

High intraspecific phenotypic variation, but little evidence for local adaptation in *Geum reptans* populations in the Central Swiss Alps

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Abstract The Alpine landscape is characterized by high spatiotemporal heterogeneity in environmental variables, such as climate and soil characteristics. This may lead to divergent selection pressures across plant populations and to local adaptation. *Geum reptans*, a widespread high-alpine clonal herb, has been the subject of several studies investigating phenotypic variation in populations across the Swiss Alps, yet so far, there is only little knowledge about local adaptation in this species from reciprocal transplantations across original field sites. Here, we reciprocally transplanted three populations of *Geum reptans* in the Central Swiss Alps, growing at close or far geographical distance from each other, and compared growth- and reproduction-related traits to investigate patterns of local adaptation. We further measured leaf morphological traits to assess potential selection at field sites, and quantified the relative importance of genetic vs. environmental variation (i.e., phenotypic plasticity) for all traits. Additionally, among and within population genetic differentiation was analyzed using microsatellite markers. Molecular diversity was high within populations, and molecular differentiation increased with geographic distance among populations, suggesting

that gene flow is maintained at close range, but decreased with distance. Although extensive phenotypic variation was found across site × population transplant combinations, our study revealed little evidence for local adaptation in *G. reptans* populations. Plant traits also showed strong plasticity, as revealed by pronounced site effects, yet no direct linear selection was detected on leaf trait values within field sites. We suggest that the glacier forelands studied here, which are representative of the habitat of large *G. reptans* populations, are too similar in environmental conditions to lead to among population intraspecific differentiation in line with local adaptation. As *G. reptans* showed a great capacity to respond plastically to environmental conditions, we cautiously advocate that the evolution of phenotypic plasticity might have prevailed over genetic differentiation for the adaptation to the relatively narrow niche of this species.

Keywords Genetic differentiation · High-alpine clonal herb · Molecular variation · Phenotypic plasticity · Reciprocal transplantation experiment

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Introduction

Alpine ecosystems are characterized by steep environmental gradients over short geographic distances (Körner 2003) and a patchy microhabitat distribution (Scherrer and Körner 2011), which offers numerous niches to alpine plant species (Aeschimann et al. 2004). These characteristics of the alpine landscape are often also associated with spatial isolation among populations and restricted gene flow (Stöcklin et al. 2009), which may allow for intraspecific population differentiation and local adaptation. Furthermore, at high elevation, plant life is challenged by low temperatures,

late snow-melting, short vegetation periods, and extreme weather events (Billings and Mooney 1968; Körner 2003). In this heterogeneous habitat, plants can adapt genetically to locally prevailing conditions (Byars et al. 2007; Gonzalo-Turpin and Hazard 2009), and/or respond to spatiotemporal variability in environmental conditions via adaptive phenotypic plasticity (Sultan 1995).

Intraspecific phenotypic variation resulting from genetic drift or natural selection (Volis et al. 2015) is common in widely distributed species (Bradshaw 1984; Joshi et al. 2001; Banta et al. 2007) and has frequently been observed in alpine plant species (Pluess and Stöcklin 2004; Giménez-Benavides et al. 2007; Byars et al. 2009; Gonzalo-Turpin and Hazard 2009; Stöcklin et al. 2009; Frei et al. 2012). Moreover, strong phenotypic plasticity is likewise common in alpine species, and has been shown to provide a potential advantage for the persistence and survival of alpine species in a heterogeneous environment (Stöcklin et al. 2009; Frei et al. 2014). While the relative role of these two non-mutually exclusive strategies (i.e., adaptive genetic differentiation and plasticity), as well as the conditions for their evolution under divergent selection, are theoretically well understood, empirical evidence is rather scarce (Baythavong 2011; Hamann et al. 2016). The spatial grain size of environmental variation, defined by the degree of environmental variation as perceived by an individual plant across its dispersal distance, is likely to determine which of these mechanisms prevails (Alpert and Simms 2002; Pigliucci et al. 2003; Kawecki and Ebert 2004). Generally, adaptive genetic differentiation is expected when spatial grain size is coarse, whereas the evolution of phenotypic plasticity is expected when spatial grain size is fine (Baythavong 2011; Richardson et al. 2014). As such, local adaptation via genetic differentiation is more likely to occur when gene flow is limited between populations, as is the case in naturally fragmented landscapes or in populations separated by great geographic distances (Kawecki and Ebert 2004; Leimu and Fischer 2008). Alternatively, adaptive genetic differentiation is unlikely to evolve when gene flow among populations is extensive, and depending on the spatial grain size, the evolution of phenotypic plasticity may prevail (Kawecki and Ebert 2004). However, certain exceptions have been documented, such as adaptive genetic differentiation despite extensive gene flow, under strong micro-geographic divergent selection (Gonzalo-Turpin and Hazard 2009; Richardson et al. 2014).

Local adaptation is also contingent on other factors such as plant mating system, longevity, and clonality due to their effects on genetic diversity and the degree of genetic differentiation of populations (Galloway and Fenster 2000; Kawecki and Ebert 2004). Selfing, as opposed to outcrossing, reduces genetic diversity within populations, thereby compromising future adaptive potential (Linhart and Grant

1996). Similarly, clonality can limit the potential for local adaptation in case of limited sexual reproduction restricting genetic diversity within and among populations, but allows for plastic foraging among ramets (van Kleunen and Fischer 2001). Additionally, clonal plants may be less locally adapted currently if long-lived genets reflect adaptation to past conditions (Leimu and Fischer 2008; de Witte and Stöcklin 2010). Nevertheless, at high elevation, clonal reproduction is common amongst alpine species, and is associated with benefits such as the ability to forage for resources, support the establishment of offspring, and buffer against environmental variation (Billings and Mooney 1968).

