Environmental filtering, not local adaptation of established plants determine the occurrence of seed- and bulbil-producing *Poa alpina* in a local flora

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Abstract

Genetic variability among subspecies, ecotypes and populations is an important component of biodiversity, particularly when habitats are characterized by steep environmental gradients across short distances as in the landscape of the European Alps. In *Poa alpina* population differentiation is high due to polyploidy, facultative apomixis and the occurrence of seed- and bulbil-producing plants. We analysed microsatellite diversity of seed- and bulbil-producing populations from two nearby alpine grassland sites in the Swiss Alps and transplanted them reciprocally to test the hypothesis that established plants are locally adapted. The seed- and bulbil-producing populations differed molecularly strongly from each other despite their proximity. The seed-producing plants from the rich, calcareous grassland site performed well at both the home and the away site. Conversely, the bulbil-producing plants from the species-poor grassland had a comparatively low productivity at both sites, but performed better at the high-quality away site compared to its home site. Thus, local adaptation of established plants could not be confirmed by reciprocal transplantation. Results suggest that the distribution of seed- and bulbil-producing *Poa alpina* is a result of habitat quality, differences in plant performance, and the requirements for successful offspring recruitment. The prevailing occurrence of bulbil-producing plants at low productivity sites is probably due to different regeneration niches for seeds vs. bulbils, i.e. an advantage of bulbils at cold sites with poor soil and short growing seasons at this elevation.

Zusammenfassung

Genetische Variabilität zwischen Unterarten, Ökotypen und Populationen ist ein wichtiger Aspekt von Biodiversität, besonders, wenn die Habitate einer Art durch steile Umweltgradienten über kurze Distanzen getrennt sind, wie dies in der Landschaft der Europäischen Alpen der Fall ist. Bei *Poa alpina* ist die Differenzierung zwischen Populationen als Folge von Polyploidie, fakultativer Apomixis und durch die Bildung von Nachkommen aus Samen oder vegetativen Bulbillen besonders gross. Wir untersuchten mit Mikrosatelliten die molekulare Diversität von zwei Populationen nah benachbarter alpiner Rasen, von denen die eine mit Samen und die andere mit Bulbillen reproduziert. Die beiden Populationen wurden reziprok transplantiert, um auf lokale Anpassung etablierter Pflanzen zu testen. Trotz ihrer engen Nachbarschaft waren die Populationen molekular stark differenziert. Die Samen produzierenden Pflanzen des nährstoff- und artenreicheren Kalkrasens wuchsen sowohl am Heim- als auch am fremden Standort sehr gut. Umgekehrt zeigten die Bulbillen produzierenden Pflanzen des ärmeren Standorts ein vergleichsweise geringeres Wachstum an beiden Orten, wuchsen jedoch am fremden Standort höherer Qualität besser als an ihrem Heim-Standort. Lokale Anpassung etablierter Pflanzen konnte folglich durch reziproke Verpflanzung nicht nachgewiesen werden. Die Ergebnisse legen nahe, dass das unterschiedliche Vorkommen von Samen bzw. Bulbillen produzierenden Pflanzen eine Folge ist der verschiedenen Habitatsqualität, der unterschiedlichen Wüchsigkeit der Pflanzen aus den beiden Populationen sowie der spezifischen Anforderungen an die erfolgreiche Etablierung von Nachkommen. Das ausschliessliche Vorkommen von Bulbillen produzierenden Pflanzen in Habitaten geringerer Produktivität ist wohl in erster Linie eine Folge unterschiedlicher Etablierungsnischen von Samen und Bulbillen, insbesondere, dem Vorteil von Bulbillen an kühleren Standorten nährstoffarmer Böden mit einer sehr kurzen Wachstumsperiode.

