

Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change

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Summary

1. Alpine plants are at high risk because of climate change. Assessing the performance of alpine plant species across different altitudes is useful for predicting how they may respond to changing climate. Adaptation and plasticity of early life stages are of particular interest since seed germination and seedling establishment could be crucial life-history stages for environmental tracking and population persistence of sexually reproducing plants.

2. To evaluate past adaptation and the potential to accommodate future climate conditions, seeds and seedlings of *Erysimum capitatum* were reciprocally transplanted between alpine and low-altitude sites.

3. When grown in a common field environment, *E. capitatum* from alpine and lower-altitude populations differed from each other in germination, size and morphological traits. Planting altitude also influenced those traits, indicating that population differentiation and plasticity to altitudinal conditions both contributed to differences in the performance of high- vs. low-altitude plants. Seeds tended to germinate more in their native habitat than in the foreign habitat. Alpine plants survived more than low-altitude plants at high altitude, and they suffered higher mortality when they were planted in low-altitude sites. The production of multiple rosettes, a characteristic morphology of alpine *E. capitatum*, was negatively associated with survival at low altitude. In contrast to alpine populations, a survival advantage of low-altitude populations in low-altitude sites was not evident in this experiment.

4. *Synthesis.* Because climate change is projected to cause alpine environments to become more similar to low-altitude environments, alpine *Erysimum capitatum* is expected to suffer reduced seedling recruitment and higher mortality as a direct response to altered environment and possibly as a result of past adaptation to high altitude. In particular, the production of multiple rosettes, an adaptive trait to the current alpine environment, would constrain plant survival should those environments come to resemble low altitude. Moreover, the limited fitness advantage of low-altitude *E. capitatum* in low-altitude conditions suggests that environmental tracking by low-altitude populations might have a limited role in maintaining future populations.

Key-words: alpine, climate change, *Erysimum capitatum*, establishment, germination, local adaptation, natural selection, phenotypic plasticity, plant development and life-history traits

Introduction

Alpine ecosystems are at the edges of climatic clines, and they are considered to be highly endangered because of

climate change. Populations that are locally adapted to alpine conditions are expected to be at especially high risk of extinction (Holt 1990; Theurillat & Guisan 2001; Dinnetz & Nilsson 2002; Thomas *et al.* 2004; Parry *et al.* 2007). Such risk can be ameliorated if traits that affect fitness exhibit adaptive phenotypic plasticity to altered climatic factors, if these traits adapt genetically in situ or if adaptive genetic variants of those traits exist elsewhere within the local species

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range (Walther *et al.* 2002; Jump & Peñuelas 2005; Parmesan 2006). Thus, for predicting population viability in alpine environments, it is important to quantify phenotypic plasticity to climatic and site factors and to determine whether extant populations exhibit evidence of local adaptation to these factors.

Reciprocal transplants between contrasting habitats are powerful tools for detecting local adaptation and phenotypic plasticity (Claussen, Keck & Hiesey 1940; Reznick & Travis 1996; Kawecki & Ebert 2004). Careful interpretation of experiments conducted along an altitudinal gradient can provide critical information on the potential of alpine populations to persist in future environmental conditions (Etterson 2004); alpine environments are expected to become more similar to below tree line environments after climate change, so natural selection at low altitude might reflect future selection in alpine environments.

Numerous studies have documented variation along altitudinal gradients in plant morphology, life-history and physiological traits (reviewed in Körner 2003), and this differentiation is often interpreted as adaptation to conditions that vary across altitude. Local adaptation to altitude has also been demonstrated with reciprocal transplants that show home-site advantages in fitness components (Gauthier, Lumaret & Bedecarrats 1998; Byars, Papst & Hoffmann 2007; Giménez-Benavides, Escudero & Iriondo 2007; Gonzalo-Turpin & Hazard 2009). However, the traits and life stages that are subject to altitudinally variable natural selection are seldom identified, even though this is critical information for predicting outcomes of altered natural selection in response to climate change (but see Byars, Papst & Hoffmann 2007).

For practical reasons, many reciprocal transplant studies use rhizome, bulbs or root clumps rather than seeds or seedlings. However, seed germination and seedling establishment are critical life-history stages that strongly influence individual fitness and population dynamics (reviewed in Donohue *et al.* 2010), including alpine plant populations (Svensson *et al.* 1993; Giménez-Benavides, Escudero & Iriondo 2007; Marcante, Winkler & Erschbamer 2009; Kim & Donohue 2011a). Moreover, it is the seed stage that colonizes during range expansion or environmental tracking. Because natural selection at later developmental stages can be opposed to that at early stages (Atchley 1987; Schluter, Price & Rowe 1991; Schupp 1995; Poorter 2007; Mojica & Kelly 2010), one cannot infer the cumulative adaptive value of traits based only on late life stages. Reciprocal transplant studies using seeds as the initial stage may provide a novel insight into the adaptation of traits to alpine environments. Here, we present

results of a reciprocal transplant study that tests local adaptation across altitude, beginning with the seed stage.

