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ORIGINAL ARTICLE

Small-scale patterns in snowmelt timing affect gene flow and the distribution of genetic diversity in the alpine dwarf shrub *Salix herbacea*

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Current threats to biodiversity, such as climate change, are thought to alter the within-species genetic diversity among microhabitats in highly heterogeneous alpine environments. Assessing the spatial organization and dynamics of genetic diversity within species can help to predict the responses of organisms to environmental change. In this study, we evaluated whether small-scale heterogeneity in snowmelt timing restricts gene flow between microhabitats in the common long-lived dwarf shrub *Salix herbacea* L. We surveyed 273 genets across 12 early- and late-snowmelt sites (that is, ridges and snowbeds) in the Swiss Alps for phenological variation over 2 years and for genetic variation using seven SSR markers. Phenological differentiation triggered by differences in snowmelt timing did not correlate with genetic differentiation between microhabitats. On the contrary, extensive gene flow appeared to occur between microhabitats and slightly less extensively among adjacent mountains. However, ridges exhibited significantly lower levels of genetic diversity than snowbeds, and patterns of effective population size (N_e) and migration (N_em) between microhabitats were strongly asymmetric, with ridges acting as sources and snowbeds as sinks. As no recent genetic bottlenecks were detected in the studied sites, this asymmetry is likely to reflect current meta-population dynamics of the species dominated by gene flow via seeds rather than ancient re-colonization after the last glacial period. Overall, our results suggest that seed dispersal prevents snowmelt-driven genetic isolation, and snowbeds act as sinks of genetic diversity. We discuss the consequences of such small-scale variation in gene flow and diversity levels for population responses to climate change.
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INTRODUCTION

Climate change, a current threat to biodiversity, is thought to alter the distribution of genetic diversity within species living in highly heterogeneous alpine environments, particularly by modifying the distribution of snow (Jay *et al.*, 2012). Climate-change-induced shortening of snow cover, for example, already had a strong influence on plant phenology and the length of the growth period (Wookey *et al.*, 1993; Jones *et al.*, 1997; Arft *et al.*, 1999; Molau *et al.*, 2005; Elmendorf *et al.*, 2012). Nonetheless, alpine habitats may still be safe places for many species in a warming world due to their small-scale topographic variability, which may provide new locations with suitable habitats within only a few meters of the current locations (Yamagishi *et al.*, 2005; Scherrer and Körner, 2011). Alternatively, such small-scale habitat variability can lead to locally adapted sub-populations even in the face of gene flow (Gonzalo-Turpin and Hazard, 2009), and such genotypes adapted to a more narrow range of conditions may respond poorly to future conditions (Linhart, 1974; Fischer *et al.*, 2000; North *et al.*, 2011). In order to predict a species' potential to persist in changing conditions, investigations of the small-scale genetic structure present within species today are needed. Until now, only a few studies have studied genetic diversity and migration at this spatial scale (Hirao and Kudo, 2008; Shimono *et al.*, 2009).

The irregular distribution of winter snow is one of the most conspicuous small-scale heterogeneities in alpine environments. Such snow patterns are caused by the interaction of wind and topography and dramatically alter the microenvironments that plants experience within a scale of a few meters (Wijk, 1986b). Snowbeds are topographical depressions that retain snow for longer periods compared with more exposed ridges that are barely covered by snow during winter. Ridges are regarded as the more stressful microenvironment because of low water availability and frequent frost events early in the spring and late in the fall (Stanton and Galen, 1997; Shimono and Kudo, 2005; Kudo and Hirao, 2006; Reisch *et al.*, 2007; Wheeler *et al.*, 2014), conditions that are thought to become more common in the future (Beniston, 2003). In comparison, late-lying

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snow in depressions offers protection against these spring and early summer frost events but leads to shorter growing seasons. This abiotic complexity caused by the irregular distribution of winter snow results in pronounced differences in plant growth and performance on a fine spatial scale (Scherrer and Körner, 2011), for example, in growth (Wijk, 1986a; Kudo *et al.*, 1999; Dietz *et al.*, 2004), germination (Wijk, 1986b; Kudo, 1992; Shimono and Kudo, 2005) and phenology (Kudo, 1992; Kudo and Hirao, 2006; Shimono *et al.*, 2009). For instance, leaf production and shoot growth in *Vaccinium vitis-idaea* and *Empetrum hermaphroditum* were found to be limited on early-snowmelt ridges (Kudo *et al.*, 1999), and germination and flowering in *Peucedanum multivittatum* were found to be delayed in the snowbeds (Shimono and Kudo, 2005; Kudo and Hirao, 2006). Such within-species differences across microhabitats can be due to genetic differentiation, phenotypic plasticity or both (Nicotra *et al.*, 2010).

