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ORIGINAL PAPER

Relationship between phenotypic differentiation and glacial history in a widespread Alpine grassland herb

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Abstract In the European Alps, alpine species were able to survive periods of glaciation by retreating to lower-lying refugia surrounding the Alps. This temporary separation of populations in refugia has often led to genetic differentiation and the appearance of phylogeographic lineages, which are still detectable after postglacial recolonisation. Recently, evidence has been accumulating that glacial history also affected differentiation of phenotypic traits, but it is yet unknown to what extent postglacial connectivity among populations influenced the signature left behind by the history of glaciation. In this study, we demonstrate differentiation in phenotypic traits among three phylogeographic lineages of the widespread subalpine grassland herb, *Geum montanum*, using a common garden approach. We observed regional differentiation in 5 out of 16 traits, which was a small portion compared with a closely related species that has been previously studied, *Geum reptans*. This may possibly be due to species-specific differences in their respective distributions: *G. montanum* has well-connected populations whereas *G. reptans* occurs more often in isolated high-alpine environments. Results of a clipping treatment, designed to address responses in phenotypic traits to stress, showed regional differentiation in the response in number of flowers produced. This is potentially due to adaptations shaped by unknown histories of herbivory during glacial survival. We suggest that glacial history may leave its mark on the current phenotypic variability even of Alpine plants that are relatively common with well-connected populations.

Keywords Adaptation · Common garden · European Alps · Fitness homeostasis · Herbivory · Trait–environment correlations

Introduction

The history of glacial advances and retreats was accompanied by the migrations of species following their climatic niches (Abbott 2008). In the European Alps, glaciations forced most species to retreat from higher elevations and survive these periods in refugia at the periphery of the Alps (Merxmüller 1954; Schönswetter et al. 2005; Parisod 2008). Survival of species in small and isolated refugia is linked with increased genetic drift due to presumed small population size and genetic bottlenecks (Knowles and Richards 2005). In line with this prediction, survival in refugia has been associated with genetic differentiation among descendent populations, which led to speciation (e.g. Kadereit et al. 2011) or to intraspecific phylogeographic lineages that are still detectable with neutral molecular markers today (Alvarez et al. 2009; Thiel-Egenter et al. 2009; Kuss et al. 2011).

Recent quantitative genetic studies suggest that phylogeographic lineages may also differ in phenotypic traits

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(Frei et al. 2012; Scheepens and Stöcklin 2011; Scheepens et al. 2013). For example, the population genetic structure of *Campanula thyrsoides*, which is a rare subalpine to alpine bell flower with isolated occurrences on calcareous soils throughout the European Alps and Jura mountains, was strongly linked to differentiation in all the tested morphological and functional traits (Kuss et al. 2011; Scheepens et al. 2013). Similarly, phenotypic differentiation was correlated with molecular differentiation in all 11 morphological and functional traits that were tested in *Geum reptans* (Frei et al. 2012), which is a high-alpine clonal herb occurring on naturally isolated glacier forefields, high-alpine screes, and crests on siliceous rocks in the European Alps, the Carpathian Mountains and on the Balkan Peninsula. These studies also observed differentiation among populations within phylogeographic lineages, which must have occurred during and since postglacial recolonisation, since the populations within a region are presumably derived from a single refugial, potentially panmictic, population (Hewitt 1996; Schönswetter et al. 2005).

Phenotypic differentiation, resulting from a history of surviving glaciation in isolated refugia, may be retained in Alpine species with currently restricted gene flow among populations due to naturally fragmented habitats (Kadereit et al. 2011). Mixing of genetic material during and since postglacial recolonisation may erase differentiation that had accumulated during glacial survival. Thus, Alpine species with more continuous distributions would be expected to show less phenotypic differentiation than species with more restricted distributions. In line with this expectation, *Campanula barbata*, which is a common subalpine grassland herb with a more continuous distribution than *C. thyrsoides* (Aeschimann et al. 2004), showed comparatively weak differentiation in phenotypic traits between phylogeographic lineages (Scheepens and Stöcklin 2011).

To further explore the hypothesis that species connectivity has a diminishing effect on the signature of glacial history, we investigated phenotypic differentiation in the widespread subalpine grassland herb, *Geum montanum*. This species is closely related to a previously studied species, *G. reptans* (Smedmark and Eriksson 2002), but has a more continuous distribution in the European Alps (Aeschimann et al. 2004). Populations of *G. montanum* in the European Alps have been shown to form three major phylogeographic lineages: a western group in the French Alps; a central group over Switzerland and Vorarlberg (Austria); and an eastern group east of Vorarlberg. This suggests that three major refugia existed during the height of the last glaciation (Thiel-Egenter et al. 2009). We applied a common garden approach to investigate whether phenotypic trait differentiation in *G. montanum* is consistent with patterns in its phylogeography associated with refugial survival. Moreover, based on its more common and less patchy distribution than *G. reptans*, which is assumed to enhance intraspecific mixing of genetic material, we would expect weaker phenotypic differentiation in *G. montanum* than in *G. reptans*. In other words, we expect a lower fraction of its phenotypic traits to show significant differentiation.