Geum reptans L. (Rosaceae) is a long-lived clonal species occurring in high-alpine environments that reproduces sexually via strictly outcrossing flowers and vegetatively via clonal ramets on stolons. This species is an ideal system to study phenotypic variation and local adaptation, as it has been the subject of numerous prior investigations describing molecular and phenotypic variation, as well as gene flow among populations in the European Alps, and the relative importance of clonal vs. sexual reproduction (Pluess and Stöcklin 2004, 2005; Weppler et al. 2006; Stöcklin et al. 2009; Frei et al. 2012). Findings from Weppler et al. (2006) suggested that the role of sexual reproduction was not restricted to the maintenance of genetic variation or long-distance dispersal, but played an equally important role for population growth as reproduction via clonal offspring (Weppler et al. 2006). Moreover, prior studies showed that genetic diversity within populations is high despite clonality (Ellstrand and Elam 1993; Pluess and Stöcklin 2004) and natural habitat isolation (Stöcklin et al. 2009). Direct measures of gene flow via seeds and pollen indicated the maintenance of considerable gene flow over short distances and low molecular differentiation among close populations (Pluess and Stöcklin 2004). Furthermore, a glasshouse experiment revealed the great capacity of *G. reptans* to respond plastically to changes in environmental conditions, especially in its reproductive behavior (Pluess and Stöcklin 2005). Finally, a common garden experiment with 20 *G. reptans* populations spanning all biogeographic regions of the European Alps revealed that phenotypic differentiation reflected the glacial history of this species shaped by founder effects and past selection, but also suggested adaptation to current climate conditions (Frei et al. 2012). However, to rigorously prove that adaptation to local conditions has occurred, reciprocal transplantation experiments across original field sites are necessary (Kawecki and Ebert 2004), which have so far never been performed with this species.

Consequently, this study aimed at complementing previous ones, by investigating local adaptation via reciprocal transplantations of *G. reptans* populations growing at close

or far geographical distances from each other in the Central Swiss Alps, and generally contributes to the body of empirical studies testing for local adaptation among alpine species. We investigated patterns of local adaptation in growth and reproductive traits by comparing the performance of sympatric and near- or far-allopatric site \times population transplant combinations. We further measured leaf morphology traits known to be particularly plastic, yet not directly related to plant fitness (Frei et al. 2012), and investigated plastic responses to environmental site effects. For all traits, we quantified the importance of genetic vs. environmental variation (i.e., phenotypic plasticity), and leaf traits were further used to investigate potential selection for mean trait values at each site. In addition, we analyzed among and within population genetic diversity and molecular differentiation using microsatellite markers.

We specifically investigate (1) whether patterns of local adaptation are present amongst the studied populations of *G. reptans* (i.e., sympatric site \times population transplant combinations outperform allopatric ones), (2) whether phenotypic plasticity is revealed in reproductive and leaf traits across sites, (3) if site-specific selection acts on leaf traits, and (4) whether genetic diversity within populations is maintained despite clonality and low molecular differentiation among close populations (i.e., because of gene flow over short distance).

Materials and methods

Study species

Geum reptans L. is a long-lived high-alpine species belonging to the Rosaceae family. It is widespread in the European Alps and extends eastward to the Carpathian mountains (Conert et al. 1995). The species occurs above 2100 m above sea level (a.s.l.) up to 3800 m a.s.l., and grows typically on moraines in glacier forelands, and on moist scree fields and mountain ridges (Aeschmann et al. 2004). *Geum reptans* is an early-successional species colonizing virgin soils after glacier retreat and usually persists until

interspecific competition becomes too strong (Weppeler et al. 2006). The species grows in rosettes with dissected compound leaves. The number of leaflet pairs on a leaf usually ranges from c. 5–15. *Geum reptans* can reproduce vegetatively by forming new rosettes (ramets) at the end of long stolons, but can also reproduce sexually via seeds borne on a single-flowered stem. Both reproductive strategies are not mutually exclusive and seem to contribute equally to population growth (Weppeler et al. 2006). The yellow flowers are proterogynous, pollinated by insects and c. 100 seeds are produced per flower (Pluess and Stöcklin 2004). Seed dispersal spectra obtained from simulations showed that most seeds are dispersed across less than 10 m, while long-distance seed dispersal over 100 and 1000 m can occur for 0.015 and 0.005% of seeds, respectively (Pluess and Stöcklin 2004; Tackenberg and Stöcklin 2008).

Reciprocal transplantations

Three large *G. reptans* populations were chosen for this reciprocal transplantation experiment growing at close or far distance from each other in the Central Swiss Alps (Table 1). For clarity, we will refer to the populations using italic font and to the sites using capital letters. Two populations, abbreviated as *Flu* growing at Flüelapass (FLU) and *Dur* growing at Dürrboden (DUR), were located at relatively close proximity from each other (c. 5 km) near Davos (canton of Graubünden, Switzerland). A third population, abbreviated as *Mut* growing at Muttgletscher (MUT), was located at a larger geographic distance (c. 110 km from Davos) near the Furkapass (on the border between the canton of Uri and the canton of Valais, Switzerland). All three sites are glacier forelands but differed in elevation and exposition (Table 1). Soil temperature was recorded (as a proxy of smoothed air temperature; Körner and Paulsen 2004) during the second growing season (July–October 2015) using one data logger buried in the soil at a depth of 5 cm at each site (Thermochrome iButton Device Model DS1921G, Maxim Integrated Products, Inc., California, USA). Mean temperature differed among sites when averaged over the time of measurement (Table 1). Precipitation