Variation in the timing of flowering between sub-populations in different microhabitats can restrict patterns of pollen-mediated gene flow and lead to small-scale genetic structure (Stanton *et al.*, 1997), regardless of whether flowering time is genetically or environmentally regulated (Stanton and Galen, 1997; Stinson, 2004; Jump *et al.*, 2009; Scherrer and Körner, 2011). Such small-scale genetic differentiation due to flowering-time divergence and restricted gene flow via pollen has been reported in the majority of studies on snowmelt-driven genetic differentiation (Stanton *et al.*, 1997; Yamagishi *et al.*, 2005; Hirao and Kudo, 2008; Shimono *et al.*, 2009). Seed dispersal, however, can counteract isolation driven by barriers to pollen flow, because seed dispersal occurs later in the season when all winter snow has melted (Kudo and Hirao, 2006). Predominant gene flow via seed may, on the other hand, result in asymmetric source/sink-like patterns driven by wind, topology and the success of seed establishment (Nathan and Muller-Landau, 2000).

Understanding patterns of genetic variation and gene flow across early- and late-snowmelt microhabitats will help to predict the response of Alpine species to climate change. Upon climate warming, snowmelt is expected to occur generally earlier (Molau *et al.*, 2005; Elmendorf *et al.*, 2012), and current late-snowmelt locations (snowbeds) likely develop season lengths more similar to current exposed ridges. Restricted gene flow and differentiation between sub-populations in different microhabitats can be associated with local adaptation (Giménez-Benavides *et al.*, 2007). In this scenario, late-snowmelt-associated genotypes of long-lived species, such as the dominant shrub species, may have difficulties to persist during warming. Early-snowmelt-associated genotypes, in contrast, would need to establish in new localities, and this could be difficult in long-lived species even if suitable localities are nearby. Alternatively, a lack of differentiation between sub-populations in different microhabitats and unrestricted gene flow between them would be more compatible with an ability of most genotypes to grow in both microhabitats and thus persist *in situ* upon climate change. Apart from differentiation and gene flow, genetic variation contained in sub-populations in early- and late-snowmelt microhabitats could also differ, due to asymmetric gene flow, for example, and this will determine the extent to which genetic variation is lost from one of the microhabitats.

In this study, we investigated the impact of small-scale snowmelt patterns on phenological differentiation, genetic diversity and gene flow in the common long-lived arctic-alpine species *Salix herbacea* (Beerling, 1998; Alsos *et al.*, 2009). Specifically, we address the following study questions: (1) do differences in snowmelt between microhabitats trigger differences in flowering time? (2) does genetic diversity differ between early- and late-snowmelt microhabitats (that is, ridges and snowbeds)? and (3) are patterns of genetic differentiation and gene flow driven by snowmelt?

MATERIALS AND METHODS

Plant material and phenological survey

S. herbacea is a dioecous, prostrate dwarf shrub with wind-dispersed seeds that grows as an extensive ramifying system (Beerling, 1998). Its estimated maximum age is 450 years (De Witte *et al.*, 2012), and its estimated maximum horizontal size is 10 m (Stamati *et al.*, 2007). Average age and genet size have been reported to be <100 years (De Witte *et al.*, 2012) and 0.96 m^2 (Reisch *et al.*, 2007), respectively. We sampled *S. herbacea* at 12 sites in the Swiss Alps near Davos in the summer of 2010 (Table 1), on three different mountains (Schwarzhorn, Jakobshorn and Wannengrat, on average 10.3 km apart). Two different altitudes (high and low, from 2100 to 2800 m a.s.l., on average 1.2 km apart) were chosen to cover the altitudinal distribution of *S. herbacea* on each mountain. A snowbed and a ridge microhabitat (on average 35.2 ± 15.5 m apart) were chosen at each altitude based on topology and vegetation. Snowbeds correspond to depressions where snow remains until relatively late in the growing season, whereas ridges are more exposed areas where snow melts relatively early. Thirty *S. herbacea* stems were randomly sampled within a $10 \times 10\,m^2$ plot at each site yielding a total of 360 samples across the four microhabitat $\times$ altitude combinations through all three mountains. Five leaves were sampled per stem and were immediately stored in empty tea bags and

Table 1 Sampling locations and date of snowmelt for the 12 *Salix herbacea* sites in the vicinity of Davos, Switzerland