Keywords: apomixis, genetic diversity, habitat quality, microsatellites, population performance, regeneration niche, reproductive mode

Introduction

The rich flora of the European Alps reflects the strength of selective forces and evolutionary processes in the alpine landscape (Aeschimann et al., 2004). The actual distribution of alpine biodiversity mirrors evolutionary processes from the time when the Alps started to take shape, from migration and differential species survival during and after glaciations (Comes & Kadereit, 1998; 2003), and was caused by the spatial and temporal heterogeneity of abiotic conditions which is particularly pronounced in the alpine landscape (Scherrer & Körner, 2010). Alpine plants evolved striking adaptations to the conditions of their habitats at high elevation, to cold temperature, short vegetation periods, and the frequent occurrence of weather-related extreme events (Chapin & Körner, 1995, Körner. 2003). In addition to species diversity, intraspecific variability is also large in alpine species, particularly when they are widespread. Landscape heterogeneity may lead to divergent natural selection, potentially resulting in adaptive genetic variation, and eventually leads to the formation of subspecies or ecotypes (Bennington & Mc Graw, 1995; Manel et al., 2012). The pattern of intraspecific variability was the focus of the pioneering studies of Turesson (1922) and Clausen et al. (1948), and since then many studies have demonstrated intraspecific specialization to particular environmental conditions in alpine plants (McGraw et al., 1987; Galen et al., 1991; Byars et al., 2007; Byars & Hoffmann; 2009; Stöcklin et al., 2009; Hautier et al., 2009; Frei et al., 2012; Scheepens et al., 2011; 2013).

Divergent natural selection is considered as the main evolutionary force causing populations to be locally adapted, i.e. to evolve traits providing them with a fitness advantage in their local habitat (Darwin, 1958; Williams, 1966). However, local adaptation may be constrained by gene flow, small population size, restricted genetic diversity or temporal variability (Bowmann et al., 2008), and diversification between species and populations may also result from non-adaptive processes such as mutations, gene flow, genetic drift, geographic isolation, hybridization, or polyploidy (Antonovics, 1976, Slatkin 1987, Young et al. 1996, Chun et al. 2009, Weiss-Schneeweiss et al. 2013). Furthermore, natural selection may have acted in the past, but the selective agents may not be present any more causing earlier fitness advantages to vanish (Harder & Johnson, 2009).

Local adaptation is frequently believed to be the cause of differentiation but less often satisfactorily demonstrated. Local adaptation can be assumed from circumstantial

evidence, i.e. by correlating population trait values from a common garden experiment with environmental conditions from the populations' origin (Linhard & Grant, 1996; Weber & Schmid, 1998; Scheepens et al., 2013). However, unequivocal evidence of local adaptation requires reciprocal transplantation experiments between a minimum of two and preferably more populations, and testing whether the outcome is compatible with divergent selection (Kawecki & Ebert, 2004; Blanquart et al., 2013). The test criteria for evaluating the outcome of reciprocal transplantations, the "local vs. foreign" and the "home vs. away" contrast are not strictly equivalent, and sometimes ambiguous, because confounding effects with habitat quality and population performance may arise. The "local vs. foreign" criterion compares the fitness of the local population at its home site to the fitness of foreign populations, while the "home vs. away" criterion compares the fitness of each population when grown at its home site to the fitness when this population is grown at away sites. In pairwise comparisons, strict evidence of local adaptation is only present when the local plants outperform the foreign plants at both sites. A recent meta-analysis by Leimu & Fischer (2008) suggests, that local adaptation is less common in plant populations than generally assumed when tests are based on the stringent criteria as mentioned above. Lack of local adaptation may result from similarity of transplant sites, high gene flow among nearby sites, high plasticity of populations, lack of genetic diversity, or founder effects during colonization (Rapson & Wilson 1988, Anderson & Geber 2010, Cheplick, 2015 for further references).

Here, we test the hypothesis of local adaptation in two populations of *Poa alpina* from two alpine grassland sites of a local flora in the Swiss Alps by reciprocally transplanting clonally produced offspring among them. The study was motivated by the observation that *P. alpina* plants from the two nearby sites differ in their reproductive mode, producing either exclusively seeds or exclusively bulbils. *P. alpina* is a common grass species in the upper montane and alpine zone of the European Alps (Müntzing, 1980; Conert et al., 1998). Population differentiation in this species is high, probably as a consequence of polyploidy, facultative apomixis of seeds and pseudo-vivipary in bulbil-producing plants (Rudmann-Maurer et al., 2007). With elevation, the occurrence of seed-producing plants is decreasing and that of bulbil-producing plants is increasing, indicating an advantage of reproduction by bulbils in harsh alpine conditions (Fischer et al., 2011; Steiner et al., 2012). The exclusive occurrence of seed-producing *P. alpina* in a species-rich calcareous grassland in a high-alpine flora while almost all plants of this