Erysimum capitatum is a rosette-forming herbaceous plant found in the western United States and Mexico (Price 1987; Rollins 1993). It occurs in a broad range of altitudes in the Rocky Mountains from 1500 m a.s.l. to over 4000 m a.s.l. It exhibits natural variation in morphological and life-history traits, such that plants in alpine habitats exhibit an iteroparous perennial life cycle, multiple basal stems and low stature, whereas those at lower altitude are semelparous perennials with fewer basal stems and taller inflorescences (Kim & Donohue 2011a; E. Kim, personal observation). During a three-year observational study, fewer seedlings emerged in alpine than low-altitude populations, but their survival rate was higher than those in low-altitude populations. Seedling survival in low-altitude populations was often below 10% and was highly variable among years.

It is unknown whether this in situ variation is genetically based or environmentally induced and whether the observed variation in morphology contributes to local adaptation. Here, we present results of a reciprocal transplant experiment between alpine and low-altitude populations of *Erysimum capitatum* to address the following: i) Do populations of *E. capitatum* show home-site advantage across altitude? ii) Are the morphological and life-history differences observed in situ caused by phenotypic plasticity to altitude, population differentiation or both? iii) Does natural selection on variable morphological traits differ across altitude and contribute to home-site advantage?

Materials and methods

EXPERIMENTAL DESIGN

Reciprocal transplant sites were established in six natural populations of *Erysimum capitatum* in the Colorado Rocky Mountains in 2005 (Table 1). One population in alpine environments (altitude > 3200 m a.s.l.) ('H1', 'H2' or 'H3' hereafter) was paired with one population at below tree line ('L1', 'L2' or 'L3', respectively, hereafter) on each of three separate watersheds. H1 and L1 are located in the Gunnison Basin, in which most land area is above 2300 m a.s.l. (Mutel & Emerick 1992). L1 was in an open meadow where sagebrush was dominant, and L2 and L3 were in open understorey habitat in a Ponderosa pine forest. All natural populations had more than fifty individuals. Hereafter, plants from alpine tundra sites are referred to as 'high-altitude populations', those from below tree line as 'low-altitude populations', and each watershed is referred to as a 'region'.

Table 1. Planting sites used in the reciprocal transplant in the Colorado Rocky Mountains

Region	Site	Location (latitude/longitude)	Altitude (m)
1	H1	Cumberland Pass (N38°42'16.84", W106°29'11.40")	3551
	L1	Gunnison Public Land (N38°36'16.75", W106°49'27.69")	2638
2	H2	Loveland Pass (N 39°39'50.03", W 105°52'48.71")	3662
	L2	Lookout Mountain (N39°43'55.15", W105°14'50.68")	2286
3	H3	Niwot Ridge (N40°03'11.59", W105°35'20.05")	3509
	L3	Boulder Open Space (N39°57' 39.95", W105°16'16.53")	1863

Air temperature and volumetric soil water content were monitored during the experiment. HOBO UA-002 loggers (Onset Computer Inc., Pocasset, MA, USA) were set up under a tree or rock to measure temperature at each site. Daily average temperature of high-altitude sites was always lower than that of low-altitude sites for each region and each month (Fig. S1). Notably, minimum temperature in early June was still below 0 °C at high-altitude sites, which is consistent with previous reports for other alpine systems (Körner 2003). Volumetric soil water content of the six natural populations was measured every 2 weeks from June to August in 2007 using a HydroSense with a 12-cm rod (Campbell Scientific, Logan, UT, USA). Soil water content of high-altitude sites was higher than that of low-altitude sites ($F = 12.46$, $P < 0.001$), although soil water content of the L1 site was similar to high-altitude sites (Fig. S1).

Two experimental plantings were conducted: one that used seeds ('Seed transplant' hereafter) and one that used seedlings ('Seedling transplant' hereafter). For the seed transplant experiment, seeds from the resident population and seeds from the corresponding high- or low-altitude populations on the same watershed were sowed in each experimental plot in August 2005. Nine blocks were established in all plots, and 20 maternal genotypes (10 from the high-altitude population and 10 from the low-altitude population) were randomly positioned within each block. Ten seeds from an individual maternal plant were sprinkled on the soil surface of a peat pot (5.6 centimetre diameter $\times$ 5.6 centimetre depth; Jiffy Products of America, Lorain, OH, USA) filled with commercial soil medium (Metromix 360; SunGro Horticulture, Agawam, MA USA); artificial soil was used to better assess effects of climatic (as opposed to edaphic) differences across sites. Each block consisted of 2 $\times$ 10 pot array in which pots were planted with 0.5 m between them. Access strips with 0.6 m wide were made between blocks. Each pot was covered by Mason jar lid rims fitted with wire mesh screens to prevent seed contamination from natural populations. The screens did not alter temperature or light levels (Donohue *et al.* 2005). Natural vegetation in the experimental plots was not disturbed other than digging holes for the peat pots. A focal plant in each pot was randomly selected, and all other plants were removed at the end of their first growing season in 2006.