Table 1 Location, geographic coordinates (latitude, longitude), elevation (m a.s.l.) and site characteristics of 3 *Geum reptans* populations sampled in the Central Swiss Alps. Pop, population abbreviation (in italic font); *n*, sample size of individuals used in the transplantations; Temp., mean temperature (°C) averaged from July–October 2015, indicative of the length of the growing season, measured with

data loggers at each site; Prec., summed amount of precipitation (mm) from July–October 2015, as obtained from the nearest weather stations to our sites (Weissfluhjoch for FLU, Davos for DUR, Gütsch ob Anderatt for MUT, respectively; MeteoSwiss 2015); Exp., exposition of the slopes of the transplantation sites

Location	Pop	Latitude	Longitude	Elevation	<i>n</i>	Temp.	Prec.	Exp.
Flüelapass (FLU)	<i>Flu</i>	46°44'54"	9°56'54"	2400	40	8.85	570	NE
Dürrboden (DUR)	<i>Dur</i>	46°42'29"	9°56'12"	2290	40	10.22	525	NE
Muttgletscher (MUT)	<i>Mut</i>	46°33'27"	8°24'39"	2480	40	7.39	465	NW

records, obtained for each site from nearby meteorological stations (MeteoSwiss 2015), also differed between sites when summed over the second growing season (July–October 2015; Table 1).

In September 2013, *G. reptans* populations were sampled from all three sites. For each population, 40 healthy mother plants were randomly chosen and three viable stolons with rosettes (ramets) were collected from each of these individuals. A minimum sampling distance of 5 m between mother plants was respected to minimize the risk of resampling genotypes (Pluess and Stöcklin 2004). Rosettes were kept in plastic bags and stored at 4 °C in the dark for a maximum of 2 days until they were planted in the greenhouse (Botanical Institute, Basel, Switzerland) in separate pots 7×7×8 cm filled with potting soil (Containererde, Ökohum GmbH, Herrenhof, Switzerland). Rosettes were grown for 9 months in the greenhouse, watered regularly to soil capacity, fertilized once a month (Wuxal, Syngenta Agro, Dielsdorf, Switzerland), and treated once with an insecticide (Spruzit[®], Neudorff GmbH, Germany) to control infestations of Aphidoidea and Aleyrodidae. Four weeks before transplantation to field sites, plants were placed outdoors (Botanical Garden, Basel, Switzerland) for acclimation.

In July 2014, plants were reciprocally transplanted into field sites as soon as the snow had melted and the growing season had started. For each population, one ramet per genet was transplanted to each of the three sites. Each site thus received a total of 120 individuals, represented by 40 individuals per population (40 genets×3 populations). Due to the MUT site being far away from the two relatively nearby sites near Davos, transplantation resulted in 3 sympatric (i.e., populations transplanted back to their site of origin), 2 near-allopatric (i.e., populations transplanted to a site at close proximity), and 4 far-allopatric (i.e., populations transplanted to a site at far distance) site×population transplant combinations. Individuals were transplanted into the local soil, in a patch within the natural populations, and experienced local intra- and inter-specific competition, reflecting natural conditions. Tagged individuals were planted in rows of 10, alternating between populations, with a minimal spacing of 20 cm between each other, and were watered once after planting to facilitate establishment.

Initial number of leaves was counted immediately after transplantation. After two growing seasons, in October 2015, we assessed whether plants had survived and reproduced. Number of leaves was counted on surviving individuals, and the number of flowers and/or stolons was counted for reproductive individuals. The total number of reproductive meristems was calculated for each individual by adding individual number of flowers and stolons. To assess the relative importance of clonal vs. sexual reproduction, we calculated the clonality of each individual as the proportion

of stolons on all reproductive meristems. For each individual, we identified the longest leaf, measured its length and width, and counted the number of leaflets. As an indicator of its leaf shape, (i.e., also called leaf aspect ratio) we calculated the ratio between leaf length and leaf width. Degree of leaf dissection was estimated by dividing the number of leaflets by the leaf length. SLA was assessed for each individual by taking four circular corings of 5 mm Ø from different mature leaves (avoiding veins), drying them at 60 °C for 48h and weighing them together. SLA was then calculated as the fresh leaf area divided by the mean dry weight of the corings (Cornelissen et al. 2003). Aboveground dry mass was harvested and dried at 80 °C for 72h before weighing.

Data analyses

All traits were analyzed with generalized linear mixed-effect models (Crawley 2007), using Type III sum of squares with the lme4 (Bates et al. 2015) and lmerTest packages (Kuznetsova et al. 2013) for R. To test for local adaptation in survival, growth- and reproduction-related traits using the sympatric vs. allopatric contrast, we tested whether the means of the three (sympatric, near-allopatric and far-allopatric) distributions significantly differed from each other. To this end, we specified models including the factors site, population, and the contrast between sympatric, near- and far-allopatric transplant combinations (Blanquart et al. 2013). Local adaptation was considered to be operating if (1) the sympatric vs. allopatric contrast was significant, and if (2) sympatric transplant combinations outperformed allopatric ones (Blanquart et al. 2013). The replication of genets within populations was accounted for by including this factor in the models as a random factor. The initial number of leaves recorded at the time of transplantation was included in the model as a covariate to account for effects of initial plant size. This factor was, however, non-significant and therefore removed from the model for all traits except the final number of leaves.