Abbreviation[a]	*Mountain*	*Microhabitat*	*Snowmelt day (DOY)*		*Latitude (N)*	*Longitude (E)*	*Altitude (m)*
			2011	*2012*			
JHR	Jakobshorn	Ridge	125	167	46°46.330′	9°51.008′	2552
JHS	Jakobshorn	Snowbed	164	189	46°46.319′	9°51.002′	2559
JLR	Jakobshorn	Ridge	138	171	46°46.273′	9°51.614′	2327
JLS	Jakobshorn	Snowbed	141	180	46°46.265′	9°51.615′	2326
SHR	Schwarzhorn	Ridge	147	171	46°44.009′	9°57.182′	2644
SHS	Schwarzhorn	Snowbed	180	208	46°43.956′	9°57.217′	2625
SLR	Schwarzhorn	Ridge	131	151	46°44.510′	9°57.836′	2359
SLS	Schwarzhorn	Snowbed	173	198	46°44.504′	9°57.829′	2339
WHR	Wannengrat	Ridge	132	136	46°47.454′	9°46.066′	2640
WHS	Wannengrat	Snowbed	181	155	46°47.461′	9°46.100′	2625
WLR	Wannengrat	Ridge	127	191	46°48.001′	9°46.918′	2349
WLS	Wannengrat	Snowbed	127	201	46°47.997′	9°46.918′	2341

[a]Site names are coded as follows: J—Jakobshorn, S—Schwarzhorn, W—Wannengrat, HR—high ridge, HS—high snowbed, LR—low ridge, LS—low snowbed.

dried in silica gel Rubin (Sigma Aldrich, Munich, Germany). In order to test the influence of snowmelt on flowering time, 359 *S. herbacea* patches outside the previous 12 sites but on the same mountains were surveyed for snowmelt and flowering time each week throughout the growth periods 2011 and 2012 and had their sex recorded. Patches comprised all *S. herbacea* stems within a 5 cm radius and were at least 6 m apart from one another.

DNA extraction and PCR amplification

Genomic DNA was extracted from silica-dried leaf material using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA concentration and purity was quantified using NanoDrop spectrophotometer ND-1000 (Saveen and Werner AB, Limhamn, Sweden). DNA samples were stored at −18 °C. Primers for the seven SSR loci used in this study (Supplementary Table S1) were initially developed for *Populus* (Yin *et al.*, 2009) and *Salix* (Stamati *et al.*, 2003) and were selected after cross-species amplification tests in *S. herbacea*, which involved a total of 23 *Populus* markers. The PCR reactions were carried out in a 10-μl final volume containing 5 ng of genomic DNA, 1X PCR buffer (10 mM of Tris-HCl pH 8.8, 50 mM of KCl), 10 pmol of each of the forward and reverse primers, 2 mM of $MgCl_2$, 100 μM of dNTPs and 0.5 U of Taq Polymerase (Fermentas, Burlington, ON, Canada). Forward primers were fluorescently labeled (6-FAM, VIC, NED and PET). A touchdown cycling program was used for the amplification of the seven SSR markers in a panel of seven samples with a hot start of 95 °C for 3 min, followed by 40 cycles of 94 °C denaturation for 30 s, an initial annealing temperature of 58 °C for 30 s and 72 °C extension for 30s. Annealing temperature dropped 1 °C per cycle for 10 cycles, followed by 30 cycles at 48 °C and a 20-min extension period at 72 °C. The PCR products were multiplexed and separated by capillary electrophoresis at the University of Fribourg, Fribourg, Switzerland, using an ABI 3130 DNA Analyzer and LIZ500 as ladder (Applied Biosystems, Foster City, CA, USA).

Data analysis

Analysis of snowmelt and flowering time. Linear models were used to assess the effect of snowmelt and altitude on flowering time and how snowmelt varies between microhabitats. Snowmelt date for each of the 12 sites was compared across years using a Pearson correlation.

Microsatellite diversity. Allele sizes were estimated in base-pairs using GeneMapper v.3.7 (Applied Biosystems). Departure from Hardy–Weinberg equilibrium and F_{is} pairwise values per marker were calculated with GENEPOP v.3.5 (Raymond and Rousset, 1995) in order to check for null alleles or microsatellite-scoring biases. Null alleles were also identified using Micro-Checker v.2.2.3 (Van Oosterhout *et al.*, 2004). The software PowerMarker (Liu and Muse, 2005) was used to determine the number of alleles (A), the number of alleles corrected by rarefaction (A_s), the expected heterozygosity (H_e) (Nei, 1987) and the observed heterozygosity (H_o) for each of the 12 sites (Anderson *et al.*, 1993). For each of the previous statistics, 95% confidence intervals (CIs) were calculated by 1000 bootstrap iterations of genotypes within sites. Additionally, A, A_s, H_e and H_o were compared between microhabitats using linear mixed models with microhabitat as fixed effect and mountain as a random effect (Venables and Ripley, 2002). Elevation was not included as there were no consistent altitudinal patterns. Overall means and standard errors for each microhabitat were extracted from identical models without intercepts (Schielzeth, 2011).

