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Changes in reproductive investment with altitude in an alpine plant

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Abstract

Aims

In perennial species, the allocation of resources to reproduction results in a reduction of allocation to vegetative growth and, therefore, impacts future reproductive success. As a consequence, variation in this trade-off is among the most important driving forces in the life-history evolution of perennial plants and can lead to locally adapted genotypes. In addition to genetic variation, phenotypic plasticity might also contribute to local adaptation of plants to local conditions by mediating changes in reproductive allocation. Knowledge on the importance of genetic and environmental effects on the trade-off between reproduction and vegetative growth is therefore essential to understand how plants may respond to environmental changes.

Methods

We conducted a transplant experiment along an altitudinal gradient from 425 to 1 921 m in the front range of the Western Alps of Switzerland to assess the influence of both altitudinal origin of populations and altitude of growing site on growth, reproductive investment and local adaptation in *Poa alpina*.

Important findings

In our study, the investment in reproduction increased with plant size. Plant growth and the relative importance of reproductive investment decreased in populations originating from higher altitudes compared to populations originating from lower altitudes. The changes in reproductive investment were mainly explained by differences in plant size. In contrast to genetic effects, phenotypic plasticity of all traits measured was low and not related to altitude. As a result, the population from the lowest altitude of origin performed best at all sites. Our results indicate that in *P. alpina* genetic differences in growth and reproductive investment are related to local conditions affecting growth, i.e. interspecific competition and soil moisture content.

Keywords: *Poa alpina* • transplantation • altitudinal gradient • genetic diversity • phenotypic plasticity

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INTRODUCTION

In alpine ecosystems, the large altitudinal differences that occur across relatively small distances, combined with small-scale differences in relief and exposure and soil properties, produce complex environmental heterogeneity affecting alpine plant life (Körner 2003). In the severe climatic environment at high altitudes, seed production and new plant establishment may be a particularly risky mode of propagation because of infrequent germination, low seedling survival (Bliss 1971; Forbis 2003; Scherff *et al.* 1994) and higher nutrient demands than for vegetative reproduction (Harper 1977; Watson 1984). Therefore, vegetative reproduction and persistence through clonal

growth is a major adaptation of arctic and alpine plants to the extreme climatic conditions and tends to increase with altitude (Bliss 1971; Klimes *et al.* 1997; Milla *et al.* 2009). Increased allocation in vegetative growth is expected to reduce the availability of resources for reproduction. Therefore, a trade-off between the allocation in vegetative growth and reproduction has been observed in several plant species (Eriksson, 1992; Harper 1977; Piquot *et al.* 1998) and in relationship with altitude (Bliss 1971; Johnston and Pickering, 2004; Klimes *et al.* 1997).

Selection pressures due to environmental conditions acting on this trade-off may lead to genetic selection in favour of a particular reproductive behaviour in a specific environment

and, hence, to local adaptations (Galen *et al.* 1991; Loehle 1987). In addition, plants may respond by phenotypic plasticity, i.e. adjustment of performance not related to genetic differences (Bradshaw, 1984). Indeed, the relationship between vegetative growth and reproduction may be highly plastic in response to different environments (Fischer and Fiedler 2002; Sultan 2000). Although local adaptation of important life-history traits in plants have been widely studied since the famous transplantation experiment of Clausen *et al.* (1940), see also (Núñez-Farfán and Schlichting 2001), its evidence is controversial: local adaptation on a small scale (Gauthier *et al.* 1998; Nagy and Rice 1997) or on a broader continental scale (Joshi *et al.* 2001; Prock and Körner 1996), little or no evidence for local adaptation (Galloway and Fenster 2000), one best performing genotype in several transplanted sites (Santamaria *et al.* 2003). Improving our knowledge on the importance of genetic and of environmental effects on the relationship between vegetative growth and reproduction and on local adaptation is of great importance to understand how plants may respond to environmental changes.

Here, we used the proportion of the number of reproductive tillers by the total number of tillers as a proxy for reproductive allocation to assess the effect of altitudinal population origin and of environmental conditions on the relationship between reproduction and vegetative growth by transplanting populations along an altitudinal gradient. We assured that altitudinal difference was the most important environmental effect that transplanted plants experienced among sites by minimizing other natural sources of variation, particularly competition and soil moisture content.