For traits related to leaf morphology (i.e., leaf shape, leaf dissection and SLA), we analyzed if plastic responses were displayed in response to environmental conditions at field sites and if these responses differed between populations. To this end, we specified models testing for differences between sites and populations, the interaction between site and population, and included genets as a random factor in the model.

The proportion of surviving, reproductive (clonal and/or sexual), and flowering individuals within each transplant combination (i.e., sympatric, near-allopatric, far-allopatric) was analyzed using a binomial distribution with a logit link function. The number of leaves, flowers, stolons, and total number of reproductive meristems were analyzed using a

Poisson distribution with log link function (zero-inflated for the number of flowers, stolons, and total reproductive meristems). The remaining traits were assessed using a normal distribution with identity link function (Crawley 2007). To normalize data and homogenize variance aboveground dry mass was log-transformed, count data (log + 1)-transformed, and ratios (clonality, leaf shape, leaf dissection, and SLA) arcsine-transformed (Crawley 2007). We report p values after Bonferroni correction (i.e., p values multiplied for nine response variables) and F -values (for fixed effects) or χ^2 -values (for random effects), the latter extracted with the “rand” function in lmerTest. Post hoc Tukey HSD multiple comparison tests were applied in the multcomp package (Hothorn et al. 2014) to detect significant differences among site \times populations transplant combinations.

Variance components were calculated for all traits by fitting site, population, their interaction and genets as random factors. We extracted variances using the “VarCorr” function from the lme4 package (Crawley 2007).

Furthermore, as strong plastic effects were found in leaf traits, a follow-up selection analysis was performed to determine if environmental conditions at each field site selected for particular mean trait values. To do so, selection gradients were calculated by means of multiple linear regressions (Lande and Arnold 1983). Leaf shape, leaf dissection, and SLA site-specific trait values were standardized to a mean of zero and a variance of 1 prior to analysis. Relative fitness was calculated by dividing the number of reproductive meristems of each genet by the site-specific mean. Standardized linear (i.e., directional) selection gradients were estimated as the partial regression coefficient from the multiple regression of relative fitness on all standardized traits (Haggerty and Galloway 2011). We report selection gradients β and p values after Bonferroni correction (i.e., p values multiplied for three response variables).

All the analyses were performed on R version 3.0.2 software (R Core Team 2013).

Molecular analyses

Leaf samples were taken randomly from 20 out of the 40 sampled mother plants of each population *Flu*, *Dur*, and *Mut* and immediately dried for DNA extraction using silica gel. Microsatellite marker development was performed by Ecogenics GmbH (Zurich-Schlieren, Switzerland), whose screening technique has previously been described in Kesselring et al. (2013). The 60 individuals were genotyped for nine microsatellite loci. A detailed description of microsatellite multiplex PCR in *G. reptans* can be found in Hamann et al. (2014). In brief, three multiplex PCRs were run. Multiplex I comprised primers for loci 015967, 011721, and 013998; multiplex II for loci 002235, 003651, and 011534, and multiplex III for loci 015615, 013198, and 007389. A

fraction of the forward primers was fluorescent labeled with ATTO-dyes or FAM. Each multiplex PCR started with a denaturation step at 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 90 s, and 72 °C for 60 s, with a final extension step at 72 °C for 30 min. Amplicons were loaded on an ABI3730 sequencer using an Eco500 size standard. Allele calling and crosschecking of genotypes were done with GeneMarker version 1.80 (SoftGenetics, State College, Pennsylvania, USA). Multiplex fingerprints in *G. reptans* have proven to be highly reproducible with an error rate of 1.4%. Nonetheless, binning of a few alleles was performed (see Table 1 in Hamann et al. 2014). The final table of genotypes was exported to GenAEx 6.5 (Peakall and Smouse 2006). GenAEx was used to check for identical multilocus genotypes among sampled individuals, and to estimate the genetic diversity within populations, calculated as the unbiased expected heterozygosity (H_e ; Nei 1973). Additionally, the same software was used to perform an analysis of molecular variance (AMOVA) with 999 permutations to analyze partitioning of molecular variance among and within populations, and to calculate population pairwise F_{ST} values based on allele frequencies.

Results

Proportion of surviving and reproducing plants

On average, 85.8% of individuals survived from transplantation into field sites until harvest two growing seasons later. Survival was, however, independent of sympatric vs. near- and far-allopatric transplant combinations ($F=0.34$, $p=0.92$). Of the surviving individuals, on average 40.6% of individuals reproduced during the second growing season via sexual and/or vegetative meristems, yet this proportion was also independent of sympatric vs. near- and far-allopatric transplant combinations ($F=0.11$, $p=0.69$). The frequency of individuals producing flowers was low with an average of only 17.5%. Nevertheless, the proportion of individuals that flowered when transplanted to a distant site (i.e., far-allopatric) was lower compared to individuals transplanted back home or to a nearby site ($F=2.27$, $p=0.03$). Indeed, only 14% of individuals flowered when grown in far-allopatric transplant combinations against 20 and 29% in sympatric and near-allopatric ones, respectively.