Genetic differentiation. Pairwise F_{ST} values among the 12 sites were obtained with GENEPOP v3.5 (Raymond and Rousset, 1995). Relatedness coefficients were computed across all pairs of individuals following Lynch and Ritland (1999). The relatedness coefficient is a measure of the inbreeding coefficient among related individuals. SPaGEDi was used to perform these calculations (Hardy and Vekemans, 2002). A Mantel test was run in R to estimate the correlation between the matrices of standardized F_{ST} and $F_{ST}/(1-F_{ST})$ according to Rousset (1997) or relatedness and mean snowmelt distance across years. The standardized F_{ST} and relatedness matrices were compared through the same methodology. A partial Mantel test was implemented to account for geographic distance, which was drawn from latitude and longitude and grid mapping in the field. F_{ST} and relatedness estimates were compared between geographic distance classes (among-within mountains, between-within altitudes and between-within microhabitats) using 1000 permutations. The grouping factor was randomly permuted in each iteration, and the difference in means was used as test statistic to compare the permuted and the observed data sets. Moreover, population structure was examined using the STRUCTURE v.2.3.3 software (Pritchard *et al.*, 2000), which was ran in UPPMAX (SNIC, Uppsala, Sweden). A total of five independent runs were used for each K value from $K=2$ to $K=12$ using an admixture model and 100 000 iterations for the burn-in and 100 000 for the MCMC analysis. The optimal number of subdivisions was determined based on the rate of change of the likelihood across different K values as described in Evanno *et al.* (2005). In addition to analyzing population structure, the genetic relationship among all samples was visualized by principal coordinates analysis using the program GenAlex v.6.1 (Peakall and Smouse, 2006).

Gene flow. Effective population sizes (N_e) and pairwise migration rates (N_em) were estimated following coalescent theory and a maximum-likelihood-based approach using MIGRATE v.3.0.3 (Beerli and Felsenstein, 1999). The computations were carried out under both the infinite-allele model and the stepwise-mutation model, and mutation rates (l) among loci were estimated from our data. Bidirectional single-locus migration rates (N_em) were estimated not only for sites within the same mountain but also across mountains. N_em estimates toward snowbeds and toward ridges were compared using 1000 permutation tests. The grouping factor was randomly permuted in each iteration, and the difference in means was used as test statistic to compare the permuted and the observed data sets. Only within-mountain comparisons were considered.

Finally, we interrogated our SSR data set for signatures of genetic bottlenecks in order to disentangle signals of recent migration from more ancient demographic processes dating back to the re-colonization after the last glacial maximum. If patterns of gene flow observed here were driven primarily by persistent departures from migration-drift equilibrium due to postglacial re-colonization, then this should be detectable in the form of genetic signals of reduced population sizes (that is, bottlenecks). We used a bottleneck test that looks for an excess in the heterozygosity in relation to allelic diversity (Cornuet and Luikart, 1996) and a second test that detects an excess in the number of alleles given the molecular allele size range for each marker (Garza and Williamson, 2001).

RESULTS

Snowmelt and flowering phenology

Snowmelt progressed earlier on the ridges than in the snowbeds in both study years (Table 1). On average, ramets from the ridges were snow-free 1 month earlier than ramets from the snowbeds (27.7 ± 9.7 days in 2011 and 24.0 ± 10.9 days in 2012). The year of 2011 was warmer and snowmelt occurred on average 29 days earlier than in 2012 (31.2 ± 8.4 days for the ridges and 27.5 ± 11.9 days for the snowbeds). This trend was consistent across sites. In 2012, snowmelt timing was similar to the long-term average of 37 years (1975–2012) from the Stillberg meteorological station at Jakobshorn (2100 m a.s.l.), which is situated in a ridge-like environment. *S. herbacea* flowered very soon after snowmelt (Figure 1), and flowers were open for 16.6 ± 0.8 days in females and 10.7 ± 1.0 days in males. Flowering time correlated with snowmelt time based on the 359 *S. herbacea* patches surveyed in 2011 and 2012 (slope = 0.805, $R^2=0.827$, P-value <0.001, Figure 1), but flowering-time differences were not explained by altitude ($R^2=0.001$, P-value = 0.435). Snowmelt was correlated across years (Pearson's correlation coefficient, $r=0.298$, Figure 2a), with the exception of two sites at Schwarzhorn that received avalanches in winter 2011/2012. These sites were removed from the following analyses and were not used for the calculation of the mean snowmelt distance across years.