We addressed the following hypotheses: (i) the relative investment in reproduction decrease with increasing altitude of population origin (genetic effect); (ii) with increasing altitude of growing site (environmental effect) and (iii) populations growing more closely to their altitude of origin have higher reproductive performances.

MATERIALS AND METHODS

The study species

Poa alpina (Poaceae) has a circumpolar, arctic and alpine distribution (Polunin 1959). This perennial plant is characteristic of nutrient-rich meadows and pastures of the subalpine and alpine zones and is a major fodder species in alpine regions (Hegi 1930; Rudmann-Maurer *et al.* 2007; Steiner *et al.* 1997). Genetically, the species constitutes an aggregate of polyploid ecotypes (Müntzing 1980), with chromosome numbers ranging in Switzerland from $2n = 22$ to 46 (Duckert-Henriod and Favarger 1987). While some individuals of *P. alpina* reproduce sexually by seeds, others reproduce vegetatively by pseudovivipary, forming plantlets in the panicles instead of seeds (Pierce *et al.* 2003). However, because it is likely that seeds are produced vegetatively by apomixis in *P. alpina*, we made no distinction in this experiment between these two modes of reproduction at the level of the inflorescences. Here, we use 'vegetative growth' for the production of vegetative tillers

and 'reproduction' for the production of reproductive tillers (inflorescences), independent of the presence of seeds or plantlets. 'Growth' includes production of both tiller types.

An intensive field survey in the neighbouring mountain ranges (Randin *et al.* 2006) during the summers 2002–04 (Guisan 2005) showed that the realized altitudinal range of *P. alpina* along the transect was between altitudes of ~1 200 and 2 950 m.

Study sites

Four sites of population origin of *P. alpina* and five experimental (growing) sites were selected along an altitudinal transect ranging from 425 to 2 156 m above sea level (a.s.l.) in the front range of the Western Alps of Switzerland (Fig. 1, Table 1). The sites were meadows or pastures (i.e. natural herbaceous vegetation or managed grasslands) with the same south-eastern exposure to minimize climatic variability between sites (Table 1). The lowest growing site, G425 (G for 'growing site' and the number for meter a.s.l.), was located in a protected area in the Rhone Valley. All others sites, G631 to S2156 (S for 'site of population origin'), were located at successive elevations. The sites of origin were all located in pastures (Table 1). Mean annual air temperature of the transect ranged from 10.0°C at G425 to 1.4°C at S2156, and mean annual sum of precipitation ranged from 1 070 mm at G425 to 1 860 mm at S2156 (Table 1). Long-term monthly means for average temperature (°C) and sum of precipitation (mm) for the period from 1961 to 90 were derived from the Swiss National Meteorological Station Network. Linear lapse rates were first calculated (i.e. rate of change along elevation) for long-term (1961–90) monthly mean temperature and monthly rainfall taken from the national meteorological networks of Switzerland (MeteoSwiss). Next, the monthly values were normalized to sea level (0 m a.s.l.), using the regression lapse rates fitted along the elevation gradient, and interpolated the 0-m data to the whole surface of both study areas using inverse distance weighted interpolations. Finally, the spatially interpolated values (representing locally adjusted regression intercepts) were re-projected to actual elevations using a 25-m digital elevation model and the regression lapse rates (for details, see Zimmermann and Kienast 1999).

Experimental design

In summer 2003, a square of 5 × 5 m at each growing site was cleared from all the vegetation and fenced. On the 13 July 2004, 50 or more flowering individuals of *P. alpina* were collected at the four sites of origin: S1280, S1701, S1921 and S2156. Individuals had to be 1 m apart to be sampled. Plants of the same source population are defined hereafter as a 'population of origin'. Tussocks from these individuals were split and one reproductive tiller for each individual was planted in a single 1 600-ml pot containing a mixture of 4/10 compost (Klasmann-Deilmann, Geeste, Germany), 2/10 sand (Decia-Vicat, L'Isle d'Abeau, France) and 4/10 standardized sterilized topsoil (University of Lausanne) and grown for 7 days in a greenhouse at the University of Lausanne. We then recorded