Fitness-related growth and reproductive traits

No significant differences were detected across the sympatric vs. allopatric contrast for any of the studied growth and reproductive traits (Table 2), suggesting that these

Table 2 Results of generalized linear mixed-effect models for the responses in growth- (aboveground dry mass, number of leaves) and fitness-related traits (number of flowers, number of stolons, total

number of reproductive structures, and clonality) in *Geum reptans* populations transplanted across field sites

	Aboveground dry mass (g)			Number of leaves			Number of flowers			Number of stolons			Total reproductive meristems			Clonality		
	Df	F/ χ^2	p	Df	F/ χ^2	p	Df	F/ χ^2	p	Df	F/ χ^2	p	Df	F/ χ^2	p	Df	F/ χ^2	p
Covariate	–	–	–	1	189.04	<10 ⁻⁴	–	–	–	–	–	–	–	–	–	–	–	–
Site	2	16.95	<10 ⁻⁴	2	39.80	<10 ⁻⁴	2	3.99	0.18	2	10.32	<10 ⁻⁴	2	12.15	<10 ⁻⁴	2	0.58	1
Population	2	9.98	<10 ⁻⁴	2	0.29	1	2	1.26	1	2	8.64	0.0018	2	4.24	0.09	2	3.21	0.36
Symp vs. Allopat	2	0.93	1	1	0.90	1	1	1.78	1	1	0.46	1	1	0.05	1	1	0.92	1
Genets	1	1.56	1	1	4.22	0.36	1	7.67	0.05	1	0.82	1	1	8.11	0.036	119	0.62	1

F- and p values report the effects of site, population, the sympatric vs. allopatric contrast calculated as fixed factors. To account for the variation among genets within populations, this factor was included in the model as a random factor, for which χ^2 - and p values are reported. The covariate (i.e., number of initial leaves at time of transplantation) was significant only for number of leaves, and removed from models for the other traits. The p values indicated in bold were significant after Bonferroni correction (at $\alpha=0.05$; p values multiplied by 9 for correction), p values in italics were significant before Bonferroni correction, and non-significant p values were truncated at 1 if >1 after correction

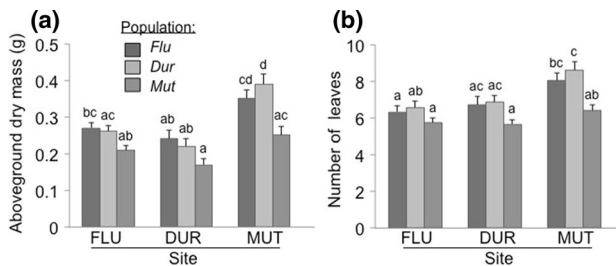


Fig. 1 Mean \pm SE of growth-related traits: aboveground dry mass (a) and number of leaves (b) in *Geum reptans* populations (*Flu*, *Dur*, *Mut*) transplanted across three sites (Flüelapass: FLU, Dürrboden: DUR, Muttgletscher: MUT). Letters reflect multiple contrast results (post hoc Tukey HSD test) between site \times population transplant combinations

fitness-related traits did not differ between populations across transplant sites.

However, highly significant site effects were detected for all fitness-related traits, except for the number of flowers and clonality (Table 2). Similarly, population effects were strong for aboveground dry mass and the number of stolons ($F=9.98$, $p < 10^{-4}$, $F=8.64$, $p=0.0018$, respectively; Table 2). Site and population effects were pronounced for aboveground dry mass as population *Dur* grew best at the MUT site, even relative to the sympatric population *Mut* (Fig. 1a). Similarly, site effects were visible for the number of leaves, which was higher in population *Flu* and *Dur* when grown at the MUT site, relative to when grown at the FLU site (Fig. 1b). The number of flowers differed between genets ($F=7.67$, $p=0.05$; Table 2), and while the number of flowers produced by population *Flu* and *Dur* tended to be lower when grown at the far-away MUT site (Fig. 2a), this site effect was not significant after Bonferroni correction (Table 2). The

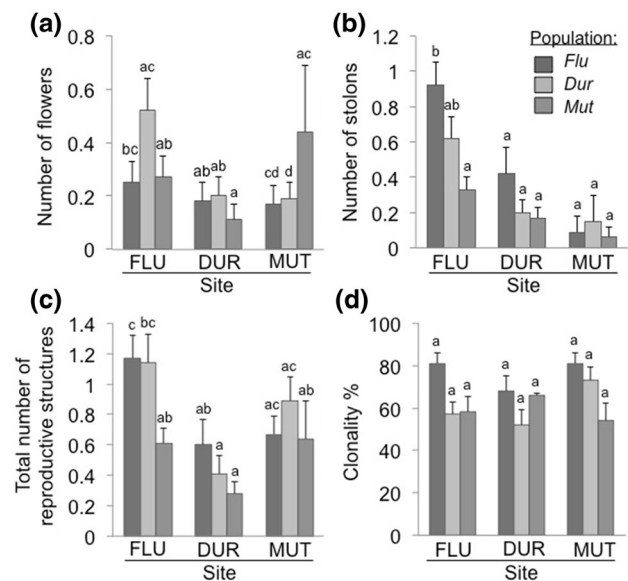


Fig. 2 Mean \pm SE of reproduction-related traits: number of flowers (a), number of stolons (b), the total number of reproductive meristems (c), and the clonality (d) in *Geum reptans* populations (*Flu*, *Dur*, *Mut*) transplanted across three sites (Flüelapass: FLU, Dürrboden: DUR, Muttgletscher: MUT). Letters illustrate multiple contrasts (post hoc Tukey HSD test) between site \times population transplant combinations

number of stolons produced by individuals was particularly high in population *Flu* when grown at its site of origin (FLU), and relative to the population *Mut* when grown together at the FLU site (Fig. 2b). Similarly, site effects on the total number of reproductive meristems were pronounced for population *Flu* (Table 2), which produced a higher number of reproductive meristems when grown at its site of origin (FLU) relative to at the DUR site (Fig. 2c). Moreover, a genet effect was revealed

for the total number of reproductive meristems ($F = 8.11$, $p = 0.036$; Table 2).