Allelic diversity

Out of the 360 ramets sampled across the 12 sites, 12 were excluded because of low DNA quality. Excluded samples were evenly

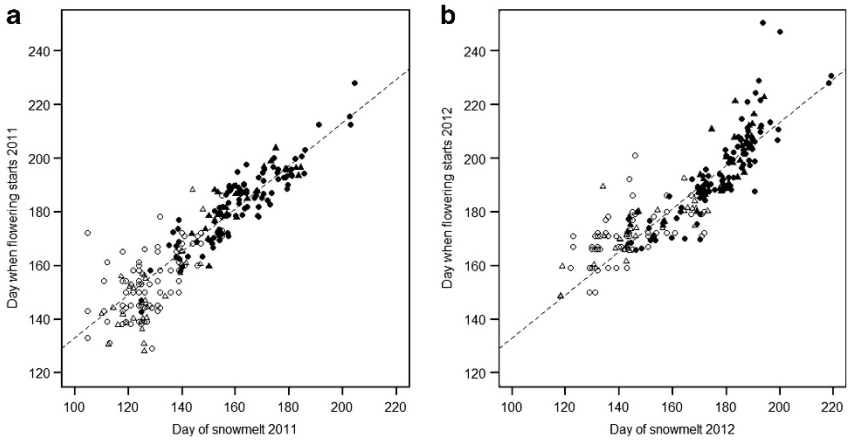

Figure 1 Day of snowmelt predicts when flowering starts for 274 female *S. herbacea* patches growing in ridges (○) and snowbeds (●) and 85 male *S. herbacea* patches growing in ridges (Δ) and snowbeds (▲) surveyed in (**a**) 2011 and (**b**) 2012. Dashed lines are regression lines ($R^2 = 0.827$, *P*-value <0.001).

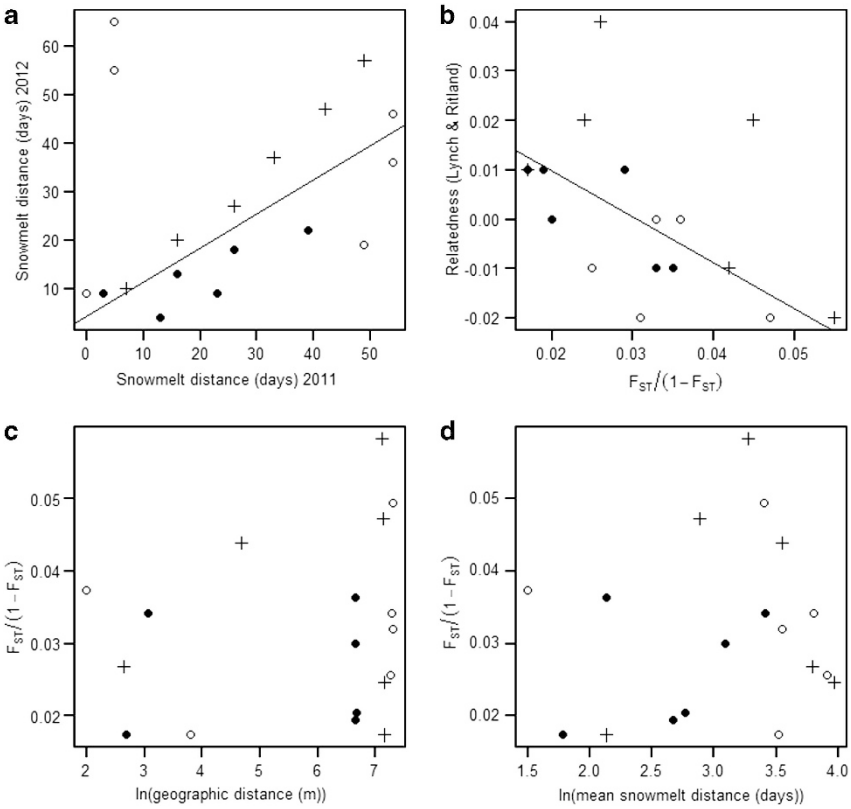

Figure 2 Correlations between genetic distance, snowmelt distance (days between snowmelt time) and geographic distance for comparisons between *Salix herbacea* growing in the 12 sites. (**a**) Snowmelt distance in 2012 vs snowmelt distance in 2011, (**b**) relatedness according to Ritland and Lynch (1999) vs $F_{ST}/(1-F_{ST})$, (**c**) $F_{ST}/(1-F_{ST})$ vs natural logarithm of the geographic distance, following Rousset (1997) and (**d**) $F_{ST}/(1-F_{ST})$ vs natural logarithm of the mean snowmelt distance. Plotting symbols refer to the mountains Jakobshorn (●), Schwarzhorn (+) and Wannengrat (○). Lines are displayed where Mantel tests were significant.