Sample sizes changed throughout experiments because of continuous mortality. Exact sample sizes are outlined in Table S1. In August 2006, all new germinants died at the L2 site, and only one plant survived at the L3 site (see Results). To better estimate post-germination performance, seedlings at the four- to six-leaf stage were planted in the same experimental plots of the seed transplant experiment in August 2006. Those seedlings were from the same maternal genotypes that were used for the seed transplant experiment. Seeds were germinated in the same type of pots with same soil medium used for the seed transplant, and seedlings were raised at a protected low-altitude location. Pots containing one seedling were planted between pots containing the seed transplants from 2005. Seedlings were watered for the first 2 weeks after planting to help establishment and prevent transplant shock. The diameters of transplanted seedlings were from two to five millimetres smaller than seedlings from the seed transplant at the H2 and H3 sites and approximately two millimetres larger than those at the H1 and L1 sites by the time they were established (Table S2).

Censuses were conducted during the growing season (June to August at high-altitude sites and May to August at low-altitude sites) every month in 2006 and approximately every 2 weeks in 2007 and 2008. The number of germinants and census-to-census survivorship were recorded. *E. capitatum* produces multiple rosettes at the pre-reproductive stage (Kim & Donohue 2011a,b). As measures of

vegetative size, the number of rosettes, mean number of leaves per rosette and maximum diameter of rosettes were measured in every census from 2006 to 2008.

STATISTICAL ANALYSIS

Statistical analyses used the SAS statistical package ver. 9.2 (SAS Institute Inc., Cary, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria). The same analyses were conducted separately for the seed transplant and seedling transplant experiments, and each region was analysed separately to accommodate unbalanced designs caused by site-specific mortality.

To test for a home-site advantage in survival, life tables were constructed using the Kaplan–Meier method, and hazard functions of the high- and low-altitude populations were compared using partial likelihood failure time analysis (R *coxme* package). Partial likelihood failure time analysis has statistical advantages in accounting for censored data compared to a linear model analysis of variance, and it does not require assumptions concerning the shape of a hazard function unlike other parametric failure time analyses (Allison 1995; Fox 2001). Survival time from germination to death was the dependent variable, and source altitude, planting altitude and the source altitude $\times$ planting altitude interaction were independent factors, and block and maternal genotype were included as random factors. Plants that were removed from the seed transplant at the end of the first growing season and plants that survived beyond the end of the experiment were treated as censored data. Hazard functions of populations were also compared within each planting site and between planting altitude.

Other measures of performance and morphology were compared across source altitude and planting altitude using mixed model analysis of variance (SAS *PROC MIXED*) with the same dependent variables as were used for the survival analysis. The proportion of seeds that germinated in each pot was examined. Differences of least-squared means between source altitudes and planting altitudes were evaluated based on Bonferroni adjustment. The proportion of seeds that germinated was arcsine-square-root transformed to satisfy normality assumptions. For focal plants, morphological traits such as mean leaf number per rosette, maximum diameter of rosettes and number of rosettes were also examined. Because of changing sample sizes (Table S1), standard analysis of variance was conducted for each month instead of repeated-measures analysis of variance, and significance was evaluated based on Bonferroni adjustment. Plant mortality during summer, especially in July and August, was extremely high at low-altitude sites, which caused an unbalanced experimental design. Because of this limitation, analysis of variance comparing source altitude effects was conducted within each site separately. The model included source altitude as an independent factor and the block and maternal genotype as random factors. Additional analysis of variance examining planting altitude effects was conducted within each source population separately for traits measured in June 2007. The number of rosettes was log-transformed to satisfy normality assumptions.

To examine associations between morphological traits and the duration of survival, Cox regression analysis was conducted (R *coxme* package). It should be noted that the hazard function is defined as the risk that the event (death) occurs at time t (Allison 1995), so a regression coefficient from Cox regression is interpreted in reverse to coefficients from standard selection analysis on survival; smaller negative values indicate higher survival. Morphological traits were independent variables, and time to death was the dependent variable.

All independent variables were standardized to have a mean of zero and standard deviation of one. Source population was included in the analyses to control for possible differences in survivorship between populations due to population differences in unmeasured traits. Block and maternal genotype were included as random factors in the model. Time to death during the summer season (from May to August at low-altitude sites and from June to August at high-altitude sites) and during the winter season (from September to the following June at high-altitude sites and from September to the following May at low-altitude sites) was analysed separately in each year. Plants that survived to the end of each season were treated as censored data. Morphological traits that were measured at the beginning of each season were used for the analysis. A univariate analysis was conducted with each independent variable separately ('Total effect'), and a multivariate analysis was conducted with all independent variables ('Direct effect') to estimate regression coefficients comparable to selection differentials (total selection) and selection gradients (direct selection) in standard phenotypic selection analysis (Lande & Arnold 1983). Both linear and quadratic terms were tested, and all two-way interactions among traits were included in the model. To facilitate the interpretation, hazard ratios of linear terms were calculated from regression coefficients. Hazard ratios in this analysis estimate a proportional change in the risk of mortality for an increase in one standard deviation in the trait value. For instance, a 0.60 hazard ratio for a trait would indicate that mortality risk decreases by 40% for an increase in one standard deviation in the trait value. To test for differences in the strength/direction of selection between planting altitudes within each region, we tested interactions between traits and planting altitude. Significant interactions would indicate significant differences in selection between planting sites.