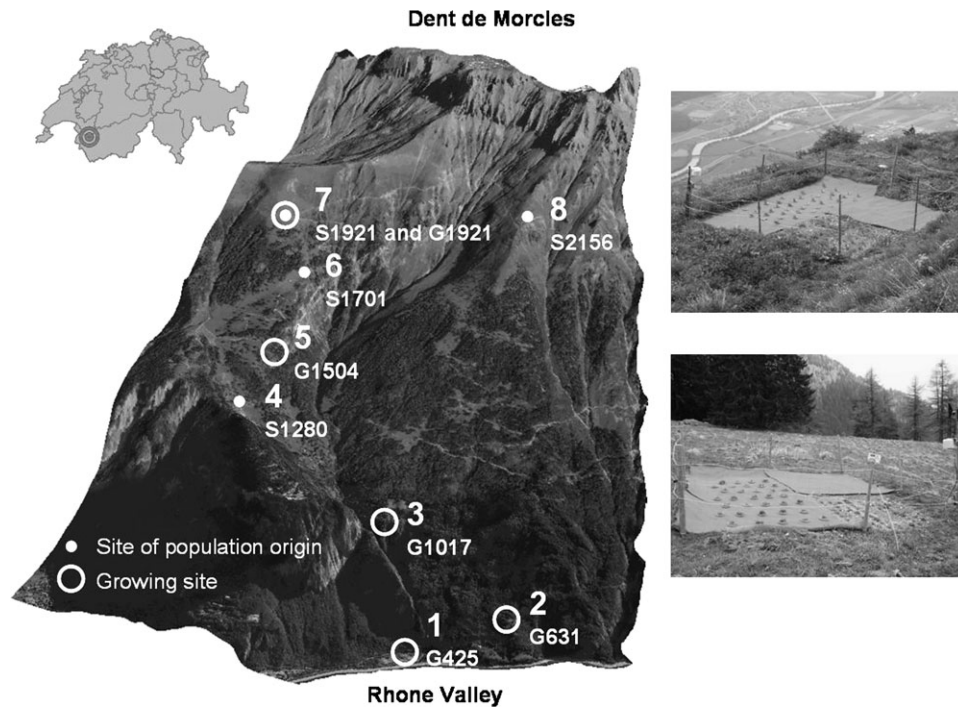


Figure 1: location of sites of population origin (S) and of growing sites (G) in the front range of the Western Alps of Switzerland. The pictures show two of the five growing sites with the carpets around the experimental pots.

Table 1: location, altitude, vegetation type, mean annual temperature and annual precipitation of the study sites (G for ‘growing site’, S for ‘site of population origin’ and the number for the altitude a.s.l.)

Sites	Latitude	Longitude	Altitude (m)	Vegetation type	Temperature ^a (°C)	Precipitation ^a (mm)
Growing site						
G425	568100	116400	425	Natural vegetation	9.98	1078.6
G631	568538	115896	631	Meadow	8.95	1160.9
G1017	568673	116870	1 017	Cow pasture	7.09	1330.3
G1452	569530	118150	1 452	Cow pasture	4.90	1531.7
G1921	570346	118518	1 921	Cow pasture	2.49	1747.7
Site of population origin						
S1280	569066	118150	1 280	Cow pasture	5.62	1469.4
S1701	570107	118242	1 701	Cow pasture	3.62	1647.6
S1921	570346	118518	1 921	Cow pasture	2.49	1747.7
S2156	570732	116943	2 156	Sheep pasture	1.32	1860.5

Climate data were derived from the Swiss national meteorological station network at different altitudes. Long-term monthly means for average temperature (°C) and sum of precipitation (mm) for the period 1961–90 were used.

^a Annual means.

the initial height of plants. This measure was used as a random term in the statistical analysis to take into account the differences in initial sizes when assessing the main treatment effects (growing site and site of population origin) and their interaction (see Statistical analyses). On 21 July 2004, pots were randomly transplanted (in rows, 30-cm intervals) to five growing sites along the transect: G425, G631, G1017, G1504 and G1921. A total of 30 individuals were transplanted to each

growing site: eight plants from each of the populations of origin S1280, S1921 and S2156 and six plants from the population S1701. Three of the five growing sites were located at lower elevations than the natural altitudinal range of occurrence of *P. alpina* in the region. To minimize plant competition, carpets were placed around the experimental pots (see Fig. 1). On each growing sites, plants were watered three times a week during the dry seasons with local water.