Genetic differentiation in phenotypic traits can result from both neutral and selective forces. It is virtually impossible to trace the selection pressures that acted during glacial survival since past environmental conditions are largely unknown. Such selection pressures may well have caused adaptation, niche differentiation and speciation (Hewitt 1996; Margraf et al. 2005). For instance, two subspecies of *C. thyrsoides* that survived in different refugia (Kuss et al. 2011) showed contrasting flowering phenology, which has been related to their refugial climate and subsequently shaped patterns in current distribution (Scheepens et al. 2011, 2013). In contrast, selection during and since postglacial recolonisation may lead to adaptation of traits to their current environment as seen, for instance, along elevation gradients (Gonzalo-Turpin and Hazard 2009; Frei et al. 2012). It is well known that temperature and atmospheric pressure decrease while radiation increases with elevation (Körner 2003, 2007). Moreover, soil depth and soil organic matter diminish with elevation above the montane zone, and climate predictability decreases with elevation (Körner 2003). These selection pressures may be related to phenotypic patterns along elevation, such as smaller stature (e.g. Gonzalo-Turpin and Hazard 2009; Hautier et al. 2009), higher investment in roots (Körner and Renhardt 1987) and earlier flowering (Sandring et al. 2007). To explore potential adaptation in *G. montanum*, we studied correlations of population trait-values in the common garden with environmental conditions at their respective sites of origin (Blanquart et al. 2013).

To investigate the response of phenotypic traits to simulated herbivory, we included a clipping treatment in our common garden study. Many subalpine and alpine pastures, habitats in which *G. montanum* occurs, may be subjected to weaker livestock grazing pressure with increasing elevation. This gradient has likely increased in strength due to ongoing land-use intensification at lower elevations and land abandonment at higher elevations (Gehrig-Fasel et al. 2007; Stöcklin et al. 2007). Even such relatively recent changes in land use have been reported to cause phenotypic differentiation via micro-evolutionary processes in *Poa alpina* (Fischer et al. 2011) and other species (Pluess 2013). In addition, pressure exerted by natural herbivores has been shown, mainly for insects, to decrease with elevation accompanied by a corresponding decline in concentration of herbivore defence chemicals in plants (Pellissier et al. 2014). We, therefore, predict differentiation in herbivore resistance within phylogeographic lineages as a result of postglacial adaptation to decreasing levels of natural and livestock herbivory with increasing elevation. In addition, the phenotypic responses to clipping may also be differentiated between phylogeographic lineages, potentially due to adaptations shaped by unknown histories of herbivory during glacial survival.

We ask whether (1) intraspecific phylogeographic lineages show phenotypic trait differentiation in *Geum montanum*; (2) phenotypic trait differentiation is less pronounced in *G. montanum* than in its congener, *G. reptans*; and (3) there is evidence for adaptation of traits to the current environment of *G. montanum* populations, particularly in response to simulated herbivory.

Methods

Sampling and experimental design

Geum montanum L. (Rosaceae) is a small hemicryptophytic herb occurring in subalpine and alpine meadows with acidic soils in most European mountain systems including the European Alps (Aeschimann et al. 2004). In late summer and autumn of 2007, we sampled seeds from five to six maternal plants in each of 16 sub-alpine to alpine populations of *G. montanum* from the European Alps. We considered all the seeds collected from the same maternal plant as a seed family. The sampled populations originate from three regions which host distinct phylogeographic lineages as detected by Thiel-Egenter and co-workers (2009) in a study on the population genetic structure of *G. montanum* (Fig. 1, Online Resource 1).

The common garden experiment was performed from 2008 to 2009. In January 2008, we sowed seed families in Petri dishes on moist filter paper and cold-stratified the seeds for 2 months at 4 °C in the dark. We then germinated the seeds in a temperature-controlled glass cabinet while keeping them moist and randomizing the position of the Petri dishes daily. After germination in mid-March 2008, seedlings were transplanted into multi-pot trays containing 54 cells (4 cm diameter) with low-nutrient soil (Anzuchterde, Ökohum, Herrenhof, Switzerland). Positions of the multi-pot trays were randomized weekly. At the end of May 2008, plants were transplanted into four blocks into the local soil of the common garden in Davos, Graubünden, Switzerland (N 46°47′06.97″, E 9°48′57.02″; Fig. 1), which is 1530 m above sea level. Plants were positioned on a 13 cm × 18 cm grid, i.e. with sufficient space between them to avoid aboveground competition. The common garden was a former mown pasture that had been ploughed to remove the vegetation layer. With some exceptions due to fewer available seedlings, each seed family was represented by eight replicates, four of which were assigned to receive a clipping treatment. In total, 698 plants were distributed among the four blocks such that each block contained two randomly positioned members of each seed family (one clipped, one untreated).