Some low-altitude plants reproduced at the L2 and L3 sites in 2008. Standard phenotypic selection analysis was conducted on fruit number (Lande & Arnold 1983). Relative fitness was calculated as fruit number divided by the mean fruit number of all reproductive plants within each site. Total selection differentials, direct selection gradients and total and direct nonlinear selection coefficients were calculated. Genotypic selection analysis was not conducted because of small sample sizes.

Results

DIFFERENTIATION AND PLASTICITY OF GERMINATION

The earliest life stage that was measured – seed germination – exhibited both population differentiation and plasticity to altitude (Fig. 1). Seeds from high-altitude populations germinated more than those from low-altitude populations at all sites except L1. Significant source altitude $\times$ planting altitude interactions were found in all regions (region 1, $F_{1, 322} = 59.99$, $P < 0.001$; region 2, $F_{1, 322} = 14.59$, $P < 0.001$; region 3, $F_{1, 322} = 5.14$, $P < 0.05$), suggesting planting altitude effect differed between high- and low-altitude populations. Seeds tended to germinate more in their native habitat than in the foreign habitat in all regions, but the difference of least-squared means between high- and low-altitude sites was significant only in the H1, L1 and L2 populations. Notably, seeds from high-altitude populations exhibited a lower proportion of germination in low-altitude environments compared to high-altitude environments.

A HOME-SITE ADVANTAGE IN POST-GERMINATION SURVIVAL

Within each region, source altitude $\times$ planting altitude interactions were significant in the seed experiment (region 1, $\chi^2 = 7.90$, d.f. = 1, $P < 0.01$; region 2, $\chi^2 = 4.00$, d.f. = 1, $P < 0.05$; region 3, $\chi^2 = 30.14$, d.f. = 1, $P < 0.001$) and the seedling experiment (region 2, $\chi^2 = 28.30$, d.f. = 1, $P < 0.001$; region 3, $\chi^2 = 4.20$, d.f. = 1, $P < 0.05$) except in region 1 in the seedling experiment ($\chi^2 = 0.03$, d.f. = 1, $P = 0.86$). High-altitude populations exhibited a home-site advantage in survival in most instances, but low-altitude populations did not (Fig. 2). In high-altitude sites, plants from high-altitude populations had higher cumulative survival compared to those from low-altitude populations in both seed and seedling transplant experiments, except at the H1 site in the seedling transplant (Fig. 2, Table 2). In low-altitude sites, even though low-altitude populations had higher survival than high-altitude populations in several instances, the source altitude effect was significant only at the L3 site in the seed transplant and L2 site in the seedling transplant. The L1 population had higher mortality than the H1 population at the L1 site, even though it exhibited higher survival than the H1 population in the summer 2007.

The low-altitude environment was associated with lower survival in many instances, especially for high-altitude plants (Fig. 2, Table 2). When plants from high-altitude populations were transplanted to low-altitude sites, their survival was lower compared to those in high-altitude sites in both seed and seedling transplant experiments. In contrast, the planting altitude effect on the survival of low-altitude populations differed among populations and between seed and seedling transplant experiments. In the seed transplant, the survival of the L2 and L3 populations was higher in high-altitude sites than in their native low-altitude sites. An opposite pattern was observed in the seedling transplant, such that the L2 and L3 populations had higher survival in low-altitude sites than in high-altitude sites. Survival of the L1 population did not differ between high- and low-altitude sites in the seed experiment, but it was lower in low-altitude site in the seedling experiment. It therefore appears that the low-altitude environment at the L2 and L3 sites is harsh for plants that experienced it as seeds and young seedlings.

POPULATION DIFFERENTIATION AND PLASTICITY IN VEGETATIVE TRAITS

Plants from native populations tended to grow more vigorously than those from foreign populations in most instances (Table S3 and Figs 1, S2 and S3), although low sample sizes caused by low germination rate or high mortality prevented these differences from being significant in many cases. At high altitude, plants from high-altitude populations had more leaves per rosette, larger rosette diameters and more rosettes than plants from low-altitude populations, except at the H1 site in the seedling experiment where no significant source altitude effect was detected throughout the experiment. At

