions to expected trends are the Svalbard and the Jasper populations. The negative slopes of reaction norms across alpine to arctic chambers for both vegetative mass and total seed mass of plants from Svalbard indicate that, relative to other populations, they did not thrive under the continuous photoperiod and cooler

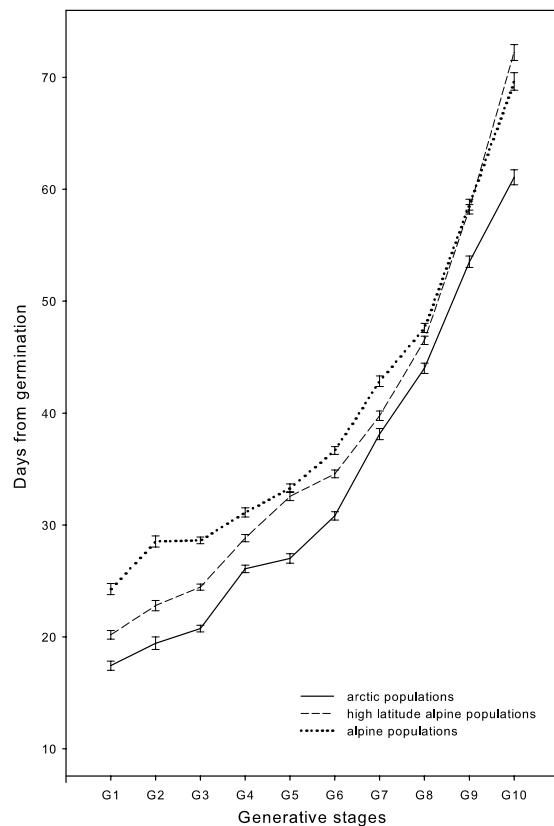


Figure 2. Generative phenology of six populations of *Koenigia islandica* grouped into one of three population categories: arctic, high latitude alpine and alpine. Mean timing of generative phenological stages (Table 2) of plants grown in simulated alpine and arctic conditions are represented.

temperatures of the simulated arctic environment; a most surprising outcome considering the high arctic origin of this population. These growth patterns, as well as most morphological traits of the Svalbard plants seem to be very similar to those of the Colorado population. Intriguing similarities between these two geographically remote populations were observed also in seed germination patterns (Wagner 2007): both required high germination temperatures and germinated slowly and incompletely, regardless of stratification treatment.

Also contrary to predictions is the finding that growth of the plants of the alpine population from Jasper was promoted, relative to other populations, in the arctic

chamber. This may be partially explained by the higher latitude of the Jasper population relative to that of Colorado. Still, individuals from Jasper accumulated a significantly higher biomass under the continuous photoperiod of this environment, an effect normally encountered in high latitude plants (Heide 1982) and confirmed here in the more northern Iqaluit, Dovre and Yukon populations.

The cases in which observed growth patterns do not fit predictions require further exploration. The simulated arctic and alpine environments used in our experiments are only crude approximations of the complex climates experienced by *K. islandica* in each location, which makes the classification into arctic and alpine sites imprecise and difficult. Moreover, the similarities in growth patterns between some populations may reflect phylogeographic relationships rather than the particular climate they experience. This may be the case for the Yukon and Jasper populations, a hypothesis that is consistent with observations that their seed germination patterns are also similar (Wagner 2007).

Reproductive allocation in plant species is usually constant within a population (Hickman 1977), with the few examples of environmentally-cued reproductive strategies found as adaptive responses to the short-term unpredictability of the environments in which the populations grow (Hickman 1975). In the case of *K. islandica*, reproductive allocation is variable among populations, and is plastic – but inconsistent among trials – within some populations; plants from Dovre in the first trial, and those from Iqaluit and Yukon in the second trial invested more in reproduction relative to other populations in the simulated arctic environment. The populations with the least growth – Svalbard, Iqaluit and Colorado – also had the smallest reproductive allocation. These observations appear to contradict the findings that populations from harsher, more northern environments have greater reproductive allocations (Potvin 1986), or in the case of plastic reproductive strategies, that populations invest more in reproduction when growing in harsher environment (Hickman 1975). However, in the present study, overall performance was reduced in the alpine chamber, a situation that may not be reflected in natural environments.