Leaf morphology

No significant interactions between site and population were detected for any of the traits related to leaf morphology (Table 3), indicating that populations did not differ in leaf morphology across transplant sites. However, highly significant site and/or population effects were detected for the leaf shape, leaf dissection, and SLA, and leaf dissection also differed across genets (Table 3).

Site and population effects in the leaf shape were particularly pronounced for population *Mut* grown at the FLU site, which had a higher leaf shape (i.e., smaller leaf width for a constant leaf length) than when grown at the DUR or MUT site (Fig. 3a), and in contrast to the two other populations at the FLU site (Fig. 3a). Similarly, site and population effects for leaf dissection were pronounced for population *Flu*, which had a higher leaf dissection ratio when grown at its site of origin (FLU) relative to when grown at the DUR site, and relative to population *Mut* when grown together at the FLU site (Fig. 3b). For SLA, the site effect was clearly visible when looking at population *Dur*, which displayed a significantly lower SLA when grown at its site of origin (DUR), relative to when grown at the FLU site (Fig. 3c).

Partitioning of genetic and environmental effects

For the growth-related traits, such as the aboveground dry mass and the number of leaves, environmental site effects explained about half of the trait variability (44.0 and 43.5%, respectively; Table 4). However, genetic effects at the level of the population or of the genets explained the remaining portion of the variance in these traits (Table 4).

For the reproduction-related traits, genetic population or genet effects explained the main part of trait variation, but environmental site effects also explained roughly a quarter of the variability in the number of stolons and total reproductive meristems (Table 4). For the number of flowers and clonality, none of the variation resulted solely from environmental effects, but was mainly explained by genet effects (Table 4).

Finally, for two of the three studied traits indicative of leaf morphology, environmental effects and genetic effects determined trait variations at a similar proportion. Environmental site effects explained 50.1% of variation in leaf shape, and 37.3% in SLA. However, the variation in leaf dissection was mostly determined by genetic effects (27.6% population, 49.9% genet; Table 4).

Table 3 Results of generalized linear mixed-effect models for the responses in the leaf shape, leaf dissection, and SLA in *Geum reptans* populations transplanted between field sites

	Leaf shape			Leaf dissection			SLA		
	Df	F/ χ^2	p	Df	F/ χ^2	p	Df	F/ χ^2	p
Site	2	12.71	<10⁻⁴	2	10.90	<10⁻⁴	2	11.64	<10⁻⁴
Population	2	8.91	0.0009	2	13.01	<10⁻⁴	2	4.71	<i>0.081</i>
Site × population	4	1.49	1	4	0.49	1	4	1.86	1
Genets	1	0.97	1	1	14.4	0.0018	1	1.62	1

F- and p values report the effects of site, population, and the site × population interaction calculated as fixed factors. To account for the replication of genets within populations, this factor was included in the model as a random factor, for which χ^2 - and p values are reported. The p values indicated in bold were significant after Bonferroni correction (at $\alpha = 0.05$; p values multiplied by 9 for correction), p values in italics were significant before Bonferroni correction, and non-significant p values were truncated at 1 if >1 after correction

Fig. 3 Mean ± SE of leaf morphology traits: leaf shape (a), leaf dissection (b), and specific leaf area (c) in *Geum reptans* populations (*Flu*, *Dur*, *Mut*) transplanted across three sites (Flüelapass: FLU, Dürrboden: DUR, Muttgletscher: MUT). Letters illustrate multiple contrasts (post hoc Tukey HSD test) between site × population transplant combinations

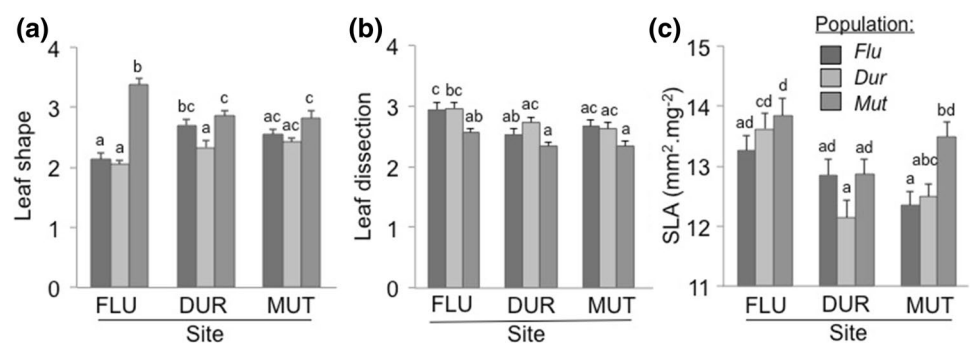


Table 4 Variance components (%) of genetic effects (Population and Genets), environmental effects (Site) and interactive genotype \times environment effects (Site \times Pop) extracted for all fitness-related traits (growth and reproduction) and leaf morphology traits from three *Geum reptans* populations transplanted across three sites in the Central Swiss Alps

	Site	Population	Site \times pop	Genets
Growth-related traits				
Aboveground dry mass	43.99	22.98	3.18	29.84
Number of leaves	43.54	34.60	0.085	21.75
Reproduction-related traits				
Number of flowers	0	0	9.1	90.9
Number of stolons	26.63	36.13	9.27	27.97
Total reproductive meristems	23.68	9.46	1.50	65.36
Clonality	0	31.62	0	68.38
Leaf morphology				
Leaf shape	50.11	32.76	0	17.13
Leaf dissection	22.45	27.63	0	49.92
SLA	37.31	11.92	10.15	40.62

Site-specific selection on trait values

Traits indicative of leaf morphology were found to be highly variable among transplant sites (Tables 3, 4). Thus, a selection analysis was performed to identify if selection for specific trait values occurred within experimental field sites. Most selection gradients calculated for SLA, leaf dissection, and leaf shape at each site were found to be non-significant, suggesting no correlation between leaf morphology and individual fitness measured as total number of reproductive meristems (Table 5). Only the leaf shape was under direct linear selection

at the MUT site where plants with a smaller ratio (i.e., wider leaves for constant leaf length) had a marginally higher fitness (non-significant after Bonferroni correction: $\beta = -0.45$, $p = 0.12$; Table 5).