Although mortality continued throughout the experiment, mortality was very high immediately after planting, so we replaced dead plants with live ones at the beginning of June 2008. These plants were smaller than those planted earlier. We, therefore, used rosette diameter, measured in June 2008, as a covariate in all models to account for initial size differences. The number of leaves was counted in July 2008. For the clipping treatment, we clipped all rosette leaves at the petiole base in July 2008 and again in May 2009. Mortality between June 2008 and May 2009 differed among regions (see "Results"), but this imbalance did not hamper further statistical analysis, because sample sizes at seed family and population levels were still sufficient.

In May 2009 we measured rosette diameter, number of rosette leaves, petiole length, full length and width of the longest leaf and number of flowers if present. In June 2009 we measured flower diameter of one fully developed flower per plant, if available, as well as the length of the tallest flowering stem. In the second half of July 2009, we harvested belowground and aboveground biomass and separated aboveground biomass into vegetative (leaves) and reproductive parts (flowering stems with flowers). The biomass was then dried for 72 h at 60 °C in an oven and weighed. We calculated the ratio between belowground and aboveground biomass and the proportion of reproductive to total aboveground biomass. Seeds were also harvested, if present and not yet dispersed, counted per individual plant and weighed with a high-precision balance (Mettler-Toledo AB204-S, accuracy ±0.1 mg). Growth rate was calculated as the difference between natural logarithms of the number of leaves measured in July 2008 and May 2009 just before the clipping treatment.

Statistical analysis

We analysed trait data for genetic differentiation within three nested levels: phylogeographic regions, populations within regions and seed families within populations. We also included response to clipping and its interaction with region, population and seed family. For these analyses, we applied linear mixed-effect models using the package lmerTest version 2.0-6 (Kuznetsova et al. 2014) in R version 3.1.0 (R Development Core Team 2013), which depends on package lme4 version 1.1-6 (Bates et al. 2014). In these models, population and seed family were treated as random factors. In all models, rosette diameter at the start of the experiment (June 2008) was included as a covariate to account for initial differences in plant size. Block was included as random effect to remove any effects of small-scale heterogeneity in the common garden. Analyses with number of flowers and

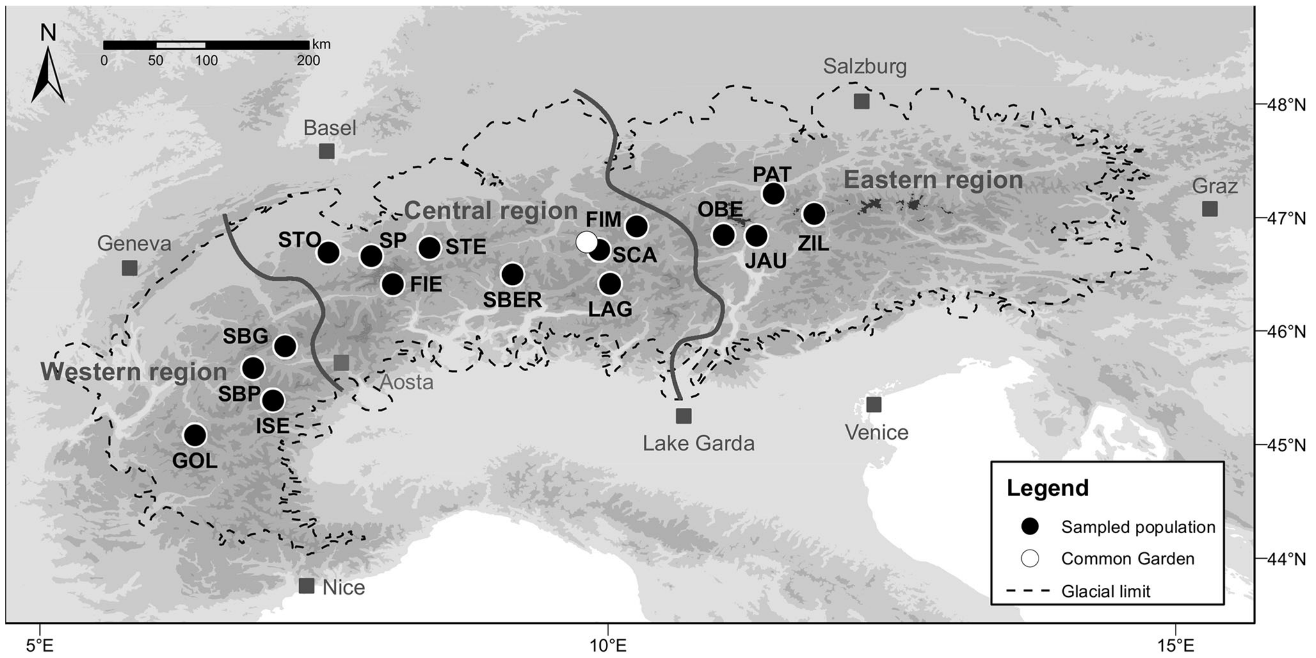

Fig. 1 Map of the European Alps with sampling populations of *Geum montanum* (*filled circles*) and the common garden site in Davos (*open circle*). The *lines* show the borders of the three phylogeographic regions adapted from Thiel-Egenter et al. (2009). The *dotted line* shows the glacial limit during the last glacial maximum (Schönswetter et al. 2005). Projection: GCS WGS 84

