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Background and Aims: The Alpine Meadow Grass Poa alpina is common in subalpine and alpine natural sites and agriculturally used land, where it is an important fodder grass. Natural factors and human land use are supposed to have been shaping its genetic diversity for hundreds of years. The species comprises sexually and vegetatively reproducing plants. The aim of this study was to investigate the effects of agricultural land use, environmental factors and the mode of reproduction on the distribution of its microsatellite diversity within and among populations and to analyse whether its genetic diversity is correlated with plant species diversity in grassland parcels.

Methods: Genetic diversity of P. alpina was assessed with five microsatellite markers for 569 plants originating from 20 natural sites and from 54 grassland parcels of different cultural tradition, land use and altitude in the Swiss Alps. Due to polyploidy and frequent aneuploidy of the species, data analyses were based on the presence of microsatellite bands.

Key Results: A low but significant differentiation was found in microsatellite bands among natural sites and agriculturally used parcels, while their microsatellite band diversity within populations did not differ. An increased differentiation was found in microsatellite bands with increasing geographic distance among parcels, and a differentiation among grazed and mown parcels, and among sexually and vegetatively reproducing populations. Band richness of sampled plants per village was higher for villages where parcels represented more different land-use types. Within populations, microsatellite band diversity was higher in grazed than in mown parcels.

Conclusions: The diversity of human land use in the Alps was associated with genetic diversity of P. alpina. Therefore, the ongoing socio-economically motivated land-use changes, which reduce the number of different land-use types, will affect the genetic diversity of P. alpina negatively.

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Microsatellite Diversity of the Agriculturally Important Alpine Grass *Poa alpina* in Relation to Land Use and Natural Environment

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Abstract

• *Background and Aims* The Alpine Meadow Grass *Poa alpina* is common in subalpine and alpine natural sites and agriculturally used land, where it is an important fodder grass. Natural factors and human land use are supposed to have been shaping its genetic diversity for hundreds of years. The species comprises sexually and vegetatively reproducing plants. The aim of this study was to investigate the effects of agricultural land use, environmental factors, and the mode of reproduction on the distribution of its microsatellite diversity within and among populations and to analyse whether its genetic diversity is correlated with plant species diversity in grassland parcels.

• *Methods* We assessed genetic diversity of *P. alpina* with five microsatellite markers for 569 plants originating from 20 natural sites and from 54 grassland parcels of different cultural tradition, land use, and altitude in the Swiss Alps. Due to poly- and frequent aneuploidy of the species, data analyses were based on the presence of microsatellite bands.

• *Key Results* We found a low but significant differentiation in microsatellite bands among natural sites and agriculturally used parcels, while their microsatellite band diversity within populations did not differ. We found an increased differentiation in microsatellite bands with increasing geographic distance among parcels, a differentiation among grazed and mown parcels, and among sexually and vegetatively reproducing populations. Band richness of sampled plants per village was higher for villages where parcels represented more different land use types. Within populations, microsatellite band diversity was higher in grazed than in mown parcels.

• *Conclusions* The diversity of human land use in the Alps was associated with genetic diversity of *P. alpina.* Therefore, the ongoing socio-economically motivated land use changes, which reduce the number of different land use types, will affect the genetic diversity of *P. alpina* negatively.

Keywords: agriculture, cultural tradition, genetic diversity, grassland, land use, microsatellites, natural environment, *Poa alpina*, rarefaction, Swiss Alps

Introduction

Genetic diversity within and among populations is shaped by the balance between genetic drift, inbreeding, recombination, gene flow, mutation, and selection (Loveless and Hamrick 1984; Hartl and Clark 1997). This balance depends on important life history traits of a plant species, such as the mode of reproduction or life form (Loveless and Hamrick 1984; Hamrick and Godt 1997; Godt and Hamrick 1998). Moreover, both natural and anthropogenic factors are important for shaping genetic diversity. Potential natural determinants of genetic diversity include abiotic parameters, such as altitude or soil conditions. Furthermore, genetic diversity may be affected by the diversity of the surrounding community. Higher plant species richness was suggested to increase genetic diversity if it increases the diversity of available niches (Odat *et al.* 2004; Vellend and Geber 2005). A potential anthropogenic determinant of genetic diversity is land use diversity, if different land management creates genetic differentiation among populations. We studied genetic diversity and its determinants for a common and important fodder plant which occurs over a large altitudinal range at natural sites and in agriculturally used grassland, the Alpine Meadow grass *Poa alpina*, in the Swiss Alps.

The species can reproduce sexually via seeds and vegetatively by producing bulbils. In an accompanying common garden study, the proportion of genotypes reproducing vegetatively via bulbils was higher among samples from higher altitudes (Weyand 2005), in line with the hypothesis of an adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Bauert 1993; Pluess and Stöcklin 2005; Weppler and Stöcklin 2005). The presence of two different reproductive modes may affect population differentiation. Furthermore, as *P. alpina* occurs across a wide geographical range, isolation by distance is likely to have shaped the distribution of genetic diversity among populations of different regions (Wright 1943). Genetic diversity within and differentiation among

populations of *P. alpina* is probably also enhanced due to the highly variable polyploidy and frequent aneuploidy within the species (Duckert-Henriod and Favarger 1987), which presumably restricts gene flow among individuals and populations and is likely to increase the ecological amplitude of the species (Briggs and Walters 1997; Brochmann *et al.* 2004; Soltis *et al.* 2004).