Measurements

During the second year of the experiment (2005), the following measurements were made at biweekly intervals: 'Vegetative traits' consisted of the tussock base diameter and the number of vegetative tillers. 'Reproductive traits' consisted of the number of reproductive tillers (one inflorescence per reproductive tiller). Survival was recorded during 2004–05, so that each individual experienced a winter season. When the total number of flowering tillers per individual exceeded seven, we measured the total length of the shoots on the smallest and the tallest flowering shoots and on five additional randomly selected shoots. The mean was calculated for statistical analyses. While we acknowledge that biomass is usually used to measure resource allocation to vegetative growth versus reproduction, biomass data taken at one time point would not consider the phenological differences along our wide altitudinal gradient. Clearly, some individuals would be at peak biomass while others would be at peak growth or senescing. In our study, we considered phenological differences among growing sites and among sites of population origin at each growing site by taking maximum plant size (tussock base diameter), maximum number of tillers (both vegetative and reproductive), maximum number of reproductive tillers and the proportion of the number of reproductive tillers by the total number of tillers as an entry for statistical analysis. By considering phenological differences along our experimental gradient, we consider our biometric data appropriate to be used as a proxy for reproductive allocation.

For each population of different origin, we estimated in each growing site and season the fecundity (m) as the average total number of inflorescences and the survival probability (p) from survival data (see Joshi *et al.* 2001). For each growing season, we calculated the effective fecundities (F) as $F = p \times m$ (Silverstone and Lovett Doust, 2001). Dominant eigenvalues were obtained from the Leslie matrices for each population of different origin in each growing site for two growing season (Charlesworth, 1994). Dominant eigenvalues correspond to the finite rate of a population increase (λ) and are an estimate of a plant's reproductive performance during the period of the experiment (Joshi *et al.* 2001). The reproductive coefficient was then calculated for each population i relative to the best sexually reproductive population at a particular growing site as $s_i = 1 - (\lambda_i/\lambda_{\max})$ (McGraw and Antonovics 1983). The most successful (best reproductive performance) population at a growing site will have a reproductive coefficient of zero, while a complete selection against reproductive performance of a population will be indicated by a coefficient equal to unity. The reproductive coefficients were also calculated for each growing site relative to the best sexually reproductive growing site for a particular population.

Statistical analyses

We used generalized linear mixed-effects models (GLMMs; (Faraway 2005; Gelman and Hill 2007; Maindonald and Braun 2007) since our design includes fixed and random effects and

our responses include variables with normal and non-normal error distributions. GLMMs are generalized linear model that include random effects. We analysed differences in continuous response variables with linear mixed-effects analysis of variance (ANOVA) using restricted maximum likelihood with the lmer function from the lme4 library (Bates 2005) for R 2.8.0 (R Development Core Team 2008). The lmer function currently does not provide F -tests for fixed effects. Instead, we reconstructed the ANOVA table according to Faraway (2005). 'Growing site', the differences due to experimental site, indicating plasticity in a measured trait; site of population origin, indicating genetic differentiation among populations of different origin; and their interaction were treated as fixed effects. 'Initial size', taking into account the differences in plant size before transplantation was treated as random effect. Data that were analysed using normal error distribution included tussock base diameter, number of vegetative tillers, number of reproductive tillers and reproductive coefficient. Data with non-normal error distributions included plant survival, which was analysed with a binomial error distribution.

In the statistical analyses, a main 'growing site' effect reveals environmental variation due to different growing sites, whereas a main site of population origin effect reveals genetic variation among populations due to different sites of origin. Interaction effects show that populations of different origins respond differently when transplanted to distinct growing sites. In the site \times population matrix (Table 5a and 5b), sorted by increasing altitude (Table 1), a home effect is reflected by low values when close to the site of population origin, whereas a distance effect is demonstrated through increasing values when further away from the site of population origin.

RESULTS AND DISCUSSION

We found strong evidence for genetic differentiation in all vegetative and reproductive traits measured among populations of *P. alpina*. Plant size decreased with increasing altitude of population origin. As the proportion of reproductive tillers increased with plant size, investment to reproduction decreased in populations from higher altitudes compared to populations from lower altitudes. We found low phenotypic adjustment due to environmental conditions at the growing sites and unrelated to altitude (e.g. interspecific competition and/or soil moisture content). As a result of this low phenotypic plasticity, the reproductive performance of the population from the lowest site of origin was higher in all growing sites.