seeds were applied to plants with one or more flowers (70 % of plants) or seeds (61 % of plants), respectively. We checked for homogeneity of variance and normality of residuals in all the models. To obtain normally distributed residuals, data transformation was necessary. We applied natural logarithm transformations to the number of flowers, aboveground reproductive biomass, belowground to aboveground biomass ratio and the proportion of reproductive to total aboveground biomass. Square-root transformations were applied to the number of seeds, aboveground vegetative biomass and belowground biomass. F-values for fixed effects and X^2-values for random effects as well as their P-values were calculated using Satterthwaite's method (Fai and Cornelius 1996).

Since most of the investigated traits reflect performance and are related to fitness, we analysed the results in terms of fitness homeostasis (Richards et al. 2006). This means that we interpret small decreases in fitness under the clipping versus non-clipping treatments as reflecting strong capacity to adjust to changing conditions; large decreases under clipping versus non-clipping would be interpreted as weak capacity for adjustment. We calculated the average trait value for each clipping × seed family combination on untransformed data. We then calculated the log-response ratio for each seed family: $\mathrm{LRR}_{\mathrm{clipping}} = \ln(X_{\mathrm{clipped}}) - \ln(X_{\mathrm{non\text{-}clipped}})$, with negative $\mathrm{LRR}_{\mathrm{clipping}}$ indicating a decreased response for clipped compared to unclipped plants. Mean and SE of LRRs were calculated from these data for each trait.

For trait-environment correlations on phenotypic traits, we used population-based averages of trait values that were calculated from seed-family averages while ignoring the clipping treatment. Environmental variables used were elevation, mean temperature and precipitation over the months May to September for the locations of population origin. The monthly mean temperature and precipitation data were obtained from the WorldClim database (http://www.worldclim.org; Hijmans et al. 2005). We also ran trait-environment correlations on the population-based responses to clipping (i.e. population averages calculated from seed-family averages of LRR with respect to clipping). Finally, to investigate the influence of regional differences, similar correlations were performed using the residuals from linear models including region as factor.

Results

Differentiation in phenotypic traits

Significant regional differentiation was found in 5 out of 16 traits: the numbers of leaves, flowers and seeds; aboveground vegetative biomass; and the proportion of reproductive to total aboveground biomass (Table 1). The number of leaves and aboveground vegetative biomass were higher in plants from western populations than in plants from central and eastern populations (Fig. 2). The numbers

Table 1 Results of linear mixed-effect models testing for differentiation in 16 phenotypic traits in *Geum montanum* grown in a common garden

Trait	*N*	Cov	Block	Clipping	Region	Pop	Sf	Cli:Region	Cli:Pop	Cli:Sf
Rosette diameter	526	135.3***	2.1	8.5*	2.0	2.5	8.7**	0.7	1.0	0.0
No. of leaves	526	119.7***	0.0	8.9**	14.4***	0.0	8.9**	0.5	0.0	0.0
Petiole length	526	70.8***	1.7	5.3*	0.3	8.6**	3.7	0.2	0.0	2.1
Full leaf length	526	85.3***	1.8	7.0*	0.3	9.0**	6.0*	0.3	0.0	2.0
Width of leaf	526	75.7***	0.0	7.8**	1.9	4.0*	11.9***	0.1	0.0	0.0
No. of flowers	491	56.1***	4.1*	30.9***	5.3*	0.3	8.9**	4.5*	0.0	0.0
Flower diameter	525	34.2***	6.6*	0.6	1.2	1.6	3.6*	1.3	0.0	0.0
Length of tallest flowering stem	484	48.1***	6.2*	5.3*	1.9	9.9**	4.9*	0.7	0.0	1.7
No. of seeds	428	12.7***	0.0	27.6***	12.4***	0.0	8.9**	2.3	0.0	0.1
Seed weight	428	8.2**	24.6***	3.8	3.3	0.3	7.5*	0.2	0.0	0.0
Abg vegetative biomass	524	126.4***	2.7	28.7***	4.6*	0.4	19.4***	1.1	0.0	0.0
Abg reproductive biomass	480	56.7***	1.4	11.8**	4.0	0.6	6.8**	0.7	1.4	0.0
Bg biomass	525	136.4***	1.2	27.8***	2.0	4.3*	8.0**	0.1	0.0	0.0
Bg to abg biomass ratio	479	12.2***	22.4***	4.7*	0.7	2.4	12.2***	1.1	0.0	0.0
Proportion reproductive to total abg biomass	479	0.6	0.0	4.3	8.8***	0.0	11.4***	0.5	0.2	0.0
Growth rate	526	55.2***	4.5*	4.0*	1.3	0.0	10.2**	0.1	0.0	0.1

Shown are F- and X^2-values and their significance is indicated with asterisks. Sf (seed family) is nested in Pop (population), which is itself nested in Region. Population and seed family are random effects (X^2-values); other factors are fixed effects (F-values); for interactions with random effects X^2-values are reported. All models were analysed using a normal distribution