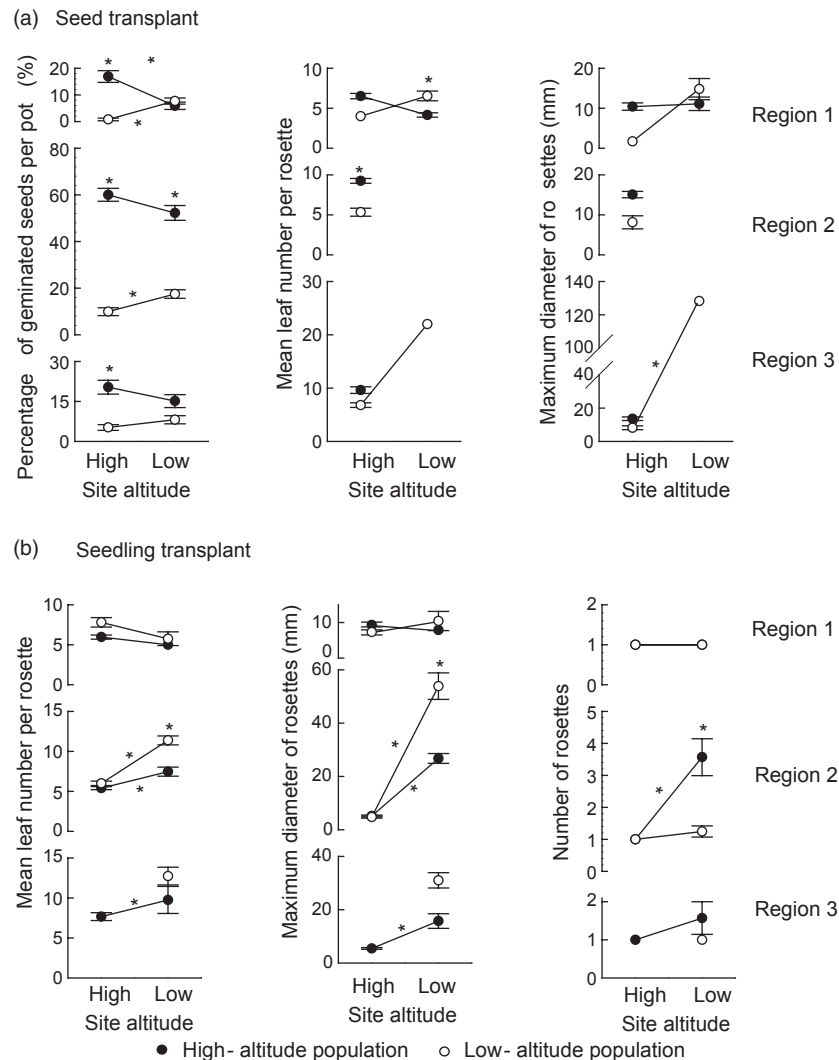


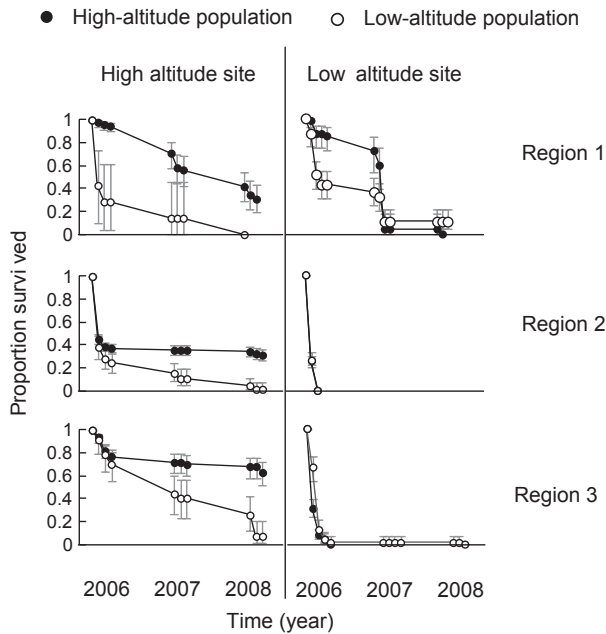
Fig. 1. Population differences in germination of the seed transplant and morphological traits of plants from the seed and seedling transplants. For morphological traits, average trait values and standard errors measured in June 2007 are given because of extremely low survival during summer, especially in July and August. Filled circle represents high-altitude populations, and open circle represents low-altitude populations. Number of rosettes for seed transplant is not given because only small proportion of plants produced multiple rosettes. *F* ratios of the source altitude effect and the planting altitude effect are given in Table S3. Morphological traits measured during experiments are presented in Figs S2 and S3. Asterisk denotes significant differences after Bonferroni adjustment. Significant differences between planting altitudes are indicated by asterisks near the line connecting the same population, and significant differences between source populations are given above the data point.

Table 2. Results of analysis of variance to compare the hazard function between source altitudes within each planting site and between planting altitudes within each population. The full model includes block and maternal genotype. Chi-square values of source altitude or planting altitude effect are given

Source altitude effect (d.f. = 1)			Planting altitude effect (d.f. = 1)		
Planting site	Seed transplant	Seedling transplant	Source population	Seed transplant	Seedling transplant
H1	16.32***	0.01	H1	12.46***	4.84*
H2	3.84+	5.90*	H2	22.18***	21.44***
H3	7.78**	12.82***	H3	89.49***	14.82***
L1	5.90*	0.18	L1	2.10	3.84+
L2	0.00	30.91***	L2	18.58***	4.88*
L3	5.76*	0.69	L3	29.27***	8.41*