Effects of plant size on reproduction

Decreasing plant size as an adaptation to increasing altitude is a well-known phenomenon. It results from a slower growth rate that may allow plants to use resources more efficiently in severe climatic environments (Bennington and McGraw 1995; Grime 1979). Moreover, a positive relationship between plant size and reproductive allocation has been demonstrated in many plant species (Samson and Werk 1986; Stöcklin and

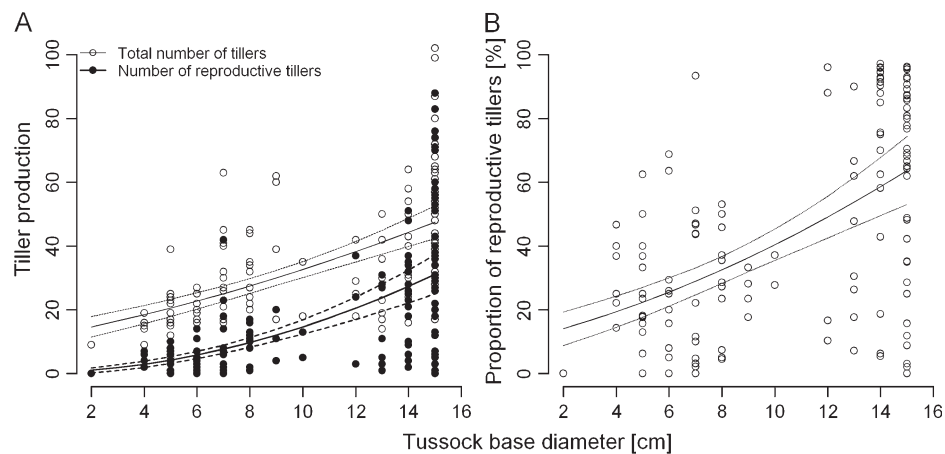


Figure 2: vegetative growth and reproduction in *Poa alpina* as a function of plant size. The solid lines represent the fit of the estimates obtained by the GLMM and the dashed lines represent the 95% confidence intervals. (A) Total number of tillers (sum of vegetative and reproductive tillers) and number of reproductive tillers (number of inflorescences). (B) Reproductive investment (number of reproductive tillers divided by the sum of vegetative and reproductive tillers).

Table 2: genetic (population origin) and environmental (growing site) effects and their interaction on plant traits

	DF den.	Population origin		Growing site		Population origin \times growing site	
		DF num.	<i>F</i>	DF num.	<i>F</i>	DF num.	<i>F</i>
Tussock base diameter	117	3	103.8***	4	5.8***	12	2.8**
Total tiller number	117	3	34.7***	4	23.0***	12	2.6**
Inflorescence number	117	3	134.4***	4	23.8***	12	4.3***
Reproductive coefficient	129	3	54.0***	4	9.7***	12	1.7

DF den., degrees of freedom for denominator; DF num., degrees of freedom for numerator. DF den. and DF num. and *F* values are from ANOVA. The reproductive coefficient is calculated from both the first and second years (2004 and 2005), and all other traits are from the second year (2005). Significance levels are represented by asterisks: ****P* < 0.001; ***P* < 0.01.

Favre 1994; Weiner 1988). As expected, the size of *P. alpina* individuals decreased with increasing altitude of origin. However, since the number of reproductive tillers increased more than the total number of tillers with the size of plants (Fig. 2A), the proportion of reproductive tillers and, therefore, the investment in reproduction increased with plant size (Fig. 2B) as has been observed in other alpine plant species (Pluess and Stoecklin 2005), demonstrating that *P. alpina* individuals may alter their reproductive behaviour with size.

The size-dependent changes in reproductive behaviour might be explained by physiological differences between reproductive and vegetative parts of the plant. Vegetative growth in *P. alpina* is mainly self-sustainable through photosynthesis in leaves and stems, while the production of reproductive units (seeds and bulbils) is more nutrient demanding and, therefore, more costly than vegetative growth (Harper 1977; Watson 1984). Additionally, alpine plants endure the difficult conditions of the alpine climate. In these circumstances, to reduce mortality, high-altitude plants should allocate a higher proportion of their resources first in persistence and storage reserves and, thus, to vegetative growth. This may result in reduced or postponed reproduction (Kozłowski 1992; Roach and Gampe 2004;

Wesselingh *et al.* 1997). In our experiment, a similar absolute number of non-reproducing tillers were formed independent of size, indicating a threshold size for reproduction. With increasing size, relatively more tillers were reproductive, indicating that the shift in reproductive behaviour in *P. alpina* is size related.