distributed across sites. On average, marker amplifications provided 97.6% of the expected data points. Of the 348 genotyped ramets, 273 were unique genets, and the largest genet was represented by 9 distinct ramets. We detected a total of 97 alleles (on average 14 alleles per locus) and no null alleles. All markers were polymorphic in all sites. There were 11 private alleles evenly distributed within microhabitats and 18 genets with at least one private allele. The total expected heterozygosity within sites ranged from 0.447 to 0.823 with an average of 0.710 across loci. The overall average inbreeding coefficient (F_{is}) across loci was significantly larger than the Hardy–Weinberg expectation ($F = 0.09$, *P*-value <0.01). As no null alleles or bottlenecks were detected, this is unlikely due to SSR artifacts or ancestral demographic events. Rather, a possible explanation is spatial association of genetically similar individuals within sub-populations. Genetic

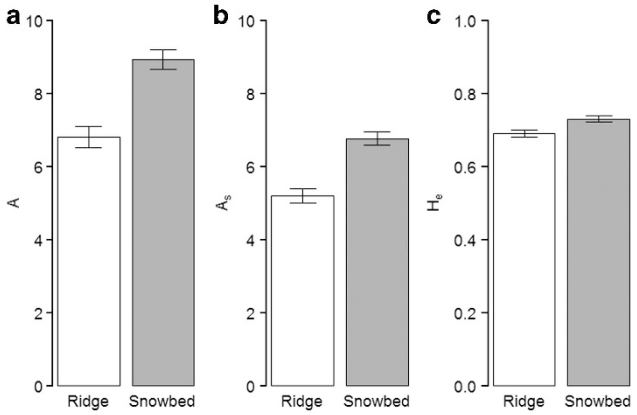

Figure 3 Means and standard errors for numbers of alleles (*A*) (**a**), number of alleles corrected by rarefaction (A_s) (**b**) and expected heterozygosity (H_e) (**c**) based on 7 microsatellite markers evaluated on 273 genets in each of the 12 *Salix herbacea* sites in the Swiss Alps.

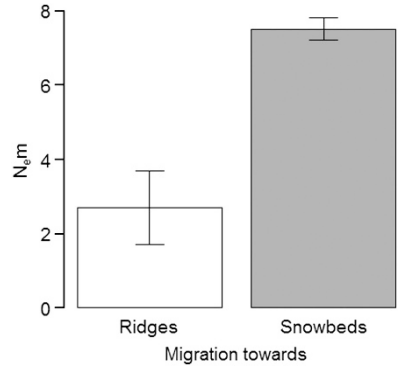

Figure 4 Directional estimates of number of migrants per generation (N_em) for *Salix herbacea* towards ridges (R) and snowbeds (S) within three mountains in the Swiss Alps.

diversity was significantly higher in the snowbeds than in the ridges (Figure 3, Supplementary Table S2) in terms of allelic richness, (8.93 ± 0.27 and 6.81 ± 0.29 for snowbeds and ridges, respectively; *P*-value = 0.007), number of alleles corrected by rarefaction (6.76 ± 0.18 and 5.19 ± 0.20 for snowbeds and ridges, respectively; *P*-value = 0.005) and expected heterozygosity (0.733 ± 0.009 and 0.690 ± 0.009 for snowbeds and ridges, respectively; *P*-value = 0.042). Observed heterozygosity did not differ between microhabitats (0.350 ± 0.007 and 0.370 ± 0.003 for snowbeds and ridges, respectively; *P*-value = 0.211).

Patterns of divergence and gene flow

Genetic differentiation among the 12 sites was generally low (average pairwise $F_{ST} = 0.034$, range 0.013–0.069) and did not differ significantly in within- or among-mountain comparisons (0.031 ± 0.003 and 0.036 ± 0.002 for within- and among-mountain comparisons, respectively; *P*-value = 0.117) or within- and between-microhabitat comparisons (0.028 ± 0.003 and 0.035 ± 0.004 for within- and between-microhabitat comparisons, respectively; *P*-value = 0.691).

Relatedness ranged from −0.04 to 0.04 (note that negative values of relatedness are a common statistical feature that ensures unbiased estimations of the inbreeding coefficient (Lynch and Ritland, 1999). Genets from the same mountain were significantly more related than those from different mountains (average pairwise relatedness = 0.002 ± 0.004, ranging from −0.02 to 0.04 for within-mountain comparisons and -0.012 ± 0.002, ranging from −0.04 to 0.02 for among-mountain comparisons, *P*-value = 0.009). Relatedness did not differ among within- and between-microhabitat comparisons (0.0008 ± 0.004 and 0.003 ± 0.006 for snowbeds and ridges, respectively; *P*-value = 0.316). As expected, standardized F_{ST} and average relatedness were negatively correlated ($r = 0.367$, *P*-value = 0.006, Figure 2b).