dgd+<0.1, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

(a) Seed transplant



(b) Seedling transplant

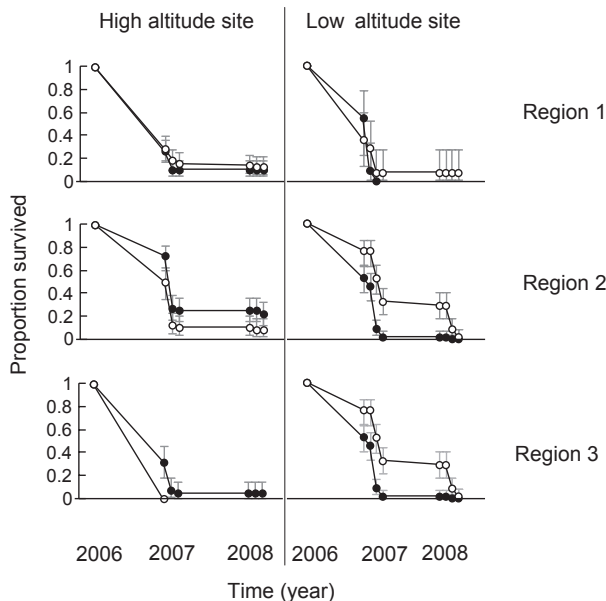


Fig. 2. Survival curves of seed and seedling transplants. Filled circles represent high-altitude populations, and open circles represent low-altitude populations, and error bar represents 95% confidence interval. Chi-square values of the source altitude effect and the planting altitude effect are given in Table 2.

low altitude, low-altitude plants had more leaves per rosettes and larger rosette diameter than high-altitude plants whenever data were available. Notably, while high-altitude plants tended to have smaller rosettes than low-altitude plants in low-altitude sites, they produced more rosettes than low-altitude plants did in the sites in which they survived (L2 and L3 sites in the seedling transplant experiment).

The planting altitude effect on the size of plants was not significant in region 1 in both seed and seedling transplant

experiment, but plants appeared slightly larger size in their native habitat (Fig. 1). In contrast, planting altitude influenced the growth of plants in region 2 and 3, with plants at low-altitude sites being larger than those at high altitude in the seedling transplant experiment. Therefore, even though conditions at the L2 and L3 sites were so harsh that almost all germinants died in the summer 2006, spring conditions the following year likely induced rapid plant growth.

NATURAL SELECTION ON MORPHOLOGICAL TRAITS

In the seed transplant experiment, regression analyses could not be performed on plants grown at the L2 and L3 sites because of high mortality. Generally, larger plants had a lower risk of mortality in both seed and seedling transplant experiments (Tables 3, 4, S5 and S6). Plants with more leaves per rosette or larger rosettes had a lower risk of death during the winter 2006 (H1, H2 and L1 sites in the seed transplant; H1, H2 and L3 sites in the seedling transplant), the summer 2007 (H1 and L1 site in the seed transplant; H1, H2 and L2 sites in the seedling transplant) and the winter 2007 (H1 site in the seed transplant). Significant stabilizing selection was detected on leaf number or rosette diameter for overwinter survival in 2007–2008 at the H3 site in the seed transplant (mean leaf number per rosette, quadratic regression coefficient = 0.76, SE = 0.21, $P < 0.001$; maximum diameter of rosettes, quadratic regression coefficient = 0.74, SE = 0.23, $P < 0.05$) and for overwinter survival in 2006–2007 at the H2 site in the seedling transplant (maximum diameter of rosettes, quadratic regression coefficient = 0.32, SE = 0.12, $P < 0.01$). While nonlinear selection was detected at the H1 site in the seed transplant experiment (during summer 2007, maximum diameter of rosettes, quadratic regression coefficient = -10.50 , SE = 5.08, $P < 0.05$; during winter 2007, mean leaf number per rosette, quadratic regression coefficient = 3.88, SE = 1.74, $P < 0.05$), survival increased or decreased monotonically with increasing plant size (Fig. S4). In the seed experiment, selection at high altitude was stronger than at low altitude in region 1 during the summer 2007 (maximum diameter of rosettes, total effect, χ^2 (trait $\times$ planting altitude) = 4.12, d.f. = 1, $P < 0.05$). A similar pattern was also found in the seedling experiment in region 2 during the winter 2006 (mean leaf number per rosette, total effect, χ^2 (trait $\times$ planting altitude) = 4.20, d.f. = 1, $P < 0.05$; maximum diameter of rosettes, total effect, χ^2 (trait $\times$ planting altitude) = 6.66, d.f. = 1, $P < 0.01$).

Notably, significant correlational selection was detected between rosette diameter and rosette number for summer survival at the L2 site in the seedling experiment (regression coefficient = 1.13, SE = 0.49, $P < 0.05$; Fig. S6). Plants with larger rosettes had high fitness only if they had few rosettes, and plants with more rosettes had high fitness only if their rosettes were small. Thus, selection actually favoured fewer rosettes, provided those rosettes were large.