For about 5000 years, the Alpine landscapes and in particular their grasslands have been shaped by human land use (Bätzing 2003). In the European Alps, *P. alpina* is one of the most important fodder grasses for cattle (Conert 1998). Therefore, *P. alpina* has been under agricultural selection pressure for hundreds of years. The species showed adaptation to anthropogenic land use variation in a common garden experiment (Weyand 2005), where plants from pastures allocated more biomass to reproduction than plants from natural sites did, while plants from meadows allocated less biomass to reproduction than plants from natural sites did. This suggests divergent selection between parcels of different land use. Higher allocation to reproduction in pastures may affect genetic diversity of *P. alpina* if it is related to higher seedling establishment. Genetic diversity in pastures could also be affected by the spatially more heterogeneous conditions created by grazing animals offering more different niches. In the Alps the relationship between land use and genetic diversity within a species is of particular interest, as due to land use changes during the last decades many meadows have been converted to pastures (Bätzing 2003) and the diversity of land use types in the landscape has decreased.

In the Swiss Alps, the cultural traditions Romanic, Germanic, and Walser contributed to a high landscape diversity through their different agricultural practices (Bätzing 2003). If differences in land use lead to genetic divergence between populations, villages with higher land use diversity may harbour higher genetic diversity of *P. alpina* than villages with lower land use diversity.

We studied the effects of natural factors and agricultural land use on genetic diversity of *P. alpina* within and among 12 villages in the Swiss Alps. Each of the three cultural traditions Romanic, Germanic, and Walser was represented by four villages. At the parcel level we studied genetic diversity within and among populations from 20 natural sites and from 54 agriculturally used grasslands at different altitudes in these 12 villages. The agriculturally used parcels were either mown or grazed and they were either additionally fertilized or unfertilized. Plant species diversity was known for all parcels from a previous study (Maurer *et al.* 2006).

As molecular markers we used five polymorphic microsatellite loci (Maurer *et al.* 2005). Microsatellites offer high resolution of genetic diversity (Schlötterer 1998). Therefore, they are ideal to investigate gene flow and genetic drift. Natural selection is unlikely to act on the investigated microsatellite loci themselves, but could affect their diversity if they were linked to loci under selection (Hartl and Clark 1997; Merilä and Crnokrak 2001).

We asked the following questions: (1) Are *Poa alpina* populations from agriculturally used grasslands genetically differentiated from natural populations? (2) Is genetic differentiation among villages and among populations related to geographical distances, to differences in land use, and to differences in reproductive modes? (3) Is genetic diversity within villages related to cultural traditions and to land use diversity? (4) Is genetic diversity within grassland parcels related to altitude, land use, and reproductive mode?

Materials and Methods

Study species

Poa alpina L. (Poaceae) is a common grass at subalpine and alpine levels in the northern hemisphere whose presence indicates high levels of nutrients and soil moisture (Conert 1998). Accordingly, it occurs in pastures and nutrient rich meadows, but also as a

pioneer species in scree slopes and in snowbeds. In the European Alps, *P. alpina* is among the most important fodder grasses due to its high contents of fats and proteins (Bachmann 1980; Conert 1998). Similar to other species in the genus *Poa* and the Poideae (Brysting *et al.* 2004), *P. alpina* constitutes a polyploid complex. Because of frequent aneuploidy and the presence of multiple B-chromosomes, chromosome numbers are highly variable (Müntzing 1980; Steiner and Heidenreich 1997). In Switzerland, reported chromosome numbers range from 2n = 22 to 46 (Duckert-Henriod and Favarger 1987), and more than 60 chromosomes were found in Scotch plants (Müntzing 1980). Chromosome numbers counted in the root tips of 25 plants of this study varied between 22 and 61 per plant. Presumably, variable polyploidy adds to genetic diversity in *Poa alpina*, as heterozygosity increases strongly with ploidy level (Brochmann *et al.* 2004).

Some plants of *P. alpina* produce seeds, while others reproduce vegetatively by forming bulbils in the panicles instead of seeds (Müntzing 1980). Such bulbils grow into little plantlets on the maternal plants, which therefore are called pseudoviviparous. Eventually, the plantlets may dehisce from the maternal plant and root (Pierce 1998). Usually, pseudoviviparous plants also develop a sexual floret at the basis of the plantlets (Philipson 1934; Müntzing 1980; Pierce *et al.* 2003). It is not known whether these sexual florets produce fertile pollen and viable seeds and whether there is gene flow between such florets and sexually reproducing plants. The mode of reproduction appears largely genetically determined, while phenotypic plasticity in the mode of reproduction plays a minor role (Schwarzenbach 1953; Schwarzenbach 1956; Heide 1989).

Study area

The study area comprises 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser (Fig. 1) and covers an area of

approximately 170 by 70 km. Each village belongs to a separate Alpine valley. To represent typical agricultural villages, the study villages were selected with the restriction that their agricultural character had only changed modestly during the last 50 years, and that they were not very touristy and did not have more than about 1500 inhabitants.