Mantel tests between F_{ST} or relatedness and mean snowmelt distance across years using only comparisons within mountains were not significant ($r = 0.279$, *P*-value = 0.062 for standardized F_{ST} and $r = 0.133$, *P*-value = 0.173 for relatedness). Neither standardized F_{ST} nor relatedness was significantly correlated with geographic distance (Figure 2c). A partial Mantel test for correlation between the matrices of relatedness and snowmelt distances, accounting for geographic distances, was also non-significant ($r = 0.274$, *P*-value = 0.059 for F_{ST} and $r = 0.140$, *P*-value = 0.127 for relatedness; Figure 2d).

Genomic admixture analysis with STRUCTURE 2.3.3 and Evanno's test (Evanno *et al.*, 2005) identified $K = 2$ as the most likely number of genetic clusters (Supplementary Figure S1a), which is by default the minimum number of clusters detectable by this method. A principal coordinates analysis (Supplementary Figure S1b) did not detect genetic differentiation between microhabitats.

No significant bottleneck was detected for different mutation models for BOTTLENECK and M_P_VAL (Supplementary Table S3). Out of the 18 different tests performed, none were significant after correcting for multiple comparisons (Verhoeven *et al.*, 2005). Therefore, estimates of numbers of migrants per generation can be attributed to recent asymmetric gene flow and not to postglacial re-colonization dynamics. Maximum-likelihood-based estimates of migration rates (N_em) among sites were high (up to 14.1 migrants per generation, ranging from 0.6 to 16.6, Supplementary Figure S2). Estimated migration rates between microhabitats within mountains were significantly asymmetric (*P*-value < 0.001, Figure 4), with higher average N_em values towards the snowbeds (average $N_em = 7.5 \pm 1.0$, 95% CI: 6.3–7.7) than towards the ridges (average $N_em = 2.7 \pm 0.3$, 95% CI: 2.2–3.3). Additionally, migration rates among mountains were generally lower compared with the estimates of migration rates within mountains ($N_em = 1.6 \pm 0.1$ average migrants among mountains compared with $N_em = 5.1 \pm 0.6$ average migrants within mountain, *P*-value < 0.001, Supplementary Figure S3).

DISCUSSION

Our results indicate that even though there is a phenological differentiation between microhabitats due to snowmelt timing, *S. herbacea* sub-populations growing in different microhabitats are not genetically differentiated. However, late-snowmelt microhabitats (snowbeds) are genetically more diverse than early-snowmelt sites, and gene flow is asymmetric toward the snowbeds. Overall, these results are consistent with snowbeds acting as sinks of genetic diversity, and seed dispersal preventing snowmelt-driven genetic isolation.

Snowmelt differences result in phenological isolation but not in genetic differentiation

We detected consistent snowmelt and flowering delays of up to 1 month in *S. herbacea* plants that grew in snowbeds where snow remains until late summer compared with plants that grew in the nearby, more exposed ridges. A 1-month shift in phenology imposes a strong barrier to pollen flow between microhabitats, as S. *herbacea*

flowers are only receptive to pollen for approximately 2 weeks (Beerling, 1998). Strong flowering time differentiation within short distances is not uncommon and has been reported in snowmelt gradients in Scandinavia for *S. herbacea* (Wijk, 1986b) and in other alpine species (Kudo, 1992; Kudo and Ito, 1992; Jones *et al.*, 1997; Kudo *et al.*, 1999; Shimono and Kudo, 2005; Hirao and Kudo, 2008; Shimono *et al.*, 2009; Wipf *et al.*, 2009; Elmendorf *et al.*, 2012). Some of these exhibit genetically differentiated, phenologically isolated subpopulations (Stanton and Galen, 1997; Yamagishi *et al.*, 2005; Hirao and Kudo, 2008; Shimono *et al.*, 2009). In comparison, we found no evidence that phenologically isolated *S. herbacea* are genetically differentiated (as indicated by Mantel tests based on F_{ST} and relatedness and by a STRUCTURE analysis). Migration estimates for *S. herbacea* indicated rampant gene flow between phenologically isolated microhabitats with N_em estimates >1, often regarded as the minimum required for ongoing panmixia (Hartl and Clark, 2007). In our study, we were thus unable to detect population differentiation using seven putatively neutral microsatellites. However, this does not preclude differentiation at other genomic regions. Narrow and scattered regions may even exhibit signatures of local adaptation while most of the genome freely recombines among ecotypes (Andrew *et al.*, 2012; Kremer *et al.*, 2012).