N sample size, *Cov* covariate (initial plant size), *Block* experimental block treatment, *Clipping* clipping treatment simulating herbivory, *Abg* aboveground, *Bg* belowground

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

of flowers and seeds were higher for plants from western and eastern populations than for plants from central populations. The proportion of reproductive to total aboveground biomass was highest in plants from eastern populations followed by those in western populations and then central populations. Three traits (petiole length, full leaf length and length of tallest flowering stem) showed significant among-population differentiation within regions (Table 1). Seed family was an important factor in explaining trait values as it was significant for all but one trait (Table 1), which indicates substantial genetic diversity within populations. Mortality was 24.6 % between June 2008 and May 2009 and differed statistically among regions (one-way ANOVA on population mean proportions of surviving plants, $F = 3.9$, $P < 0.05$) with survival in the western, central and eastern regions being 92, 64 and 73 %, respectively.

Effects of simulated herbivory

Clipping had a significant negative effect on trait values in 13 out of the 16 observed traits (Table 1). The only significant interaction observed was for clipping × region in the number of flowers (Table 1; Fig. 3). In this interaction effect, plants from western and eastern populations had a strong negative response to clipping, whereas plants from central populations showed no response to clipping. Plants from central populations also generally had lower flower number. The mean LRRs (Table 2) indicate that clipping had variable effects on traits in that more severely detrimental effects were found for biomass traits and the number of flowers (range −0.399 to −0.257) than for other measured traits (range −0.163 to 0.064).

Trait–environment correlations

Only belowground biomass was related to the elevation of plant origin ($r = 0.53$; $P < 0.05$; Online Resource 2). This positive correlation between belowground biomass and elevation indicates increasing investment in roots with higher elevations. The investment in belowground relative to aboveground biomass was not positively correlated with elevation but negatively correlated with mean temperature at site of origin ($r = -0.56$; $P < 0.05$; Online Resource 2). Finally, the number of seeds was negatively correlated with mean precipitation at the site of population origin ($r = -0.69$; $P < 0.01$; Online Resource 2). Even after correcting for regional differences, the correlation between the relative investment in belowground to aboveground biomass and temperature was present ($r = -0.57$, $P < 0.05$; Online Resource 3). In contrast, the correlations between belowground biomass and elevation ($r = 0.22$, $P = 0.41$), and the number of seeds and the proportion of