species are bulbil-producing in all other habitats of the surrounding vegetation suggested the hypothesis of a selective advantage being responsible for this distribution. We therefore used seed-producing plants of *P. alpina* from the nutrient-rich calcareous grassland site (Festucetum), and bulbil-producing plants from a nearby low-productivity grassland on acidic soil (Nardetum) for a reciprocal transplantation experiment to test for local adaptation of established plants and for a microsatellite analysis to detect their level of genetic differentiation.

We asked the following specific questions: (1) In the molecular analysis: do the two nearby populations of *Poa alpina* differ in their genetic diversity, and how much are they differentiated from each other? (2) By reciprocal transplantation: can we detect local adaptation, i.e. is there a “local vs. foreign” or a “home vs. away” advantage or both of plants grown at these sites. (3) Can the distribution of seed- and bulbil-producing *P. alpina* plants be explained by local adaptation of established plants, by habitat quality, or plant performance?

Material and methods

Study species

Poa alpina L. (Poaceae) is a common arctic-alpine plant in the Northern Hemisphere (Conert, 1998). In the Alps it occurs in pastures, nutrient-rich meadows and as a pioneer species on glacier foreland, scree slopes, and snowbeds. Due to its high contents of fats and protein it is considered one of the most important fodder grasses in subalpine and alpine grassland (Bachmann, 1980; Conert, 1998). *P. alpina* is a polyploid complex, with highly variable, aneuploid chromosome numbers and multiple B-chromosomes (Müntzing, 1980; Steiner & Heidenreich, 1979). In Switzerland chromosome numbers range from 22 to 61 chromosomes per plant (Duckert-Henriod & Favarger 1987; Rudmann-Maurer et al., 2007). Plants of *P. alpina* produce either seeds, or reproduce vegetatively by forming bulbils in the panicles that grow into little plantlets on the parent plant, which are therefore called pseudo-viviparous (Müntzing 1980, Pierce 1998). The plantlets may dehisce from the parent plant and can eventually root and establish quickly (Pierce, 1998). The reproductive mode is largely genetically determined (Rudmann-Maurer et al. 2007; Steiner et al., 2007). Pseudo-vivipary is

probably evolving locally from seminiferous populations as proposed by Wilkinson and Stace (1991) and demonstrated by Chiurugwi et al. (2011) for *Festuca vivipara*. According to Müntzing (1933) seeds of seminiferous plants are either produced sexually or apomictically. Steiner et al. (2012) found between 7 to 8% of the offspring from seed-producing *Poa alpina* to differ genetically from their mother and therefore to be produced via sexual reproduction. The frequency of seed-producing plants decreases and that of bulbil-producing plants increases with elevation suggesting an advantage of reproduction by bulbils in harsher and colder conditions (Fischer et al. 2011; Steiner et al., 2012).

Study site

The study locations are two S- and SE-exposed natural grassland sites near the Furka Pass (46°34'N 8°25'E) at almost identical elevation (2390 and 2430 m a.s.l.). The two sites selected for the reciprocal transplantation differ in their soil type, vegetation, and productivity. Mean annual precipitation at the study location is c. 1600 - 1900 mm, with a growing-season length between 2.5 and 3.5 month, and a mean growing-season temperature (mid Jun-Aug) of c. 7.5 °C. (measured by a weather station at the study location). The more productive site is a species-rich grassland with calcium- and nutrient-rich, relatively deep soil, on a S-exposed and steep slope, dominated by *Festuca violacea* and a pH around 5 (Festucetum). *Poa alpina* plants at this site are almost exclusively seed-producing. The other site, in less than 200 m distance, is a species-poor low-productivity grassland with an acidic soil, on a SE-exposed flat slope, dominated by *Nardus stricta* and *Carex curvula* and a pH around 4 (Nardetum). Here, *P. alpina* is almost exclusively bulbil-producing. Due to differences in slope and exposition, snow melt at the calcareous grassland site is usually three to four weeks earlier and, accordingly, the growing-season length is longer and maximal development of the vegetation is reached earlier.