There are several reasons why population differentiation could be absent in the presence of phenological barriers as observed here. First, seed dispersal may overcome pollen dispersal as the mechanism for inter-microhabitat gene flow. This seems to be feasible given that seed dispersal is not extensively constrained by snowmelt gradients—many of the late snow patches have melted by the time the first seeds are released at early-snowmelt sites. *S. herbacea* seeds are wind-dispersed, resulting in extensive and long-distance gene flow (Alsos *et al.*, 2012; Kremer *et al.*, 2012). Second, intermediate sites not surveyed in this study may act as bridges for gene flow between microhabitats in the well-defined snowbeds and ridges. Third, a disruption of the microhabitat differentiation in years with extreme weather could create gene flow across an otherwise stable phenological barrier. Because some *S. herbacea* clones may live for several centuries (Beerling, 1998; De Witte *et al.*, 2012), such a sporadic connection may be more frequent in the dwarf willow than in other plant species. Finally, the absence of population differentiation in the presence of phenological barriers may be due to recent ancestry. However, the systematic absence of recent bottlenecks across mountains, altitudes and microhabitats allows us to rule out that low population differentiation and asymmetric gene flow are remnants of plant colonization of alpine areas since the last glacial period (Alsos *et al.*, 2012). Thus, the most likely explanations for the lack of population differentiation in the presence of phenological isolation between microhabitats are seed dispersal and climate variation between years.

Alpine snowbeds are sinks of genetic diversity

We found higher genetic diversity levels in *S. herbacea* growing in snowbeds as compared with *S. herbacea* growing on the more exposed ridges. Moreover, gene flow was strongly biased towards snowbeds, a pattern that has not been reported previously. Genetic diversity patterns in related *Salix* and *Populus* species have thus far been analyzed at larger geographic scales (Alsos *et al.*, 2009; de Carvalho *et al.*, 2010; Tiffin *et al.*, 2010; Kelleher *et al.*, 2012), or molecular markers have been mainly used to estimate clone size in alpine *Salix* species (Rossi *et al.*, 2006; Reisch *et al.*, 2007; Stamati *et al.*, 2007; Alsos *et al.*, 2012; De Witte *et al.*, 2012). The diversity and migration biases observed in our study suggest that snowbeds are collecting and maintaining the genetic diversity from the surrounding ridges and other adjacent snowbeds, whereas the ridges are less able to capture variants that originate in other places. It is doubtful that this asymmetric gene flow pattern is driven by phenological isolation. A more likely explanation is that asymmetric gene flow is a consequence of wind dispersal of seeds toward snowbeds, which are often located in topographical depressions (Nathan and Muller-Landau, 2000). This pattern may be exacerbated by greater establishment from seeds in snowbeds, as ridges likely represent more hazardous microenvironments for seed germination because of frequent frost events and low water availability early in the spring and late in the fall (Stanton and Galen, 1997; Shimono and Kudo, 2005; Kudo and Hirao, 2006; Reisch *et al.*, 2007; Wheeler *et al.*, 2014). Thus, the overall asymmetric gene-flow pattern, which can be regarded as a source/sink system across *S. herbacea* growing in different microenvironments, may be mainly driven by small-scale environmental differences associated with topography.

Disentangling the effects of wind-dispersal and seed establishment as causes of the asymmetric gene flow is essential in the light of climate-change research. Abiotic factors that drive seed dispersal such as topography and local wind dynamics may remain constant in a changing environment. That is not the case for other environmental factors. A potential effect of warmer springs and late winters is that snow may retreat earlier and fall later, such that the snowbeds start experiencing more ridge-like conditions. If this is the case, more frequent frost events early in the season—the so-called paradox of a colder spring in a warmer world (Wipf *et al.*, 2009; Wheeler *et al.*, 2014)—and dry periods late in the season may prevent seed germination or establishment even in the formerly well-protected snowbeds. Therefore, snowbeds may cease acting as sinks and preservers of genetic diversity in alpine *S. herbacea*.

In conclusion, we observed that microhabitat heterogeneity induces phenological differentiation but not small-scale genetic structure, and we suggest that this likely results from seed dispersal. Asymmetric gene flow toward the snowbeds makes them sinks and reservoirs of genetic diversity. However, this function likely depends on the presence of protective late-lying snow that may become rarer under future climate conditions.

DATA ARCHIVING

Data are available from the Dryad Digital Repository: doi:10.5061/dryad.9v7bn.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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