Some low-altitude plants reproduced in L2 (88%, $n = 17$) and L3 (79%, $n = 14$), but no plants reproduced in the H1, H2 and L2 sites, and only 3 of 40 H3 plants reproduced in

Table 3. Results of Cox regression analysis of the seed transplant at the H1 and L1 sites. Results for the other sites are presented in Table S4 because of lack of data for low-altitude transplant sites. Hazard ratios of standardized variables are given. Regression coefficients of the number of vegetative rosettes had values of zero or extremely large standard errors because only a small proportion of plants had multiple rosettes in all transplant sites, so their hazard ratios are not given in the table. Source altitude was included as a fixed factor, and block and maternal genotypes were included as random factors in the model. *N*, sample size. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	Winter 2006–2007		Summer 2007		Winter 2007–2008	
	Total	Direct	Total	Direct	Total	Direct
(a) H1 site	<i>N</i> = 54		<i>N</i> = 40		<i>N</i> = 32	
Mean leaf number per rosette	0.49*	3.41	0.10*	0.56	0.24*	0.70
Maximum diameter of rosettes	0.20**	0.03**	0.06***†	0.09	0.19*	0.27
(b) L1 site	<i>N</i> = 40		<i>N</i> = 29		<i>N</i> = 6	
Mean leaf number per rosette	0.41	1.07	0.59	0.98	‡	‡
Maximum diameter of rosettes	0.17*	0.15	0.54*	0.55	‡	‡

†Significant difference between high and low altitude in regression analysis.

‡Regression analysis was not conducted because of small sample size.

Table 4. Results of Cox regression analysis of the seedling transplant. Hazard ratios of standardized variables are given. Source altitude was included as a fixed factor, and block and maternal genotypes were included as random factors in the model. Except at the L2 site in summer 2007, hazard ratios of the number of vegetative rosettes were not given because only a small proportion of plants had multiple rosettes. Results of H3 and L3 sites are presented in Table S5. *N*, sample size. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	Winter 2006–2007		Summer 2007	
	Total	Direct	Total	Direct
(a) H1 site	<i>N</i> = 147		<i>N</i> = 41	
Mean leaf number per rosette	0.81	0.86	0.33***	0.60
Maximum diameter of rosettes	0.74*	0.80	0.24***	0.30**
(b) L1 site	<i>N</i> = 25		<i>N</i> = 10	
Mean leaf number per rosette	1.11	0.91	0.49	0.68
Maximum diameter of rosettes	1.20	1.29	0.43	0.51
(c) H2 site	<i>N</i> = 111		<i>N</i> = 69	
Mean leaf number per rosette	0.80†	1.08	0.56***	0.75
Maximum diameter of rosettes	0.59*†	0.55	0.44***	0.54**
(d) L2 site	<i>N</i> = 127		<i>N</i> = 75	
Mean leaf number per rosette	0.99	1.09	0.86	0.92
Maximum diameter of rosettes	0.88	0.83	0.40***	0.43**
Number of rosettes	‡	‡	0.80	0.83

†Significant difference between high and low altitude in regression analysis.

‡Regression model did not converge mainly because of small sample size or lack of variation.

the H3 sites. Thus, the life cycle of L2 and L3 populations can be completed more quickly in their native sites. In L3, plants with more leaves (regression coefficient = 0.49, SE = 0.09, $P < 0.01$) and larger rosettes (regression

coefficient = 0.45, SE = 0.11, $P < 0.05$) produced more fruits, but no relationship between any trait and fruit production was found in L2.

Discussion

Erysimum capitatum exhibited home-site advantage in the form of higher survival at their native sites, especially for plants from high altitude. High- and low-altitude populations differed in seed germination, growth and morphology. Phenotypic plasticity in response to altitude was also detected. Thus, differential plant performance observed in natural populations across altitude is attributable to direct environmental effects (plasticity) as well as population differentiation caused by genetic differentiation and/or maternal environmental effects. Plants at low altitude suffered higher mortality, but showed faster growth and reproduction of those that survived. Such trends are consistent with the previous observational study (Kim & Donohue 2011a).

LOCAL ADAPTATION TO HIGH ALTITUDE

Erysimum capitatum from high-altitude populations exhibited a survival advantage compared to low-altitude populations when grown in their native high-altitude sites. In addition, plants from alpine populations survived more at high altitude than at low altitude. These results suggest local adaptation of alpine *E. capitatum* to its native alpine environment with respect to survivorship.

Seeds from alpine *E. capitatum* tended to germinate to higher levels than those from low-altitude populations, especially under alpine conditions. This difference in germination proportion could be the result of differences in seed viability, such that seeds might suffer higher mortality at foreign sites. Alternatively, seed dormancy may differ between populations from high vs. low altitude, and high-altitude conditions may promote germination more strongly than those at low altitude. For instance, a long and wet chilling period is known to be

effective at stimulating germination in alpine plant species with physiological dormancy (Bliss 1971; Baskin & Baskin 1998; Giménez-Benavides, Escudero & Iriondo 2007). Notably, germination occurred more under conditions favourable for survival and was suppressed under less favourable conditions, which is consistent with adaptive germination cueing (Donohue *et al.* 2010).

In this experiment, survival advantages of alpine plants were evident at the early developmental stage. Given that survival and growth of juvenile plants significantly contribute to projected population growth rate of some alpine herbaceous plants and alpine *E. capitatum* in particular (Svensson *et al.* 1993; Marcante, Winkler & Erschbamer 2009; Kim & Donohue 2011a), adaptation at early developmental stages might play an important role in the persistence of those alpine plant populations. Studies that do not use plants at early developmental stages may miss important episodes of natural selection.