Sampling, plant propagation and reciprocal transplantation

In July 2012 we randomly sampled 22 *Poa alpina* plants in each of the two study sites described above. To reduce sampling of genetically identical individuals as much as possible, the minimum distance among samples was 2 m. We transplanted the 44 plants into multitrays containing 28 cells of 6 cm diameter in low-nutrient soil, (Anzuchterde, Ökohum, Herrenhof, Swizerland), clipped them to a standard size of 2 cm above ground

and brought them into a glasshouse at the Botanical Institute in Basel, Switzerland (260 m a.s.l.) to reduce carry-over effects. After two months, the plants consisted of several tillers, which we isolated from each other to obtain on average four clonal offspring from each initially sampled genotype. The re-potted plants remained in the glasshouse in Basel until the end of October, when they were cut back again to a standard size of 2 cm above ground and brought back to the study location on the Furka Pass. The clonal offspring from each plant of both populations were randomly assigned to the two sites and transplanted in several rows and two blocks per site into the local soil of the two transplant sites, i.e. the rich calcareous grassland (Festucetum) and the nutrient-poor grassland on acidic soil (Nardetum). In total, 137 offspring from 37 genotypes were reciprocally transplanted, 67 originating from the rich calcareous grassland (Festucetum), and 70 from the nutrient-poor grassland (Nardetum), respectively. The two sites (Festucetum and Nardetum) received 68 and 69 plants, respectively, and c. half of the transplanted plants in each site came from the two populations. Mortality after the first overwintering did not depend on site or origin. We found 26 dead plants, and 4 plants did not increase in biomass or died later. The remaining 107 plants from 31 genotypes were used for measurements.

We harvested aboveground biomass at the end of the first growing season in September 2013 by cutting leaves 0.5 cm above soil level. Only very few plants had reproduced by that time. In 2014, we harvested aboveground biomass again in the same way at peak flowering, on 15th July in the Festucetum, and on 24th July in the Nardetum. At this time all plants were reproducing and we counted the number of inflorescences of each plant. Harvested plant material was dried for 48 hours at 90 °C and weighed.

Data analysis

We performed linear mixed-model analyses with transplant site and origin as well as their interaction as fixed effects and genotypes nested within origin and its interaction with transplant site as random effects using the lmerTest package of R (R Development Core Team, 2009). lmerTest allows F-tests for fixed effects and likelihood-ratio tests for random effects using stepwise model reduction and comparison. Type 3 sums of squares and Satterthwaite approximations for degrees of freedoms were used for calculations. Untransformed data were used (Crawley, 2007).

Molecular analysis

Maurer et al. (2005) developed microsatellite primers for five loci of *P. alpina*. Details on the five polyploid loci, PCR conditions, and horizontal Spreadex[®] gel electrophoresis (Elchrom Scientific, Cham, Switzerland) of the PCR amplicons are described in Maurer et al. (2005), and in Steiner et al. (2012), respectively. In brief, DNA was extracted from silica-dried leaf material of the 22 plants from the Festucetum (seed-producing individuals) and the 22 plants from the Nardetum (bulbil-producing specimens). PCR was done with the illustra[™] puReTaq Ready-To-Go PCR Beads (GE Healthcare, Buckinghamshire, UK). The beads give reliable PCR products in the resolution of microsatellite banding pattern using Spreadex gels. In a pre-analysis, four unrelated individuals together showed a total of 154 bands at the five microsatellite loci. In a repetition PCR run of the same individuals, five mismatch bands were found, i.e. $5/154 = 0.03 = 3\%$. This means an acceptable replication rate in the banding pattern of 97%. Molecular diversity among the two study populations was analysed following the procedure for polyploid microsatellite data of *P. alpina* described by Rudmann-Maurer et al. (2007). In all 44 individuals, the presence/absence of alleles were 1/0-coded as for dominant markers, and GenAlEx ver. 6.2. (Peakall & Smouse, 2006) was used for detecting identical multilocus genotypes and to perform an analysis of molecular variance (AMOVA). Genetic diversity was measured as the number of allelic bands per individual genotype and this measure was used to test for differences in genetic diversity among populations and among seed- and bulbil-producing populations. The 1/0 data matrix was also used for the reconstruction of an unrooted, individual-based UPGMA dendrogram (see Appendix A). The UPGMA clustering based on Jaccard index was run on the online platform 'DendroUPGMA' at the University of Rovira i Virgili (URV), Tarragona, Spain (Garcia-Vallvé & Puigbo, 2002).