Altitudinal and environmental effects on growth and reproduction

Populations responded differently to the environmental conditions of the different growing sites (Table 2, significant interaction between population origin and growing sites). As an example, the proportion of reproductive tillers generally decreased with increasing altitude of population origin (Fig. 3A); however, the shape of the reproductive investment over growing sites (lines in Fig. 3B) differed among populations of different origin. Investment in reproduction for plants from the lowest population origin (S1280) was highest in the lowest growing sites (G425, G631) and decreased with increasing altitudes of growing sites. Plant population originating from site S1701 had the highest investment in reproduction at growing sites of medium elevation (G1017, G1504). Finally, plants

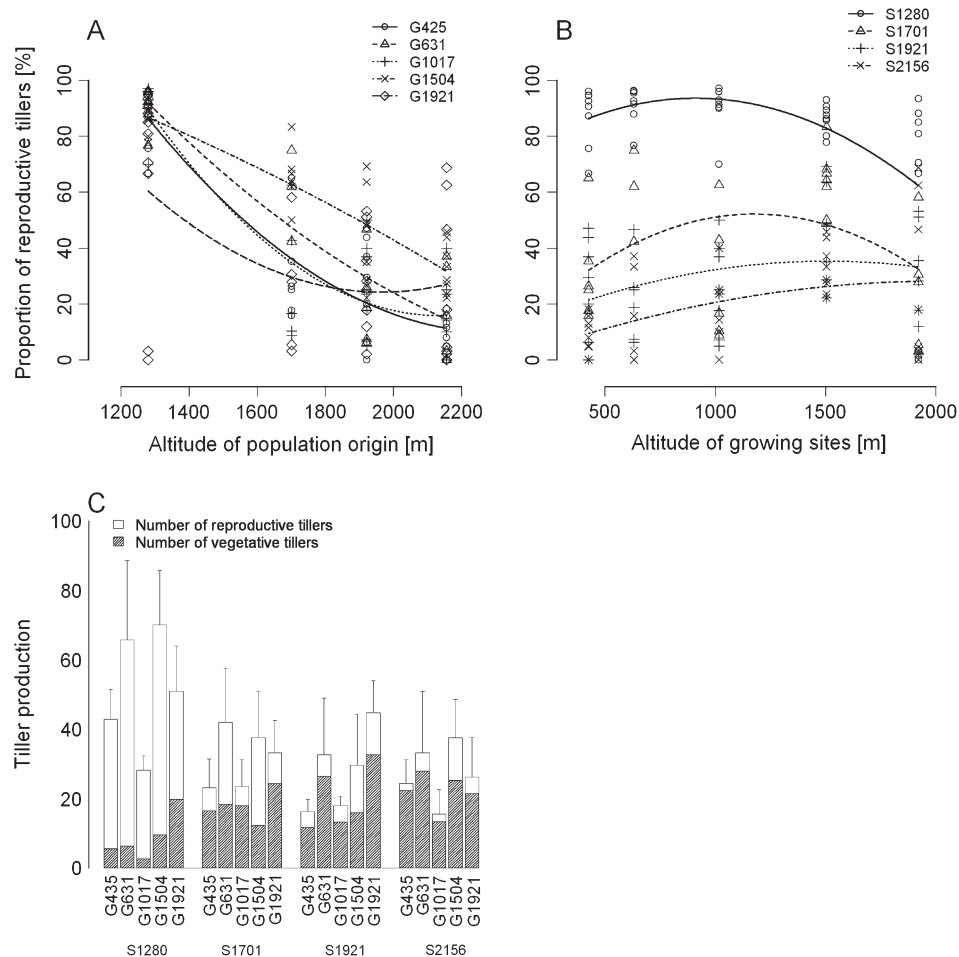


Figure 3: altitudinal effect of population origin and growing site on vegetative growth and reproductive investment in individuals and populations (lines) of *Poa alpina*. (A) Reproductive investment (number of reproductive tillers divided by the sum of vegetative and reproductive tillers) as a function of altitude of population origin and (B) as a function of the altitude of growing sites. (C) Mean number of reproductive and vegetative tillers and their sum (\pm standard error for the sum) across altitude of population origin (S) and altitude of growing sites (G).