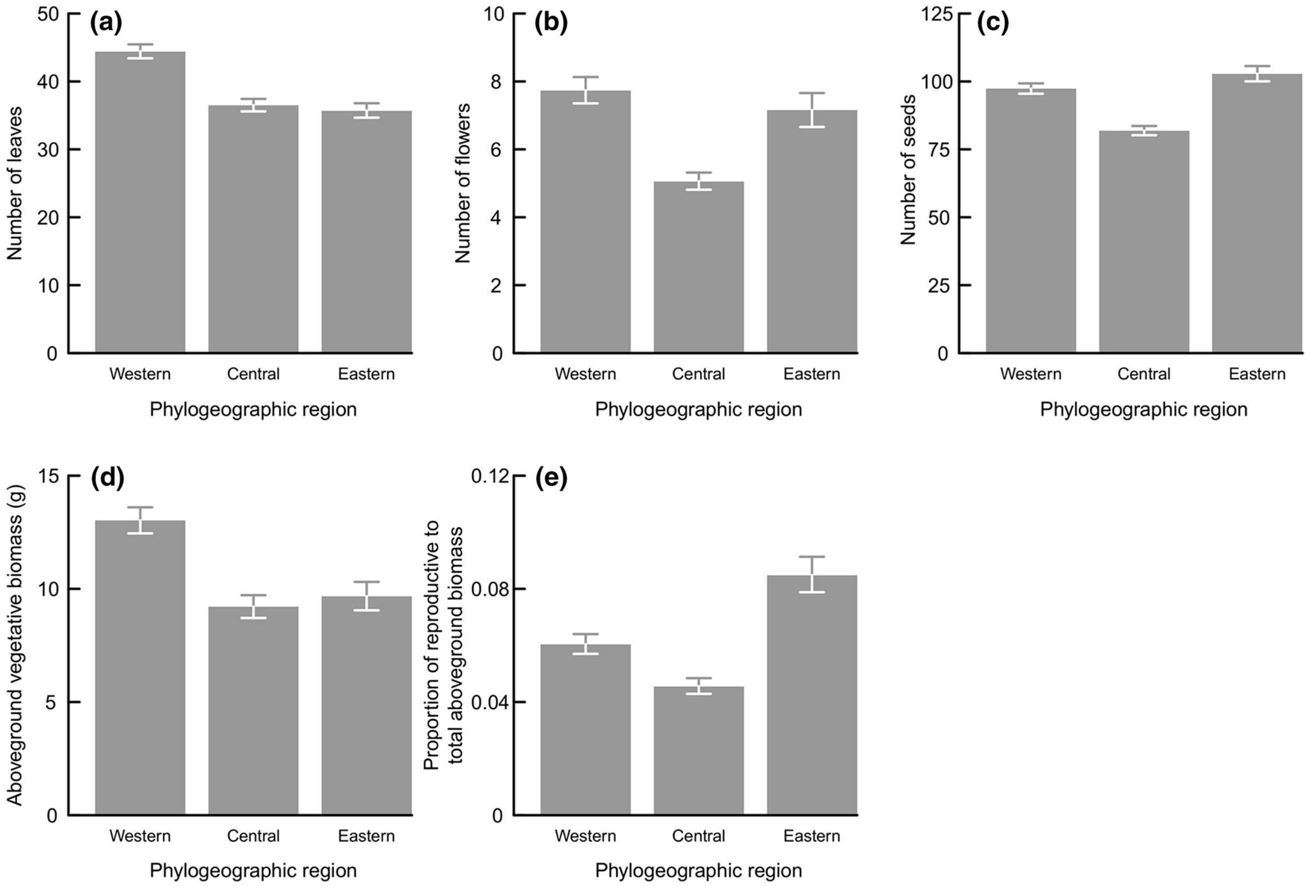

Fig. 2 Means ± SE for **a** number of leaves, **b** number of flowers, **c** number of seeds, **d** aboveground vegetative biomass and **e** the proportion of reproductive to total aboveground biomass measured in a common garden experiment with *Geum montanum* plants from 16 populations spread across three phylogeographic regions in the European Alps. Means ± SEs were back-transformed from square root-transformed data for number of seeds and from natural log-transformed data for the proportion of reproductive to total aboveground biomass. SEs were calculated based on population means, which were based on seed-family means

aboveground to belowground biomass with precipitation disappeared ($r = -0.18$, $P = 0.49$; $r = -0.34$, $P = 0.87$), whereas a correlation between growth rate and temperature appeared ($r = -0.50$, $P < 0.05$; Online Resource 3).

With respect to fitness homeostasis (i.e. the capacity to compensate after clipping), only 2 out of 16 analysed traits showed a significant relationship between population-averaged log-response ratio to clipping and elevation (Fig. 4; Online Resource 4): aboveground vegetative biomass ($r = -0.52$; $P < 0.05$) and belowground biomass ($r = -0.60$; $P < 0.05$). Their negative relationships indicate that plants showed decreased capacity to compensate for clipping with increasing elevation of origin. After correcting for regional differences, the correlation of elevation at origin with aboveground vegetative biomass was not significant anymore ($r = -0.35$, $P = 0.19$), whereas the correlation of elevation at origin with belowground biomass was still significant ($r = -0.56$, $P < 0.05$; Online Resource 5).

Discussion

Phenotypic differentiation

Five traits of *Geum montanum* showed differentiation consistent with its phylogeographic regions. This supports the idea that glacial survival in isolated refugia may cause not only phylogeographic differentiation in neutral molecular markers among populations throughout the European Alps but also phenotypic trait differentiation corresponding to the phylogeographic regions. Compared with the related species *G. reptans* (Frei et al. 2012), our study species *G. montanum* showed much weaker regional differentiation in phenotypic traits. We found 5 out of 16 traits to be regionally differentiated, whereas in *G. reptans* we found significant regional differentiation in all 11 investigated traits. These results are similar to comparisons between the rather rare *Campanula thyrsoides* with isolated populations and the common and widely distributed congeneric species *C. barbata*. All eight

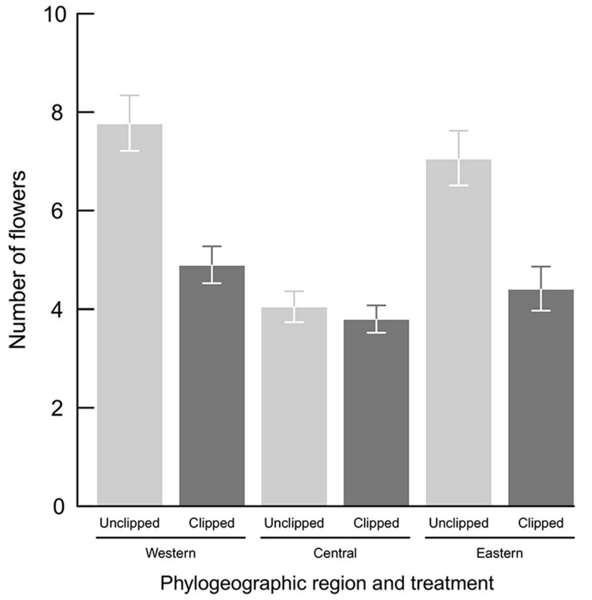

Fig. 3 Clipping × region interaction for number of flowers in *Geum montanum* grown in a common garden with a clipping treatment simulating herbivory. Means ± SEs were back-transformed from natural log-transformed data and SEs were calculated based on population means, which were based on seed-family means