Results

Genetic diversity

Among the 44 sampled plants of *Poa alpina*, altogether 44 distinct allelic bands were found at the five microsatellite loci, between 6 and 9 per locus. We found 40 multilocus phenotypes, 21 among the 22 plants from the Festucetum, and 19 among the 22 plants from the Nardetum.

The genetic diversity, measured as the number of allelic bands per individual, did not differ between the seed-producing plants from the *Festucetum* (13.1 ± 0.5 , mean $\pm$ s.e.), and the bulbil-producing plants from the *Nardetum* (13.5 ± 0.5 , $t=0.6$, $df\ 33.1$, n.s.).

In the AMOVA analysis we detected high molecular differentiation between the two populations (Table 1). 35% of the variation came from the variation in microsatellite bands among populations, and the remaining 65% from within-population variation. The high differentiation is reflected by a high number of private alleles observed. Nine private alleles were found in the plants from the *Festucetum* and eight in the plants of the *Nardetum*. In the UPGMA-dendrogram of the banding pattern the plants from the two populations appear in separate clusters (see Appendix A).

Habitat quality and population performance

All plants were on average significantly larger and reproduced more on the calcareous grassland site (*Festucetum*) compared to growth on the grassland on acidic soil (*Nardetum*, Fig. 1, Table 2). 28 of 31 genotypes performed better when grown on the calcareous grassland site. Across both populations, mean biomass of plants in the first year (2013) was 226 ± 21 mg when grown in the *Festucetum* vs. 107 ± 8 mg when grown in the *Nardetum*, and in the second year (2014) biomass was 754 ± 75 mg vs. 261 ± 22 mg, respectively. All plants reproduced at both sites.

Performance was dependent on the origin of plants, indicating differences in intrinsic population performance (Table 2). Across both sites, biomass of seed-producing plants from the rich calcareous grassland was 47% and 45% larger than the biomass in the first and second year, respectively. The mean number of inflorescences in the second growing season was 4.4 ± 0.5 for the plants of the *Festucetum*, and 2.0 ± 0.2 for those from the *Nardetum*.

Local adaptation

In the first year (2013) the site $\times$ origin interaction for biomass was significant (Table 2). This was due to the large advantage of seed-producing plants at their home site compared to the bulbil-producing foreign plants (71% higher biomass). However, in 2014 the interaction was not significant anymore (Table 2), and the aforementioned advantage of the seed-producing plants at their home site was smaller (36%). Qualitatively similar results were obtained for fitness expressed as numbers of

inflorescences: *P. alpina* from the Festucetum had 5.8 ± 0.5 inflorescences at its home and 2.9 ± 0.6 at the away site compared to 1.1 ± 0.5 of *P. alpina* from the Nardetum at the home site and 2.9 ± 0.5 at the away site (Fig. 1 C).

The seed-producing plants from the Festucetum performed always better at their home site compared to the performance at the away site. Also, plants of this population performed better at their local site compared to the foreign population. However, the opposite was the case for the bulbil-producing plants from the Nardetum, which performed always better at the away site and were mostly outperformed at their local site by the foreign plants (Fig. 1 A, B, C). Only in the first year was the performance in the Nardetum similar for both populations (2013, Fig. 1 A), while in the second year the foreign population performed better than the local population in the Nardetum (2014, Fig. 1 B, C). Thus, the “home vs. away” and the “local vs. foreign” criterion was satisfied only for the population from the Festucetum, but not for the population from the Nardetum.

Overall, genotypes did not differ in their growth and reproduction but in the second year they developed differently when their growth was compared among the two study sites (Table 2). Results at the genotype level were very consistent and similar to those at the population level. 13 of the 16 genotypes originating from the Festucetum, and all 15 genotypes from the Nardetum performed better when grown on the calcareous site. Also, most genotypes of the Festucetum outperformed the best Nardetum genotypes at the Nardetum site, indicating that established genotypes were not locally adapted.