While this experiment documented significant population differentiation, we cannot distinguish between genetic differentiation and maternal environmental effects, since we used seeds collected from natural environments. Environmental conditions can influence seed characteristics, which in turn influence plant performance. It should be noted, however, that source altitude effects on survival were significant even when seedlings rather than seeds were used, and population differences were still apparent after a complete growing season in some cases, suggesting that genetic differentiation likely contributes to population differentiation, at least in survival at these high-altitude sites.

PLANT PERFORMANCE AT LOW ALTITUDE

Even though alpine environments are often considered to be extreme among stressful environments, low-altitude conditions appear to be more stressful to alpine plants. Low soil water content during the summer seems to impose higher mortality of alpine plants in the low-altitude L2 and L3 sites. First, plants at those sites suffered high mortality during the summer, in which soil water content was consistently low in this experiment as well as previous *in situ* observations of natural populations (Kim & Donohue 2011a). In both experiments, plants showed a characteristic drought response, such that leaves often turned yellow and curled over the apical meristem during summer (E. Kim, personal observation). In addition, experimental studies of *E. capitatum* populations further showed drought as a critical factor that induces the differential survival of high- and low-altitude genotypes (Kim & Donohue 2012), which was also observed in this experiment. Decreasing soil water content in lower-altitude environments was also reported in Mediterranean mountain environments (Giménez-Benavides, Escudero & Iriondo 2007; Gonzalo-Turpin & Hazard 2009), suggesting that summer drought and consequent short summer growing seasons may be common conditions to which low-altitude plants must adapt.

In *E. capitatum*, rosette number and size were associated with plant survival during summer at one low-elevation site.

This result is consistent with a recent study that demonstrated a negative effect of multiple rosettes on survival under drought conditions (Kim & Donohue 2012). Lower leaf area may reduce water loss (Chaves, Maroco & Pereira 2003), and producing fewer rosettes may be a more energetically efficient way to accomplish this than to reduce leaf size of multiple rosettes.

IMPLICATIONS FOR RESPONSES TO CLIMATE CHANGE

Average temperature is projected to increase in alpine environments in response to climate change, which would influence diverse environmental factors. For instance, increased temperature might induce earlier snowmelt, longer growing season and higher evapotranspiration rate of soil water, which would consequently lower soil water content in the summer growing season (Taylor & Seastedt 1994; Harte *et al.* 1995; Calanca 2007; Lambrecht *et al.* 2007; Solomon *et al.* 2007; Inouye 2008; Anderson *et al.* 2012). In the southern Rocky Mountains where reduced precipitation is expected (U.S. Global Change Research Program 2009), such a decrease in soil water content would be accelerated. Thus, at least in the experimental sites of this experiment, environmental conditions in alpine environments are likely to become more similar to those at lower elevations with higher temperature and low soil water contents as global climate change progresses.

If climate change causes alpine environments to become more similar to present lower-altitude environments, the results suggest that alpine *E. capitatum* is expected to suffer reduced seedling recruitment and higher mortality. First, given the critical role of wet chilling period in stimulating germination of alpine plant species with physiological dormancy, increasing temperature at higher altitude could potentially reduce seed germination in high-altitude populations by changing critical germination cues. Germination cueing to environments that no longer exist may actually restrict the opportunity for adaptation to changed conditions (Donohue *et al.* 2010). Secondly, novel drought stress would impose higher mortality of alpine plants at the early developmental stage. In particular, the production of multiple rosettes, which is a developmental prerequisite of iteroparity in alpine *E. capitatum*, would cause high mortality in drier conditions (Kim & Donohue 2011b). Iteroparity, a life-history strategy with repeated reproduction, is common in alpine environments and is considered to be adaptive there (Bliss 1971; Körner 2003). Such past adaptation to alpine environments would therefore be selectively disadvantageous in novel, drier conditions.

Low-altitude populations, with their own adaptations, may contribute to the persistence of high-altitude populations via dispersal and gene flow, such that standing genetic variation may contribute to the maintenance of future populations. However, potential environmental tracking by low-altitude genotypes is likely to have a limited effect since even low-altitude populations had lower survival under low-altitude conditions. Therefore, even with environmental tracking, climate change is expected to cause population decline.

Evolutionary adaptation to novel environmental conditions may be necessary for the persistence of populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Number of plants of which morphological traits were measured.

Table S2. Results of analysis of variance to test for morphological differences between seed transplants and seedling transplants.

Table S3. F ratios from the analysis of variance for the morphological traits of seed transplants and seedling transplants.

Table S4. Results of Cox regression analysis of the seed transplants at the H2 and H3 sites.

Table S5. Results of Cox regression analysis of the seedling transplant at the H3 and L3 sites.

Figure S1. Temperature and soil water content in the transplant sites.

Figure S2. Population differences in morphological traits of plants from the seed transplant within each planting site.

Figure S3. Population differences in morphological traits of plants from the seedling transplant within each planting site.

Figure S4. Non-linear relationships between morphology and fitness for plants in the seed transplant.

Figure S5. Non-linear relationships between morphology and fitness for plants in the seedling transplant.

Figure S6. Relationship between relative fitness and morphological traits in the L3 site during the summer 2007.