Study design and sampling

We searched for *P. alpina* in parcels of land chosen for a vegetational survey of grasslands (Maurer *et al.* 2006) situated at three altitudinal levels, at the valley bottom (c. 1000 m asl), at intermediate altitudes (c. 1500 m asl), and at the alp level (c. 2000 m asl) in each village. We searched for *P. alpina* L. in those of 216 grassland parcels where, according to local farmers, the type of land use had never changed. These parcels were characterised by a combination of land use (mown or grazed) and fertilisation (fertilised or unfertilised). In each parcel where *P. alpina* occurred, we sampled eight plants at interdistances of five meters to minimize the probability of sampling the same genotype more than once. Altogether, we sampled plants from 54 agriculturally used grassland parcels, 13 meadows and 41 pastures, of which 19 were additionally fertilised and 35 were not. Because *P. alpina* did not occur in all parcels and not all land use types were applied in each village, we could not find plants of all combinations of land use and fertilisation per village. However, the occurrence of certain land use types revealed no geographical pattern that could have confounded differences in land use with differences due to geographic distance. In the same way as in the agricultural parcels, we sampled eight plants from each of 20 natural sites above tree line that had never been used agriculturally. The sampled plants were also used for a common garden experiment (Weyand 2005). We obtained single genotypes by separating collected plants into four single tillers which we planted in the corners of 7 cm x 7 cm pots. After two months of growth in a greenhouse we discarded three of the four plants and kept one randomly selected plant per

genotype. After two more months we collected leaf samples of each plant. Because some of the plants died we analysed 415 plants of *P. alpina* from 54 agriculturally used parcels and 154 plants from 20 natural sites, usually eight plants per parcel, and a few times only seven or six.

Microsatellite analysis

We dried collected leaf samples immediately with silica gel. Then, we ground about 30 mg of the material in Eppendorf tubes with a glass bead in a shaking mill. We extracted DNA according to a Rogers & Bendich (1994) protocol modified by Steinger (1996), except that we incubated samples with CTAB buffer and mercaptoethanol at 65° C.

We screened all plants for variation at five polymorphic microsatellite loci (Maurer *et al.* 2005). We amplified DNA with 10 µl reaction volumes containing 10 ng genomic DNA, 0.5 µl each of the fluorescence-labelled forward primer and of the reverse primer, 5 µl Hotstar *Taq* Mastermix (Qiagen, Hombrechtikon, Switzerland), and 3 µl of sterilized H_2O. After a preliminary denaturation step at 95° C for 15 min., we amplified DNA with polymerase chain reaction (PCR) on a PTC-100 Programmable Thermo Controller (MJ Research Inc.) for 30 cycles of 30 s denaturing at 95° C, 30 s of annealing at locus-specific temperatures (Maurer *et al.* 2005) and 30 s of extension at 72° C, with a final 8 minute extension step at 72° C. We mixed 1 µl of the PCR product with 10 µl of a 75:1 solution of formamide and GeneScan-500 (ROX) size standard (Applied Biosystems, Foster City, CA, USA). We determined fragment lengths by capillary gel electrophoresis with an ABI PRISM 310 Genetic Analyser using GeneScan 2.1. (Applied Biosystems, Foster City, CA, USA). Plants were combined in random order for PCR and the sequencer-runs. Microsatellite bands were binned using Genotyper 2.1 (Applied Biosystems, Foster City, CA, USA) by correcting peaks manually after automated scoring, and we controlled the assignment of each peak to the corresponding

band. In each PCR-run at least one blank sample was added to control for a possible contamination of the samples. Preliminary tests of repeated PCR with a sample of eight plants showed a very high accuracy of the produced band-pattern. Thus, band-scoring appeared to be the most possible source of a potential genotyping error. Therefore, we independently scored a sample of 50 plants (8.8% of all analyzed individuals) twice for each of the five primers and obtained a genotyping error of 1.48%.

Due to their polyploidy, plants could show more than two microsatellite bands per locus. In a subset of 25 analyzed plants the number of bands was positively correlated with the number of chromosomes, but variable chromosome numbers due to aneuploidy and frequent B-chromosomes do not allow to assess ploidy levels (Maurer *et al.* 2005). Therefore, our data does not conform to standard statistics for codominant microsatellite markers of diploid organisms, such as observed and expected heterozygosities, and tests for deviation from Hardy-Weinberg equilibrium. Consequently, all analyses in this study are based on a presence-absence matrix of all bands across all plants.

Analysis of differences between natural sites and agriculturally used grassland parcels

To test for differentiation between natural and agricultural sites, we partitioned molecular variation among natural sites and agriculturally used parcels, among parcels, and within parcels with analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) based on the pairwise Euclidean distance matrix of the presence of microsatellite bands in individuals of all 74 parcels. Then, to test whether regional differentiation was similar for natural and agricultural sites, we partitioned molecular variation among villages, among parcels, and within parcels separately for the plants of the 54 agriculturally used parcels and for those of the 20 natural sites.

Analysis of differences among villages and among agriculturally used grassland parcels

To assess potential isolation by distance, we calculated a Mantel test (Mantel 1967; Manly 1997) using the matrix of Euclidean genetic distances among villages and among parcels, based on the relative abundance of each band per parcel, and the matrix of geographical distances among villages and among parcels. Furthermore, we calculated the Mantel test for the parcels with exclusively sexually and vegetatively reproducing *P. alpina* plants separately.