Discussion

By reciprocal transplantation of a seed- and a bulbil-producing population of *Poa alpina* from nearby grassland sites we could not detect local adaptation of established plants. Nevertheless, plants from the two populations differed genetically in performance, with the seed-producing plants from the Festucetum yielding higher biomass and having more inflorescences compared to the bulbil-producing plants at both transplantation sites in 2014. Difference in habitat quality caused plants from the two populations and almost all genotypes from both origins to perform better in the calcareous soil of the

Festucetum. These results indicate that habitat quality in combination with intrinsic plant performance and factors not investigated here explain the different distribution of seed- and bulbil producing *P. alpina* at the Furka Pass, but not local adaptation. Despite taking measures during propagation of plants to reduce carry-over effects, we cannot completely exclude that such effects have affected our results. However, such effects, if present, are expected to diminish over time, as has been found in other studies (Ouborg et al. 1991, Schmid & Dolt 1994), and in a study of ourselves using clonal offspring of *Poa alpina* (Fischer et al. 2011). Therefore, we suggest that it is unlikely that carry-over effects were of great importance with respect to our results.

Molecular diversity and performance of seed- and bulbil-producing Poa alpina

The microsatellite analysis revealed that seed- and bulbil-producing populations of *Poa alpina* were equally diverse but strongly differentiated from each other in their allelic composition and by a high number of private alleles. The two populations are thus largely independent units with no or only little genetic relationship. On the one hand, seed-producing *P. alpina* plants are facultative apomicts (Müntzing, 1933), and the percentage of sexually produced seeds is relatively high with c. 8% compared to what is known from other apomictic species (Asker & Jerling 1992, Steiner et al., 2012). The residual sexuality in *P. alpina* in seed-producing genotypes seems high enough to purge deleterious mutations and to allow for natural selection (Asker & Jerling, 1992; van der Hulst et al., 2003). Evidence for adaptation in *P. alpina* to human land use (Fischer et al., 2011) is a clear indication of ongoing natural selection in this species. On the other hand, bulbil-producing genotypes are an evolutionary dead-end because they reproduce exclusively asexually by bulbils and never produce seeds (Darlington, 1958). The high number of multilocus genotypes in the bulbil-producing population suggests that the formation of bulbil-producing cytotypes from seminiferous plants happens relatively frequently. Occasional gene flow from bulbil-producing cytotypes to seed-producing facultative apomicts is not completely excluded, since pseudo-viviparous plants may occasionally develop a sexual (male) floret at the base of vegetative bulbils (Müntzing, 1980, Pierce et al., 2003). However, this is rarely the case in the field, if at all (Rudmann-Maurer, 2007; Fischer et al., 2011, and personal observation). Their strong genetic differentiation may explain why the two populations differ so much in their performance. It is conceivable that the residual sexuality in seed-producing cytotypes allows for the selection of high-performing genotypes, while this is not the case in the

bulbil-producing plants, which produce exclusively clones. Similar to our results, Steiner et al. (2012) found in several common gardens at different elevation that bulbil-producing *P. alpina* plants were always less vigorous than seed-producing ones. Thus, lower performance of bulbil-producing cytotypes in *P. alpina* seems to be a general phenomenon and may be a consequence of their asexuality and genetic separation from seminiferous plants. The increase of bulbil-producing plants with elevation can thus hardly be explained by the performance of established plants and may therefore depend on other advantages, possibly through better establishment rates of bulbil-derived offspring under the relatively cold and wet conditions at high elevation and due to a short growing season, as has been discussed for pseudo-viviparous grasses in general (Lee & Harmer, 1980) and also suggested from results of Fischer et al. (2011) and Steiner et al. (2012).

Reciprocal transplantation pattern

The outcome of the reciprocal transplantation of seed- and bulbil-producing *P. alpina* between nearby grassland sites was clearly not in line with the hypothesis of local adaptation of established plants. In the pairwise comparisons of several performance traits neither the “local vs. foreign” nor the “home vs. away” criteria were fulfilled. As a consequence, not divergent natural selection but other processes and the intrinsic differences between the origin of the two populations must be the cause of their different distribution.