Table 2 Overall mean ± SE of the log-response ratios to clipping ($LRR_{clipping}$) in 16 phenotypic traits in *Geum montanum* grown in a common garden sorted by descending LRR value

Trait	$LRR_{clipping}$
Belowground to aboveground biomass ratio	0.064 ± 0.029
Flower diameter	−0.036 ± 0.049
Growth rate	−0.062 ± 0.025
Number of leaves	−0.064 ± 0.027
Full leaf length	−0.067 ± 0.034
Width of leaf	−0.068 ± 0.024
Rosette diameter	−0.070 ± 0.026
Petiole length	−0.081 ± 0.046
Seed weight	−0.096 ± 0.051
Length of tallest flowering stem	−0.098 ± 0.040
Number of seeds	−0.121 ± 0.028
Proportion of reproductive to total aboveground biomass	−0.163 ± 0.082
Belowground biomass	−0.257 ± 0.061
Number of flowers	−0.272 ± 0.070
Aboveground vegetative biomass	−0.277 ± 0.065
Aboveground reproductive biomass	−0.399 ± 0.129

investigated traits for *C. thyrsoides* were regionally differentiated (Scheepens et al. 2013), whereas only two out of six traits for *C. barbata* were regionally differentiated (Scheepens and Stöcklin 2011). Although we need to emphasise that evidence from two species pairs is not enough to draw compelling conclusions and that further tests will be necessary, we suggest possible reasons for the results from both species pairs of common versus rare species in the European Alps.

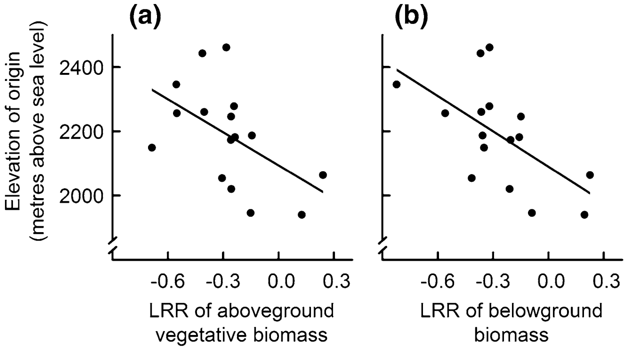

Fig. 4 Log-response ratios (LRRs) of **a** aboveground vegetative biomass and **b** belowground biomass to the clipping treatment correlated with elevation of population origin for *Geum montanum* grown in a common garden

First of all, the currently more common occurrence of *G. montanum* and *C. barbata* compared to their congeners should increase connectivity, which may have caused a partial reduction of genetic differentiation accumulated during glaciation. However, the levels of phylogeographic differentiation prior to recolonisation are unknown and it is possible that they may have been initially low compared with their more isolated congeners. Our results, therefore, only suggest the possibility of reduced glacial signatures, and molecular studies are needed to reconstruct temporal changes in genetic differentiation through time.

Second, it is important to consider whether life history may explain the observed differences among the studied species pairs. In contrast to the exclusively sexual reproduction of *G. montanum*, *G. reptans* can reproduce clonally as well. However, since sexual reproduction is also common in *G. reptans*, we exclude the potential of clonal reproduction as an important factor in explaining differences in regional differentiation. Furthermore, seed morphology of both species pairs (Hess et al. 1972, 1976) and, therefore, seed dispersal capacity, is similar.

We found regional differentiation in the numbers of leaves, flowers and seeds, aboveground vegetative biomass and the proportion of reproductive to total aboveground biomass. This may be the result of neutral processes during glacial survival but may also have occurred due to selection processes (Hewitt 1996; Leinonen et al. 2008). Phenotypic patterns do not suggest any home-site advantages for populations originating from the same phylogenetic region as the location of the common garden (central group), with generally lower trait values for central populations than for populations of the other regions.

Populations within regions showed differentiation in petiole length, full leaf length and length of tallest flowering stem. This differentiation must have occurred during and since postglacial recolonisation, reflecting either neutral or adaptive processes.

Differentiation among seed families was high, overall, which indicates substantial genetic diversity within populations. It may well be that the wide niche occupied by *G. montanum* is supported by high genetic diversity within populations (Van Valen 1965; Banta et al. 2012). This high genetic variation among seed families may buffer the species against the impact of future climatic changes (Davis and Shaw 2004).

Our grouping of the sampled populations into three regions depends on a phylogeography of *G. montanum* based on neutral genetic marker data from three samples per 22.5 km × 25 km (Thiel-Egenter et al. 2009). An alternative approach would have been to screen neutral genetic markers of the 16 studied populations. However, we emphasise the broad coverage of the study by Thiel-Egenter et al. (2009) and deem the strong phylogeographic structure in their results accurate enough for our regional grouping.