To examine potential differentiation among parcels of grassland of different land use (mown vs. grazed) and fertilisation (fertilised vs. unfertilised), we partitioned molecular variation among these groups, among parcels within these groups, and within parcels with AMOVA including plants of the 54 agriculturally used parcels. Furthermore, we used AMOVA to examine potential differentiation between 10 parcels from which all sampled plants exclusively reproduced pseudoviviparously in the common garden (Weyand 2005), and 22 parcels from which all sampled plants exclusively produced seeds.

In addition to AMOVA, we measured among-parcel differentiation for microsatellite band richness $\rho_{ST(n)}$ among all 54 agriculturally used populations and also among meadows and pastures separately following El Mousadik & Petit (1996) and Petit *et al*. (1998). To obtain $\rho_{ST(n)}$ first we calculated the expected band richness $r'_{T(n)}$ of a random sample of n=6 plants out of all 415 plants and $r'_{S(n)}$ of a random sample of n=6 plants for each parcel. Then, we calculated the differentiation for band richness as $\rho_{ST(n)}=1-r'_{S(n)}/ r'_{T(n)}$ (where S represents the single parcels and T the total population of all sampled plants) for each locus separately and the mean across the five loci.

Analysis of genetic diversity within villages and within parcels

We measured genetic diversity within villages and within parcels as band richness $r'_{(n)}$ for each locus following El Mousadik & Petit (1996), except that we used plants as sample units instead of genes. We used the rarefaction procedure of Hurlbert (1971) to estimate band richness for a standardized sample size of n plants. As rarefaction sample size we used $n = 16$ for villages and $n = 6$ for parcels. For each locus we calculated the expected number of different bands $r_{(n)}$ in a sample of n plants according to the formula

$\hat{r}_{(n)} = \sum_i \left[1 - \binom{N - N_i}{n} \Big/ \binom{N}{n} \right]$ (where N_i represents the number of occurrences of the i^{th} band among the N sampled plants of a population and n the standardized sample size). We subtracted one from the band richness $r_{(n)}$ to obtain the corrected band richness $r'_{(n)}$, because a village or a parcel with only one single band is considered to be monomorphic. Then, we calculated mean band richness over all five loci, for simplicity further on called band richness.

We tested potential effects of the village characteristics latitude and longitude, ratio of the numbers of meadows and pastures in a village, altitude, number of land use combinations (combination of land use and fertilisation) investigated in a village, and culture on band richness per village with linear models and analysis of covariance (ANCOVA). For the two significant variables (number of land use combinations and culture) we calculated ANCOVAs with sequential sums of squares including both variables. For these analyses only plants from agriculturally used parcels in each village were included. Furthermore, we tested whether band richness per village was correlated with the accumulated number of plant species obtained from vegetation records in the corresponding parcels of each village.

To analyse within-parcel genetic diversity, we investigated effects of cultural tradition, altitude, land use, and fertilisation on band richness per parcel and on the mean number of bands per plant for each parcel with hierarchical analysis of covariance (ANCOVA) with

sequential sums of squares. We tested effects of culture against remaining variation among villages and of all other factors against variation due to remaining differences among parcels. To account for differences among parcels because of different soil conditions or solar radiation, we used pH values and aspect of each parcel as covariates. However, as these covariates did not qualitatively change the results, we present results without covariates. Furthermore, we tested whether there was a difference in within-parcel diversity between populations from agriculturally used parcels and populations from natural sites. As there was no difference we present results including only the agriculturally used parcels.

Furthermore, we tested whether microsatellite diversity was affected by the abundance of *P. alpina* in the parcels. We calculated Spearman's rank correlations with the mean abundance of *P. alpina* of two plots (5 m x 5 m) per parcel and the measures of microsatellite diversity mean number of bands, band richness, and mean Euclidean genetic distance of each parcel to all other parcels.

To test whether genetic diversity of *P. alpina* was correlated with plant species diversity, we calculated Spearman's rank correlations between mean plant species richness of two plots (5 m x 5 m) per parcel and mean number of bands per plant and parcel, and between total plant species richness of two plots per parcel and band richness per parcel.

We did all statistical analyses with the software R (R Development Core Team 2004). For Mantel tests we used the R-package vegan (Oksanen *et al.* 2006) and for AMOVAs the R-package ade4 (Chessel 2004).

Results

Overall microsatellite diversity

Among the 569 plants of *Poa alpina*, altogether we detected 209 bands at the five microsatellite loci, between 25 and 61 per locus. We detected between one and eight bands

per plant and locus, with a mean of 3.35. In total we detected 531 multilocus-microsatellite phenotypes among all 569 plants and 386 multi-locus microsatellite phenotypes among the 415 plants from agriculturally used parcels.

Differentiation between natural and agriculturally used grassland parcels

Natural and agriculturally used grassland parcels were genetically differentiated as low, but highly significant, 1.1 % of the variation in microsatellite bands resided between natural and agriculturally used grassland parcels (AMOVA, $P < 0.004$).