Molecular differentiation

No identical multilocus genotypes (clonal offspring) were found across the 60 analyzed plants. We detected a mean number of alleles per population and locus of 7.18 ± 0.49 , with a range of 3–11 alleles per locus. The mean genetic diversity (estimated as the unbiased expected heterozygosity) across all studied populations and loci was $H_e = 0.72 \pm 0.02$. The genetic diversity within populations ranged from 0.69 to 0.74 and did not significantly differ among populations. Low inbreeding was revealed by $F_{IS} = 0.16 \pm 0.08$ across all populations and loci. AMOVA revealed that 11% of molecular variance was found among populations ($p = 0.001$; Table 6), and 89% within populations (Table 6). Population pairwise F_{ST} values suggest that little molecular differentiation resided between the two close populations near Davos (*Flu* and *Dur*; $F_{ST} = 0.016$). However, higher F_{ST} values were found when comparing populations *Flu* and *Dur* to *Mut*, the more distant population at Muttgletscher ($F_{ST} = 0.068$ and $F_{ST} = 0.073$, respectively), suggesting higher molecular differentiation among distant populations.

Table 5 Standardized linear selection gradients (β) and their level of significance (p value) estimated as the multiple regression coefficients of relative fitness (i.e., total number of reproductive meristems) on standardized mean trait values at each field site

	Flüelapass (FLU)		Dürrboden (DUR)		Muttgletscher (MUT)	
	β	p	β	p	β	p
Leaf shape	-0.151	1	-0.173	0.39	-0.45	<i>0.12</i>
Leaf dissection	-0.148	1	-0.031	1	-0.083	1
SLA	0.359	1	0.011	1	-0.048	1

The p value in italics was significant before Bonferroni correction (at $\alpha = 0.05$; p values multiplied by 3 for correction), and non-significant p values were truncated at 1 if >1 after correction

Table 6 AMOVA results showing the molecular variance among and within populations

Source	df	SS	MS	Est. Var.	%	p
Among populations	2	56.70	28.35	1.01	11	0.001
Within populations	57	460.10	8.07	8.07	89	–
Total	59	516.80	–	9.08	100	–

Discussion

Molecular differentiation and gene flow among populations

High genetic diversity was found within the three populations ($H_e=0.72$). Our sampling method was designed in order to avoid picking the same genetic individual twice, by implementing at least 5-m distance between sampled individuals. This method was apparently successful since no identical multilocus genotypes were found, which also suggests that clonal ramets of *G. reptans* establish predominantly at close proximity to their mother plants (Pluess and Stöcklin 2004; Hamann et al. 2014). Furthermore, this result suggests that the clonality of *G. reptans* did not cause a loss of genotypic diversity within populations, and is in line with previous findings reported in Pluess and Stöcklin (2004), and ultimately corroborates the consensus that populations of clonal species are often as genetically diverse as populations of non-clonal plants (Ellstrand and Elam 1993; Widen et al. 1994). While sexual reproduction probably plays an important role for recruitment during founding stages of a population after glacier retreat (Cannone et al. 2008) and for the preservation of genetic diversity (Weppler et al. 2006), the long lifespan of clonal ramets and potential immortality of genets undoubtedly contributes to the maintenance of genotypic diversity in *G. reptans* (de Witte et al. 2011).

Molecular differentiation was substantial among populations (11%; Table 6). However, pairwise population F_{ST} comparisons revealed that molecular differentiation was low between the two populations growing at close proximity near Davos (*Flu* and *Dur*), yet both these populations differed strongly from the population *Mut* growing at a larger geographical distance at the MUT site. This suggests that gene flow is maintained over distances of c. 5 km, despite the fact that these two populations are located in neighboring valleys, which is nonetheless in accordance with a prior studies on pollen and seed dispersal distances (Pluess and Stöcklin 2004; Tackenberg and Stöcklin 2008).

Little evidence for local adaptation

No differences were found in growth or reproductive traits between populations transplanted back to their home site or to foreign sites (i.e., sympatric vs. allopatric contrast). The frequency of individual survival and reproduction also did not differ across the sympatric vs. allopatric contrast, and only the frequency of flowering was lower in far-allopatric transplant combinations. Hence, our results suggest only little evidence for local adaptation in the studied *G. reptans* populations from the Central Swiss Alps, even when

separated by relatively large geographic distances, where gene flow is probably restricted.

Evidence for local adaptation has been found in a number of alpine plant populations (Gonzalo-Turpin and Hazard 2009; Fischer et al. 2011; Giménez-Benavides et al. 2011; Hamann et al. 2016); however, an extensive meta-analysis and another recent study suggest that local adaptation may be less common than frequently assumed (Leimu and Fischer 2008; Hirst et al. 2016). Extensive gene flow among populations has been recognized as a main hindrance for local adaptation (Kawecki and Ebert 2004). Given the low level of molecular differentiation found in our study among populations at close proximity, this could potentially explain the lack of phenotypic differentiation between the two populations growing at the sites near Davos (*FLU* and *DUR*), but fails to do so for the more distant population at the *MUT* site. Nevertheless, the two nearby populations are c. 5 km apart, making genetic swamping very unlikely.