Consistent with previous research (Heide and Gauslaa 1999), *K. islandica* was found to be completely day-length neutral in flowering requirements; both low and high latitude populations flowered completely and abundantly in both arctic and alpine environments. This is in

Table 4. Multivariate analysis of variance for repeated measures for vegetative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions. Stages 0 and 6 (Table 2) were not included in the analysis.

Source	Value	Exact F	DF	DenDF	Prob > F
Between subjects:					
chamber	F = 0.07	2.81	1	42	0.10
population	F = 0.36	5.04	3	42	0.004
chamber × population	F = 0.03	0.45	3	42	0.72
Within subjects:					
stage	F = 155.24	679.18	8	35	<0.0001
stage × chamber	F = 0.69	3.04	8	35	0.01
stage × population	Wilks' λ = 0.40	1.56	24	102.11	0.065
stage × chamber × population	Wilks' λ = 0.50	1.15	24	102.11	0.31

Table 5. Multivariate analysis of variance for repeated measures for generative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions. Stages 0, 2 and 5 (Table 2) were not included in the analysis.

Source	Value	Exact F	DF	DenDF	Prob > F
Between subjects:					
chamber	F = 0.02	0.54	1	30	0.466
population	F = 0.41	6.09	2	30	0.006
chamber × population	F = 0.05	0.79	2	30	0.46
Within subjects:					
stage	F = 200.40	687.09	7	24	<0.0001
stage × chamber	F = 0.71	2.45	7	24	0.048
stage × population	Wilks' λ = 0.09	7.86	14	48	<0.0001
stage × chamber × population	Wilks' λ = 0.36	2.30	>14	48	0.016

agreement with the global distribution of this species; its probable origin in low latitude alpine environments of the Himalayas (Hedberg 1997), from where it spread into the arctic regions (Mesíček and Soják 1973, P. Hollingsworth pers. comm.) and into the mountain ranges of the rest of the northern hemisphere. Its day-length neutrality also permitted it to cross the short-day regions of equatorial mountains by mountain-hopping (Heide 2002), and establish itself in southernmost South America.

The significant differences in both vegetative and generative phenologies seem to be more closely correlated with the arctic or alpine origin of the populations than are the morphological and life-history characteristics. The succession of vegetative and generative phenological stages appeared to be clinally delayed from the most northern to the most southern populations. The early development and flowering of the high arctic Svalbard and arctic Iqaluit populations, and late development and flowering of the alpine Colorado population is consistent with the results of other studies, in which more northern populations developed and flowered earlier (Chapin and Chapin 1981, Potvin 1986, Winn and Gross 1993, Li et al. 1998). Remarkably, the Colorado population, which showed similar growth to the arctic populations from Iqaluit and especially Svalbard, had a markedly dissimilar phenology compared to these arctic populations. In fact, this low latitude alpine population had the latest flowering and the longest life cycle of all analysed populations. We speculate that this could be an adaptation to a longer and more predictable, even if relatively cold, growing season, as well as the relatively short photoperiod typical of these southern latitudes.

Trends with respect to habitat of origin are further clarified when phenological stages are plotted for the environmental category of each population. The rapid development and early flowering of the arctic populations could be an adaptation to the shortness and increased unpredictability of the growing season (Simons and Johnston 2003). By flowering soon after the first leaves are produced, these populations could take advantage and yield a few seeds even in the shortest and most unpredictable growing seasons (Cohen 1971). Interesting also is the tendency of true alpine populations to “accelerate” the post-flowering portion of development: they produce mature seed and senesce more rapidly than do high latitude alpine populations.

The results of this study provide clear evidence for ecotypic differentiation among arctic and alpine populations of the annual *K. islandica* in morphology, life-history traits and phenology. The reciprocal environment analysis provides evidence for local adaptation, but cannot be used to assess the adaptive significance of trait differences among populations originating along a complex gradient of arctic to alpine habitats. Molecular data on the phylogeographical relations among populations might further clarify the complex interactions between the observed ecotypes and their environment. Strong population differentiation implies that these arctic-alpine plants would be particularly sensitive to climate change; thus, this system merits more intensive study.

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