Genetic diversity among villages

Regional differentiation was slightly higher in natural sites, as 8.4 % of the variation in microsatellite bands in natural sites resided among villages, while the corresponding proportion was 6.8 % for agriculturally used sites (AMOVA, for both $P < 0.001$, Table 1). We found no statistically significant relationship of genetic distances with geographic distances (Mantel test with plants from agriculturally used parcels, $r_M = 0.22$, $P = 0.16$) among pairs of the 12 villages.

Genetic diversity within villages

Band richness per village, based on a standardized sample size of 16 plants, was between 11.2 and 16.6 bands, with a mean of 14.5, and it was higher in villages with higher numbers of land use combinations among the sampled parcels ($P < 0.05$, Fig. 2 a). Furthermore, cultural traditions affected genetic diversity within villages because in villages with Walser tradition, band richness was higher than in Germanic villages (Tukey HSD, $P < 0.05$, Fig. 2 b). When both these significant variables were fitted in a model with sequential sums of squares, the effect of cultural tradition only was significant when introduced into the

model before the number of land use combinations (introduced first: $P < 0.05$, second: $P = 0.15$), while the significant effect of the number of land use combinations was independent of the fitting sequence. Moreover, band richness per village was positively correlated with the accumulated number of plant species occurring in the parcels with *P. alpina* per village ($r = 0.73$, $P < 0.01$, Fig. 2c).

Genetic diversity among agriculturally used parcels

Of the variation in bands, 25.1 % resided among parcels (AMOVA, $P < 0.001$) and 18.4 % among parcels within villages (AMOVA; $P < 0.001$, Table 1). Parcel differentiation for band richness $\rho_{ST(n)}$ was 0.25 across all five loci (Table 2), in line with the AMOVA result. Differentiation for band richness was higher among the 13 meadows than among the 41 pastures (Table 2).

We found a tendency to isolation by distance among agriculturally used parcels, as geographically more distant parcels tended to be genetically more distant (Mantel test, $r_M = 0.12$, $P = 0.057$, Fig. 3 a). The pattern was significant among parcels with exclusively sexually reproducing plants ($r_M = 0.22$, $P < 0.05$), but not among parcels with exclusively vegetatively reproducing plants ($r_M = 0.19$, $P = 0.213$). When we partitioned variation in microsatellite bands among parcels according to land use, we found 1.18 % to reside between mown and grazed grassland parcels (AMOVA, $P < 0.02$), and only 0.02 % between fertilised and unfertilised grassland parcels (AMOVA, $P > 0.42$).

Of the variation in microsatellite bands, 4.2 % resided between the 22 parcels with exclusively seed producing samples of *P. alpina* and the 10 parcels with exclusively pseudoviviparous ones (AMOVA, $p < 0.001$).

Overall, the results of this section indicate substantial regional genetic differentiation of *P. alpina* among different villages and genetic differentation among parcels with different

reproductive modes of *P. alpina*. Genetic differentiation among mown and grazed was less pronounced.

Genetic diversity within agriculturally used parcels

Of the variation in microsatellite bands, 74.9 % resided within parcels (AMOVA, $p < 0.001$; Table 1). Band richness, based on a rarefaction sample size of six plants, varied between 5.12 and 11.62 per parcel and locus (Table 2), and the mean number of bands per plant was between 2.88 and 4.44 per locus (Table 2) and 16.8 across all loci.

Band richness increased with increasing parcel altitude (Table 3, Fig. 4), while the mean number of bands per plant was independent of altitude (Table 3). Cultural traditions did not affect genetic diversity within parcels (Table 3).

Land use affected within-parcel microsatellite diversity, as the mean number of bands per plant was 3.0 % higher in pastures than in meadows ($F_{1,53} = 7.32$; $P < 0.05$, Table 3, Fig. 5). Moreover, band richness tended to be higher in pastures than in meadows ($F_{1,53} = 4.04$; $P = 0.07$, Table 3).

Within-parcel microsatellite band diversity did not appear to be affected by more pronounced genetic drift in smaller populations of *P. alpina,* because the mean number of bands per plant ($P = 0.3714$), band richness per parcel ($P = 0.6486$), and mean Euclidean genetic distance of each parcel to all other parcels ($P = 0.6599$) were independent of mean abundance of *P. alpina*

Correlations between mean plant species richness in two plots per parcel and the mean number of bands or total plant species richness in two plots per parcel and band richness were not statistically significant ($P = 0.1875$ and $P = 0.9031$, respectively), indicating that genetic diversity of *P. alpina* was not affected by plant species diversity of the 54 agriculturally used grassland parcels.

Discussion

Genetic differentiation among natural and agriculturally used parcels

The small but significant microsatellite band differentiation among natural sites and agriculturally used parcels suggests that human land use not only caused a divergence in genetic diversity among populations of *P. alpina* from mown and grazed grasslands, but also differentiation from natural habitats. This is in line with an accompanying quantitative genetic experiment, where plants from pastures and from meadows both differed from plants from natural sites in biomass allocation to reproduction (Weyand 2005). To our knowledge, only one study considering populations from natural sites and different agriculturally used parcels of land has been done, which investigated *Sesleria albicans* (Reisch *et al.* 2003). Unfortunately, the authors did not separate natural from agricultural populations but rather analysed, whether there was general variation in genetic diversity among habitat types, which prevents a comparison with their results.