Table 3: means (\pm SE) of plant traits of *Poa alpina* for each population of different origin averaged over all growing sites

Site of population origin	Altitude (m)	Tussock base diameter		Total tiller number		Inflorescence number	
		Means \pm SE	%	Means \pm SE	%	Means \pm SE	%
S1280	1 280	14.25 \pm 1.41	0	51.58 \pm 20.56	0	42.75 \pm 20.07	0
S1701	1 701	13.56 \pm 2.20	5	31.00 \pm 12.26	40	13.40 \pm 10.99	69
S1921	1 921	8.83 \pm 4.10	38	27.89 \pm 14.49	46	8.36 \pm 6.59	80
S2156	2 156	6.27 \pm 1.48	56	26.95 \pm 12.74	48	5.32 \pm 5.27	88
Mean %			33		45		79

The highest value for each plant trait of a population is given in bold. The percentage indicates how much lower the trait value of a population is relative to the best population for that trait. Mean percentage indicates overall variation from genetic differences due to population origin.

originating from the highest elevations of S1921 and S2156 had increasing investment in reproduction with increasing altitudes of growing sites (highest values at G1921).

Altitudinal effects on growth and reproduction

We found pronounced differences in growth and reproduction among populations of *P. alpina* related to the altitudinal origin

of populations indicating a genetic differentiation among populations ($P < 0.001$, Table 2). On average, values of plant traits decreased linearly with increasing altitude of population origin (Table 3). Plants originating from the highest altitude were 56% smaller, produced 48% less tillers and 88% less inflorescences compared to the plants originating from the lowest altitude. As the number of reproductive tillers decreased faster with

Table 4: means (\pm SE) of plant traits of *Poa alpina* for each growing site averaged over all plants of different origin

Growing site	Altitude (m)	Tussock base diameter		Total tiller number		Inflorescence number	
		Means \pm SE	%	Means \pm SE	%	Means \pm SE	%
G425	425	9.93 \pm 4.56	17	27.31 \pm 12.25	41	13.34 \pm 15.80	53
G631	631	11.00 \pm 4.29	9	46.09 \pm 23.68	0	27.68 \pm 27.82	1
G1017	1 017	9.48 \pm 4.15	21	21.14 \pm 7.31	54	9.93 \pm 10.37	65
G1504	1 504	12.03 \pm 3.85	0	44.10 \pm 20.91	4	28.10 \pm 22.29	0
G1921	1 921	10.46 \pm 3.96	13	39.18 \pm 14.63	15	14.86 \pm 15.95	47
Mean %			15		29		41

SE, standard error. The highest value for each plant trait at a growing site is given in bold. The percentage indicates how much lower the trait value at a site is relative to the best growing site for that trait. Mean percentage indicates overall variation from differences due to environmental conditions of growing sites (plasticity).

Table 5: reproductive coefficients (derived from the finite rate of population increase, see Materials and methods for details) for *Poa alpina* populations transplanted into near or distant sites on an altitudinal gradient

Site of population origin	G425	G631	G1017	G1504	G1921	Means \pm SE
a. Growing site						
S1280	0.000	0.000	0.000	0.000	0.000	0.00 \pm 0.00
S1701	0.400	0.623	0.477	0.251	0.426	0.44 \pm 0.13
S1921	0.549	0.628	0.406	0.385	0.310	0.46 \pm 0.13
S2156	0.563	0.720	0.522	0.379	0.407	0.52 \pm 0.14
Means \pm SE	0.38 \pm 0.26	0.49 \pm 0.33	0.35 \pm 0.24	0.25 \pm 0.18	0.29 \pm 0.20	
b. Growing site						
S1280	0.141	0.011	0.236	0.000	0.260	0.130 \pm 0.12
S1701	0.313	0.503	0.467	0.000	0.433	0.343 \pm 0.20
S1921	0.370	0.401	0.262	0.000	0.170	0.241 \pm 0.16
S2156	0.395	0.553	0.411	0.000	0.293	0.330 \pm 0.21
Means \pm SE	0.305 \pm 0.11	0.367 \pm 0.25	0.344 \pm 0.11	0.000 \pm 0.00	0.289 \pm 0.11	

SE, standard error. A coefficient of zero indicates that a population is the fittest one at a particular site with a selection advantage over all other populations at that site. (a) Averaged across origin of populations (S). (b) Averaged across growing sites (G).

increasing altitude of population origin than the production of tillers, the proportion of reproductive tillers decreased with increasing altitude of population origin. On average, 83% of the tillers from plants originating from the lowest altitude were reproductive, while only 20% of the tillers from plants originating from the highest altitude were reproductive (Fig. 3A).