Obviously, the calcareous grassland is qualitatively a much better habitat for both *P. alpina* populations. At a closer look, this is not surprising: the soil in the Festucetum is deeper, more nutrient-rich, snow-melt is three to four weeks earlier, solar insolation and soil temperature are higher due to exposition and slope, and the overall duration of the growing season is c. one month longer (Christoph Mullis, unpublished data). Vice versa, in the nutrient-poor grassland on acidic soil both populations performed poorly, which can be explained by the combined effect of low nutrients, late snow-melt and the shorter growing season.

The almost exclusive occurrence of high-performing seed-producing genotypes in the Festucetum might result from several non-exclusive reasons. Firstly, despite the missing evidence for local adaptation between the two sites, hidden adaptation of seed-producing genotypes to their home-site might have occurred. The residual sexuality in facultative apomicts allows continuously for the formation of new genotypes that can

then be subject to natural selection. The higher vigour of the plants from the Festucetum could thus result from directional selection of high-performing genotypes in the calcareous grassland site itself. Such directional selection cannot be revealed by reciprocal transplantation and remains a speculation. Secondly, the seed-producing genotypes could be high-performing generalists which were selected in the past at other locations due to their high fitness and which were dispersed and established easily at the high-quality site. From our study we cannot distinguish between the above two possibilities. Whatsoever, they do not explain, why pseudo-viviparous plants are missing in the Festucetum. Bulbil-producing plants are likely to be bad competitors in the species-rich, productive calcareous grassland because of their low vigour. In addition, the bulbils, which remain well attached to their reproductive stems for a long time, are not far dispersed, which may make them a rare occurrence outside of their local habitat. Winkler et al. (2010) observed that during primary succession seeds are more successful than bulbils with increasing vegetation density. Hence, dispersal limitation of bulbils together with their low performance are the likely causes for the absence of bulbil-producing *P. alpina* in the Festucetum.

More difficult to explain is why seed-producing plants are missing in the Nardetum, despite their much better performance compared to the bulbil-producing local genotypes. A first reason could be the rarity of seed-producing plants in the local flora of the Furka Pass (Hefel & Stöcklin, 2010). Grassland on calcareous soil where seed-producing *P. alpina* plants occur is limited to a small area in this region. Silicious bedrock with grassland dominated by *Nardus stricta* and *Carex curvula* or scree vegetation where *P. alpina* is almost exclusively bulbil-producing prevail in the flora of the Furka Pass. Therefore, despite grass seeds being dispersed relatively far by wind (Cheplick, 1998), the seed rain from seed-producing *P. alpina* may be relatively low in the Nardetum.

Furthermore, if seeds arrive and successfully germinate they have still a disadvantage compared to bulbils. After germination and establishment it takes seeds usually three years to become reproducing (Matthews, 1992). Bulbils consist of already three to four leaves when released from the maternal plant, they have a higher nutrient capital, are non-dormant and able to establish immediately. Plants grown from bulbils can reproduce already in the second year (Lee & Harmer, 1980). The aforementioned advantages of bulbils are probably the main reason for the high occurrence of bulbil-

producing plants in cold, wet and short growing-season conditions at higher elevation. If a seed-producing *P. alpina* would get established in the Nardetum, late snow-melt and the short growing season might still limit successful establishment compared to the already more frequent bulbils.

The local occurrence of seed- and bulbil-producing Poa alpina

Understanding species distribution patterns is among the most challenging questions in plant ecology. Historical processes such as migration, speciation, or cytotype formation in a polyploid complex as *Poa alpina* determine the pool of species, subspecies or ecotypes in a particular region (Zobel, 1992). The local occurrence is then determined by environmental filtering, the availability of suitable habitats and the potential of plants to colonize them (Keddy, 1992; Maire et al., 2012). The colonizing capacity of species is determined by their fundamental and realized niche (Hutchinson, 1957).