Genetic differentiation among villages and among populations

Probably because of the large 170 km east-west range comprising different Alpine valleys, genetic differentiation among grasslands was pronounced and explained 25 % of the variation, and pairs of parcels tended to be genetically isolated by distance. The tests using parcels with exclusively sexually and exclusively vegetatively reproducing plants show that the pattern including all parcels is weakened by the lack of isolation by distance in plants reproducing vegetatively by bulbils. Previously, in *P. alpina* isolation by distance had been studied and observed only within a population in Norway (Bjørnstad *et al.* 1995). In addition to geographic distance, variable polyploidy, which can restrict gene flow (Briggs and Walters 1997), may have contributed to population differentiation in *P. alpina.* Plants of *Poa alpina*

were highly variable in chromosome number (between 22 and 61 in this study) due to frequent aneuploidy and multiple B-chromosomes (Müntzing 1980; Duckert-Henriod and Favarger 1987; Maurer *et al.* 2005).

Differentiation due to land use

Different habitat conditions can lead to genetically based ecotypic differentiation in grass species (Stapledon 1928). In our study, mowing and grazing over hundreds of years apparently led to genetic differentiation between mown and grazed populations of *P. alpina,* while fertilisation had no effect. This corresponds with the results of a common garden experiment using the same genotypes, which showed a divergence in biomass allocation between plants from mown and grazed parcels, but no difference between plants from fertilised and unfertilised parcels (Weyand 2005). Although the effect of mowing and grazing on genetic divergence between parcels was smaller than effects of isolation by distance or reproductive mode, it adds to the evidence that land use not only affects biodiversity at the plant community level, but also at the level of genetic diversity within species (Odat *et al.* 2004). The result is especially remarkable, as it suggests that land use affects biodiversity independently of regional differences. Land-use induced ecotypes have also been reported for the grassland forbs *Rhinanthus alectorolophus* (Zopfi 1993) and *Euphrasia rostkoviana* (Zopfi 1998). As in the common garden there was no difference in reproductive modes among plants from meadows and pastures, and as the reproductive mode appeared to be largely genetically determined (Weyand 2005), mowing and grazing are likely to act on genetic diversity of *P. alpina* directly rather than via its reproductive mode.

For the observed higher population differentiation among populations of meadows than among populations of pastures there are two mutually non-exclusive explanations. Firstly, endozoochorous or exozoochorous seed transport by cattle could increase gene flow among

pastures. Secondly, the result could indicate more diverse habitat conditions among meadows than among pastures. Land use intensity of the investigated meadows with *P. alpina* varies between mowing every second year to twice per season and may thus be responsible for differentiation among mown parcels as different mowing intensity was reported to exert differential selection in *Festuca pratensis* (Kölliker *et al.* 1998). In contrast, among pastures land use intensity rather varies among seasons than among parcels, which results in more uniform selection, and may thus contribute to the weaker genetic differentiation among pastures than among meadows. A third alternative explanation of higher genetic drift among meadows than among pastures can be ruled out, because abundance of *P. alpina*, which determines the importance of genetic drift, was neither correlated with within-parcel genetic diversity nor with genetic distance of a parcel to all other parcels.

Differentiation due to reproductive mode

The observed substantial genetic differentiation among parcels with exclusively seed-producing samples of *P. alpina* and those with exclusively pseudoviviparous ones suggests that gene flow between seed-producing plants of *P. alpina* and pseudoviviparous ones, which produce a sexual floret at the basis of their inflorescence (Philipson 1934; Müntzing 1980), is rather low. Furthermore, the lack of isolation by distance among parcels with exclusively pseudoviviparous plants is an indication that gene flow is largely restricted to sexually reproducing plants.

Genetic diversity within villages

In Walser villages more different land use combinations with *P. alpina* tended to be present than in Romanic and Germanic villages (result not shown). Most likely, this is due to the combination of the alpine to subalpine altitudinal distribution of *P. alpina* with the higher

altitudes of Walser villages than of villages of the other traditions. Due to settlement history, the later arriving Walser people had to settle at higher altitudes than Romanic and Germanic people (Bätzing 2003). At the valley bottom of villages at lower altitudes, *P. alpina* was not present in all types of parcels. Accordingly, this reduced the number of investigated land use combinations in Romanic and Germanic villages and probably caused the observed dependence of the statistical significance of cultural tradition on band richness of *P. alpina* within villages on the fitting sequence. The higher genetic diversity of *P. alpina* in villages where *P. alpina* occurred in a larger number of different land use combinations is in line with the observed microsatellite differentiation among parcels of different land use. Moreover, it corresponds to the result of a study of plant species diversity of the same 12 villages, which revealed a significantly positive relationship between plant species richness per village and the number of different land use combinations present in the villages (Maurer *et al.* 2006). Accordingly, genetic diversity of *P. alpina* was also positively correlated with the total number of plant species recorded in all the parcels per village where the *Poa* plants had been sampled.