Trait–environment correlations

None of the traits with a significant population effect in the models were correlated with environmental factors and it, therefore, remains unclear whether differentiation in these traits could have resulted from natural selection. The increasing belowground biomass with higher elevation, as well as increasing belowground to aboveground biomass ratio with decreasing temperature of population origin, may be a result of selection for increased investment in roots at higher elevations, where growing conditions are less favourable (Körner 2003; Gonzalo-Turpin and Hazard 2009). For instance, plants have been shown to increase allocation of resources to roots at higher elevations, which may be a response to reduced mycorrhizal infection (Körner and Renhardt 1987). Less developed soil organic structure at higher elevations (Körner 2003), which may lead more easily to water stress, may also select for stronger root foraging capacity.

Three out of four traits showing significant correlations with environmental factors did not correlate significantly when correlations were performed with the residuals of models testing for a regional effect, suggesting that environmental differences between the sampled localities among the regions affected the correlations at least to some extent. Since the localities differed only in precipitation among regions (one-way ANOVA, $F = 5.5$, $P < 0.05$) and not in elevation and temperature (one-way ANOVAs, $F = 2.5$, $P = 0.12$; $F = 0.0$, $P = 0.99$, respectively), regional patterns in precipitation may have driven regional adaptation reflected in the number of seeds and proportion of reproductive to total aboveground biomass. However, it remains unclear how the observed regional patterns of these traits would be adaptive for differential precipitation. The significant correlations in the relative investment in belowground to aboveground biomass and in growth rate with temperature, when using the residuals of models testing for a region effect, strengthen the interpretation that these relationships are not influenced by regional effects and, therefore, likely arose during and since postglacial recolonisation, since they depend on temperature variability among populations within regions.

Response to simulated herbivory

The plastic responses to clipping in 2009 can be seen as mainly passive responses, also known as 'apparent plasticity' (Pigliucci and Hayden 2001; Wright and McConnaughay 2002; Ghalambor et al. 2007), due to decreased energy status after clipping in 2008 and should, therefore, be interpreted as decreased individual fitness. Despite a substantial number of seed families showing positive responses to clipping, overall means at the population level were negative. Thus, the variation in the plastic responses among populations and regions is of interest here as it indicates different capacities at the population and regional levels to overcome the negative effects of clipping (or even to respond to it with overcompensation). This was the case for number of flowers, which showed stronger compensation after clipping for central populations than for western and eastern populations. Such regional variability in fitness homeostasis is hard to explain by invoking differences in selection pressure, especially because the mechanisms behind this differentiation may well lie in selective processes experienced during glacial survival, about which virtually nothing is known. One could speculate that the refugium that harboured the phylogeographic lineage recolonising the central region had a higher level of herbivory than refugia that sheltered the other two lineages.

Pellissier et al. (2014) showed that variation in herbivore defence by *Plantago lanceolata* along elevation gradients is linked to both phenotypic plasticity and genetic adaptation to levels of herbivory. Accordingly, we observed a genetically determined decrease in capacity to retain fitness after herbivory with increasing elevation of origin in two important fitness-related traits, aboveground vegetative biomass and belowground biomass. Therefore, our results may likewise indicate adaptation to weaker selection for fitness homeostasis due to decreasing levels of natural and livestock herbivory with increasing elevation (Pellissier et al. 2014). These effects may be enhanced by ongoing land abandonment at higher elevations (Gehrig-Fasel et al. 2007). Since such differentiation with elevation in the response to clipping must have been shaped after postglacial recolonization, our results reveal and exemplify pronounced recent phenotypic evolution. However, it is noteworthy that, despite such recent evolution, regional differentiation in line with phylogeographic lineages was retained.

Local adaptation to as well as selection under herbivory regimes has been observed in many studies (e.g. Sandring et al. 2007; Pluess 2013; Pellissier et al. 2014), but evidence for local adaptation through variable levels of phenotypic plasticity is scarce. Genetic variation in controlling phenotypic plasticity to clipping was found in a study on *Campanula thyrsoides*, where plants from populations in the Western Alps showed lower susceptibility to clipping than plants from populations in three Alpine regions to the east. Drivers of this regional differentiation in trait responses to clipping could not be identified, mainly due to lack of information on herbivore pressure in the studied regions during glaciations and in the historical past (Scheepens et al. 2013). Pellissier et al. (2014) were able to explain patterns of insect herbivory and herbivore resistance with variation in temperature and presence of mycorrhizal fungi along elevation. Since our observations indicate that temperature (and precipitation) was not correlated with fitness homeostasis for any trait, herbivory, itself, may be the main driver of differences in susceptibility to herbivory.

Conclusion

The results of this study do not contradict the hypothesis that glacial history may cause regional differentiation in phenotypic traits in *Geum montanum*, similarly to what we have found in other species. The response in number of flowers produced after a clipping treatment was regionally differentiated, which is potentially due to adaptations shaped by unknown histories of herbivory during glacial survival. The observed regional differentiation in phenotypic traits was less pronounced than in its congener, *G. reptans*. We think this is due to the more common occurrence of our study species, causing higher gene flow among populations and thereby diminishing regional differentiation. We suggest that glacial history may have left its mark on current phenotypic variability of Alpine plants, even in relatively common species with well-connected populations.

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Conflict of interest The authors declare that they have no conflict of interest.

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