While it is possible that local adaptation may take more time than allowed in our experiment to express depending on plant longevity (Bennington et al. 2012; Hirst et al. 2016), the most likely explanation for the lack of local adaptation in our study is related to the narrow habitat niche of *G. reptans*. This species grows at high elevation, typically in glacier forelands, close to the glacier snout, and in moist scree fields (Aeschimann et al. 2004). Consequently, it is likely that environmental conditions are very similar in these habitats, regardless of geographic distance, which may explain the lack of intraspecific differentiation (Cannone et al. 2008; Cheplick 2015). Indeed, differences in elevation, temperature, precipitation, and exposition recorded in our study (Table 1) might not be substantial enough to lead to divergent selection. Supporting this interpretation, the selection analysis for mean leaf traits at different sites showed only little direct linear selection on these traits (Table 5), corroborating the fact that there was no divergent selection across the studied sites. Since only three populations from the Central Swiss Alps were studied here, it is important to note that adaptive genetic differentiation may in fact be found across larger geographic ranges, and such genetic differentiation may well be in line with this species' glacial history and postglacial recolonization (Frei et al. 2012).

An alternative, not mutually exclusive, explanation for the lack of local adaptation in our study could be that highly plastic phenotypic responses to local environmental conditions may overcome the need for genetic differentiation among populations, especially in perennial herbs (Antonovics and Primack 1982; Bazzaz 1996; Cheplick 2015; Hirst et al. 2016). Indeed, our study revealed that *G. reptans* had a great capacity to respond plastically to environmental conditions (Tables 2, 3), which can represent a means to maximize plant performance in heterogeneous

environments (Alpert and Simms 2002; Stöcklin et al. 2009; Nicotra et al. 2010). This may be especially true when considering the relatively narrow range of environmental conditions in the glacier forelands studied here, which may be within the limits that plants can adjust to by means of plastic responses (Alpert and Simms 2002). However, future studies should investigate the adaptive value of trait plasticity in contrast to genetic differentiation in more detail and across the entire geographical and ecological range of *G. reptans*.

Phenotypic differentiation: environmental vs. genetic effects.

While populations transplanted back to their home sites did not outperform populations transplanted to foreign sites, our experiment revealed certain differences in site characteristics (Tables 2, 3). Especially plants grown at the MUT site had a higher aboveground dry mass and produced a greater number of leaves compared to when grown at the other sites (Fig. 1). Variations in these traits were generally strongly driven by environmental conditions (Table 4). While we mentioned earlier that environmental conditions in glacier forelands are relatively similar, they can differ in the time lapse since glacier retreat and hence in their successional stage (Cannone et al. 2008). Indeed, the MUT site, where glacier retreat started in the mid 1990s, is at an earlier successional stage than the two sites near Davos, where glacier retreat started in the late nineteenth century (Schweizerisches-Gletschermessnetz 2015). Hence, this site is still at an early-successional stage, and might allow for better growth of pioneer and early-successional species, such as *G. reptans*, relative to sites at a later-successional stage where interspecific competition increases (Cannone et al. 2008). Similarly, the number of stolons and of total reproductive meristems produced by individuals was lower at the DUR site (Fig. 3), where higher competition might have hindered optimal reproduction.

Leaf morphology also varied greatly in response to environmental conditions at transplant sites. Variation in SLA equally reflected environmental and genetic differences among genets, and variation in the leaf shape predominantly resulted from plastic responses to environmental site conditions (Table 4). All these traits can help optimize light capture and gas exchange (Wright et al. 2004; Poorter et al. 2009), and may have positive repercussions on plant fitness if rapidly adjustable across diverse environments.

While genetic population and genet effects explained a large part of phenotypic variation in reproductive traits (Table 4), the reproductive output of individuals also varied between transplantation sites (i.e., plasticity). The low frequency of flowering individuals, and the high relative proportion of reproduction via clonal ramets (Fig. 2d)

were probably related to the young age and small size of our experimental plants as found in prior studies (Pluess and Stöcklin 2005; Weppeler et al. 2006). Pluess and Stöcklin (2005) also revealed a great size-dependent plasticity in the reproductive strategy of *G. reptans*, which ensures population persistence and reproduction across a range of habitat conditions, and corroborates our hypothesis that phenotypic plasticity might prevail over genetic differentiation in *G. reptans* growing in glacier forelands in the Swiss Alps.

Conclusion

Our study revealed only little evidence for local adaptation of *G. reptans* populations growing on the studied glacier forelands in the Central Swiss Alps, even though extensive molecular differentiation was found between the far-away populations. We suggest that the niche of this species is relatively narrow, and restricted to similar environmental conditions in glacier forelands and moist screes. Moreover, both growth- and reproduction-related traits, as well as leaf traits exhibited strong phenotypic plasticity, which may overcome the need to adapt by means of genetic differentiation. Since only a limited number of populations were studied here, we cautiously advocate that selection could have led to the evolution of phenotypic plasticity rather than genetic differentiation, and encourage future studies to investigate the adaptive value of phenotypic plasticity across the natural range of this species.

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Compliance with ethical standards

Conflict of interest Data and findings presented in this manuscript have not been published and are not under consideration for publication elsewhere. All the authors have approved this submission and all persons entitled to authorship have been named. The authors have no conflict of interest to declare.

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