Genetic diversity within populations

In contrast to findings with *F. pratensis* populations (Kölliker *et al.* 1998), genetic diversity of *P. alpina* was not affected by fertilisation. Nevertheless, agricultural land use significantly affected within-population genetic diversity of *P. alpina.* Populations originating from pastures were genetically more diverse, as they showed more bands per plant and tended to have higher band richness than meadow populations. Because samples of *P. alpina* from meadows and those from pastures did not differ in their reproductive modes (Weyand 2005), variation in reproductive mode cannot be responsible for these effects of land use on genetic diversity. Rather, this may be due to higher recruitment in grazed sites, either because of the

higher biomass allocation of plants from pastures to reproduction (Weyand 2005), or because of the higher probability of establishment of seedlings and pseudoviviparous plantlets in pastures, which offer more vegetation gaps as safe sites for establishment (Grubb 1977). Moreover, selection in mown sites may be more uniform than in spatially more heterogeneous grazed sites, which may reduce genetic diversity more strongly in meadows than in pastures. Accordingly, in *Festuca pratensis* molecular genetic diversity was the lower, the more intense the cutting regime was (Kölliker *et al.* 1998). Furthermore, genetic diversity could have been enhanced in pastures because of higher gene flow due to seed transport by cattle.

Parcels from higher altitudes had higher band richness. In contrast, the number of bands per plant, which did not significantly differ between exclusively pseudoviviparous and exclusively seed-producing samples, did not increase with altitude and neither did the number of chromosomes per plant (data not shown). A potential biological explanation for this set of results could be the differentiation between pseudoviviparous and seed-producing samples of *P. alpina* in combination with an increase in pseudoviviparous reproduction from 20 % in valley genotypes to 50 % in alpine genotypes observed in the common garden (Weyand 2005) and a resulting accumulation of bands typical for each of the two reproductive modes with increasing altitude.

Correlations between genetic diversity of Poa alpina and plant species richness

A high niche diversity has been suggested to maintain higher genetic diversity in plant species (Odat *et al.* 2004; Vellend and Geber 2005) and can also increase species richness, at least in nutrient-rich habitats (Gigon and Leutert 1996; Proulx and Mazumder 1998; Austrheim and Eriksson 2001; Maurer *et al.* 2006). Therefore, higher genetic diversity of *P. alpina* in parcels with higher plant species richness might have been expected. However, in our study, genetic diversity of *P. alpina* was not correlated with plant species richness.

Conclusions

The genetic complexity of the polyploid and aneuploid *Poa alpina* constrained our study of molecular diversity to an analysis of the presence of microsatellite bands. Nevertheless, the distribution of genetic diversity within and among populations of *P. alpina* turned out to be affected by land use diversity in villages and by the specific land use within parcels. Higher genetic diversity within pastures than within meadows, genetic differentiation among populations from meadows and pastures, and higher genetic diversity within villages with more diverse land use imply two important conclusions. First, they demonstrate that the ongoing socio-economically motivated land use changes in the Swiss Alps do not only affect diversity at the landscape and community levels, but they also change genetic diversity within species. Moreover, promoting genetic diversity cannot be achieved by just maintaining the single type of land use associated with highest within-population diversity, but requires the maintenance of a high diversity of land use types.

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Figure legends

FIG. 1. Map of Switzerland with the 12 study villages and their cultural traditions. ● = Romanic, ■ = Germanic, ▲ = Walser

FIG. 2. Relationship between microsatellite band richness of *Poa alpina* per village sample and the number of land use types investigated per village (ANOVA-model with sequential sums of squares, $P < 0.05$), cultural tradition of 12 villages in the Swiss Alps (Tukey-HSD, $P < 0.05$), and number of plant species recorded in the same parcels per village ($P < 0.01$). Band richness is based on a standardized sample size of 16 plants. G = Germanic tradition, R = Romanic, W = Walser. In the Figure with the number of land use types three data points are hidden by others.

FIG. 3. Relationship of pairwise Euclidean genetic distance between populations of *Poa alpina* from 54 agriculturally used parcels of grassland in the Swiss Alps with pairwise geographic distances (Mantel test, $r_M = 0.12$, $P = 0.057$).

FIG. 4. Relationship between microsatellite band richness, based on a rarefaction sample size of six plants, and altitude of 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps (ANCOVA-model with sequential sums of squares, $P < 0.05$).

FIG. 5. Relationship between number of bands per plant and type of land use among 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps (ANCOVA-model with sequential sums of squares, $P < 0.05$).