Metapopulation theory and available evidence suggests that at a given moment only a fraction of suitable habitats will be occupied, because of dispersal and microsite limitations (Hanski & Gilpin, 1997, Munzbergova & Herben, 2005). Thus, divergent selection of populations in different habitat conditions is only one among many processes explaining the current distribution of species. It is therefore not surprising that local adaptation is less frequent as commonly assumed (Leimu & Fischer, 2008). Local adaptation has been detected at very small spatial scales, for instance among closely located subpopulations of trees (Sork et al. 1993), along altitudinal gradients (Galen et al. 1991, Byars et al. 2007), or along a snow-melt gradient (Kawai & Kudo 2011). However, because adaptive processes are inherently slow, constrained by gene flow and scale-dependent, local adaptation is more likely to be found at the continental or regional scale than at the small spatial scale (Joshi et al., 2001; Becker et al., 2006). This may apply even more to a species composed of a mixture of cytotypes which in *P. alpina* are formed at an unknown rate. The strong molecular differentiation of the seed- and bulbil-producing populations at the Furka Pass resulted in a distinguished clustering of the studied genotypes (see Appendix A) indicating clearly separated gene pools and a different evolutionary history which is probably less related to selection than to unknown factors of cytotype formation in the past. However, the process of cytotype formation still remains a mystery (Matzk & al., 2005).

As we were not able to detect local adaptation of established plants by our reciprocal transplantation, the most parsimonious explanation for the striking separation of seed- and bulbil-producing *P. alpina* on the Furka Pass is environmental filtering by the distinct habitats of the two study sites as revealed by our experiment. The supposed filtering process is certainly related to the difference in the intrinsic quality of seed- and bulbil producing plants, which is accentuated in their respective habitats on the Furka, and probably related to the different regeneration niche, i.e. the different dispersal and establishment properties of their propagules, that is to say their seeds and bulbils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXXX.

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Fig. 1. Reciprocal transplantation of seed- and bulbil-producing populations of *Poa alpina* between a species-rich grassland on calcareous soil (Festucetum) and a nearby low-productivity grassland (Nardetum) on the Furka Pass in the Central Swiss Alps. (A) Biomass (mean $\pm$ se) in 2013, the first year after transplantation; (B) Biomass (mean $\pm$ se) in 2014, including reproductive stems; (C) Number of reproductive stems (mean $\pm$ se) in 2014 (dark shaded bars: plants originating from the Festucetum; light shaded bars: plants originating from the Nardetum).

Table 1. Analysis of molecular variance (AMOVA) of two populations of *Poa alpina* from a calcareous grassland site (Festucetum, 21 genets) and a nearby site on acidic soil (Nardetum, 19 genets) from the Furka Pass in the Swiss Alps

Source of variation	df	Mean squares	Estimated variance	Variation (%)	Fixation index
Between populations	1	64.72	2.96	35	$\Phi = 0.347^{**}$
Within populations	38	5.59	5.59	65	
Total	39	7.11	8.55		

Df, degrees of freedom. Significance of Φ is based on 1'000 permutations: $^{**}P < 0.01$. Significance for within population variation cannot be calculated since we used a data matrix of dominant markers, and therefore the within population variance corresponds to the error-term.

Table 2. Results of linear mixed-effect models testing for the effects of reciprocal transplantation of *Poa alpina* between two grassland sites on growth (total aboveground biomass in 2013 and 2014) and no. of inflorescences (2014) at the Furka Pass in the Central Swiss Alps.

Source of variation	Biomass 2013	Biomass 2014	No of inflorescences
Transplant site	30.20 ***	29.17 ***	20.57 ***
Population Origin	7.17 **	4.30 *	18.25 ***
Transplant site x Population origin	5.17 *	0.92	1.09
Genotype (random)	0.19	n.a.	0.00
Genotype x Transplant site (random)	1.65	11.00 ***	1.53

Shown are F- and X²-values and their significance is indicated with asterisks. Genotype is nested in Population origin. Genotype and Genotype x Transplant site are random effects (X²-values); other factors are fixed effects (F-values). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Appendix A

Individual based UPGMA dendrogram using presence/absence of alleles from five microsatellite loci in 44 genotypes of *Poa alpina* from a species-rich grassland on calcareous soil (Festucetum) and a nearby low-productivity grassland on acidic soil (Nardetum) on the Furka Pass in the Central Swiss Alps.