Environmental effects on growth and reproduction

Reproductive and vegetative traits also differed significantly among growing sites ($P < 0.001$, Table 2), indicating phenotypic plasticity induced by environmental conditions at the growing sites; however, the variation in these traits was less pronounced across growing sites and surprisingly unrelated to the altitude of growing sites ($P > 0.33$, Table 4). This indicates that conditions not related to altitude were mostly responsible for plastic responses. In general, plants from a particular population origin produced a comparable number of inflorescences and tillers across altitudes of growing sites

(Fig. 3C). Therefore, no significant relationship was observed between the proportion of reproductive tillers and altitude of growing sites ($P = 0.98$, Fig. 3B).

Numerous studies have shown population differentiation in response to small-scale environmental heterogeneity (McGraw 1987; Rochow 1970; Schaal 1975; Waser and Price 1985), which was sufficient to change the reproductive outcome of plants. Young *et al.* (2002) showed higher importance of vegetative growth of *Rutidosis leirolepis* with increasing altitude. In our study, as expected, across populations of different origin, *P. alpina* invested relatively more resources in reproduction at low altitude and relatively more in vegetative growth at higher altitudes. As discussed above, these differences in the investment in reproduction and vegetative growth of plants were mostly an effect of differences in plant size determined by population origin and, hence, genetic effects.

Surprisingly, little change in reproductive investment was observed across growing sites, and these environmentally

induced effects were also not linearly related to the altitude of growing sites. Hence, each population origin exhibited a phenotype that was largely maintained in different growing sites with apparently little variation due to local environmental conditions, suggesting a strong genetic and a low environmental effect of altitude on growth and reproduction of *P. alpina*. The observed plasticity was probably also not related to abiotic conditions such as soil nutrient or moisture content, slope or exposition, nor was it related to intra- or interspecific competition since these conditions were controlled or removed in all growing sites. Therefore, in our experiment, environmental effects among growing sites are probably related to changes in temperature. However, the differences among the abiotic conditions of growing sites turned out to have a relatively low impact, indicating that temperature per se is not a strong driver of environmentally induced variation.

Local adaptation

A significant main effect of growing sites and population origin ($P < 0.001$) was also shown for the reproductive coefficient (Table 2). Averaged across populations (Table 5a), selection was strongest against populations from the highest site of origin S2156 (reproductive coefficient: 0.52 ± 0.14 , mean \pm SEM), while the population from the lowest site of origin (S1290) performed best at all growing sites (0.000 ± 0.00). Averaged across growing sites (Table 5b), negative selection was strongest at site G631 (0.37 ± 0.25), whereas all plants performed best at site G1504 (0.000 ± 0.00).

We expected to detect the best reproductive performances for populations growing close to their home sites indicating adaptation to the particular altitudinal condition of their origin, i.e. temperature. However, because of the low effect of growing sites, each population largely kept its characteristic phenotype once transplanted to other altitudes. As a result, the overall performance of the population originating from the lowest altitude was best at all growing sites. This best performing ecotype could possibly have resulted from a selective pressure not related solely to altitude, such as different grazing pressures or an adaptation to particular nutrient condition of a site. However, as we found a linear decrease in reproductive investment with altitudinal origin, it is more likely that the best performance of this population results from a higher growth rate at low altitude that was maintained at all growing sites. As competition was minimized at different growing sites and soil moisture conditions kept constant, it is likely that the putative ecotypic differences among populations are related to changes in the competitive environment and seasonal soil moisture content that dominates at different altitudes.

CONCLUSIONS

Our results suggest that populations of *P. alpina* from different altitudes invest resources according to differences in their size. Due to a negative relationship between plant size and altitude, this results in a higher investment in reproduction in popula-

tions from low altitude and in vegetative growth in populations from higher altitudes. In contrast to the genetic differentiation due to altitudinal origin, phenotypic plasticity of *P. alpina* was low and unrelated to altitudinal change. Our results suggest that genetic population differentiation in this common grassland species most likely result from altitudinal differences in interspecific competition and/or soil moisture content since the variation due to temperature alone was weak.

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