Fig. 1

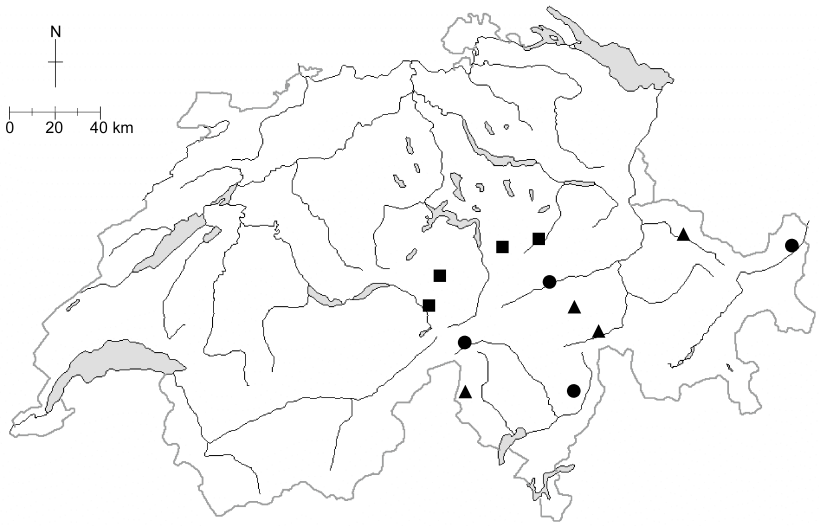

Fig. 2

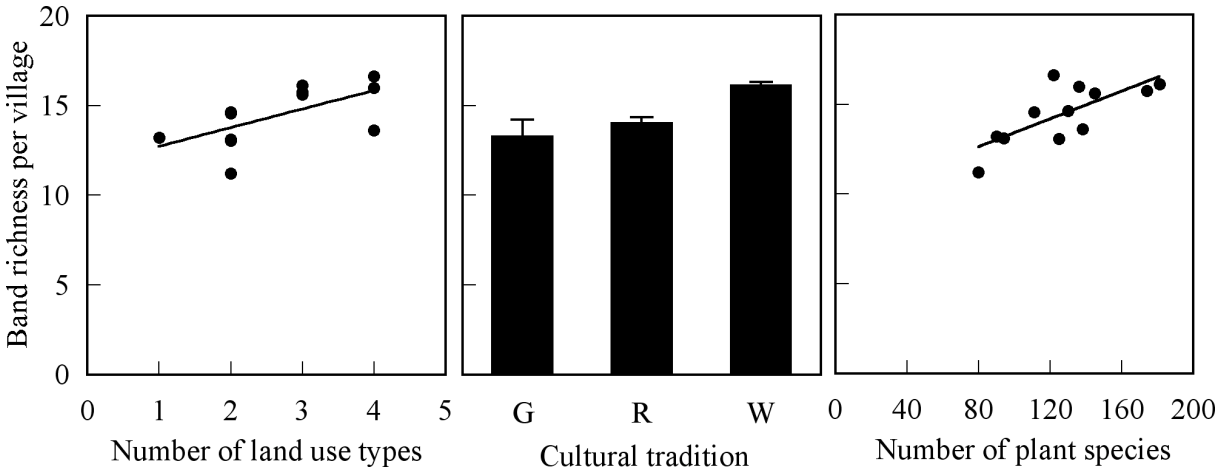
Band richness per village
20
15
10
5
0
0
1
2
3
4
5
Number of land use types
G
R
W
Cultural tradition
0
40
80
120
160
200
Number of plant species

Fig. 3

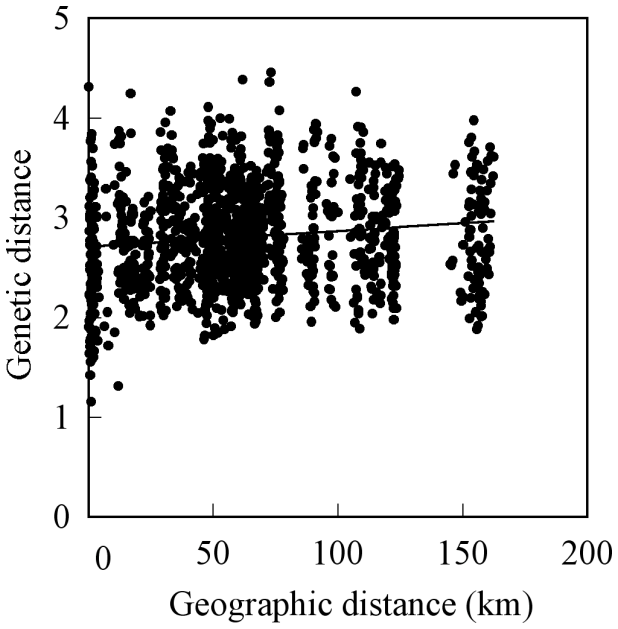
5
4
3
2
1
0
Genetic distance
0
50
100
150
200
Geographic distance (km)

Fig. 4

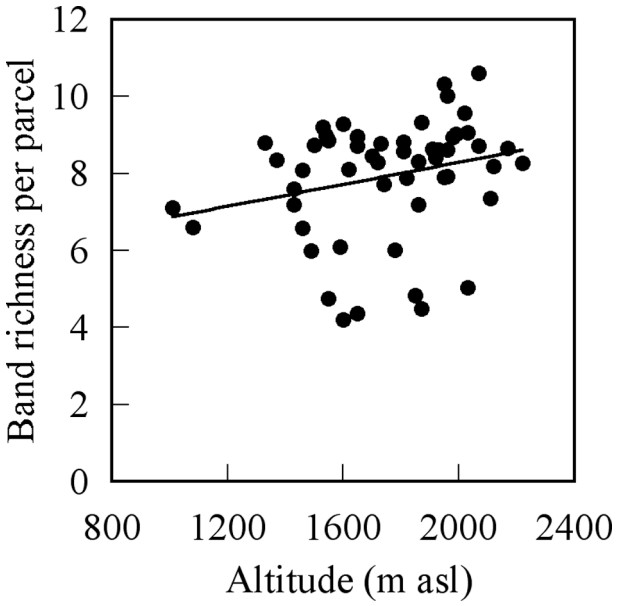
Band richness per parcel
12
10
8
6
4
2
0
800
1200
1600
2000
2400
Altitude (m asl)

Fig. 5

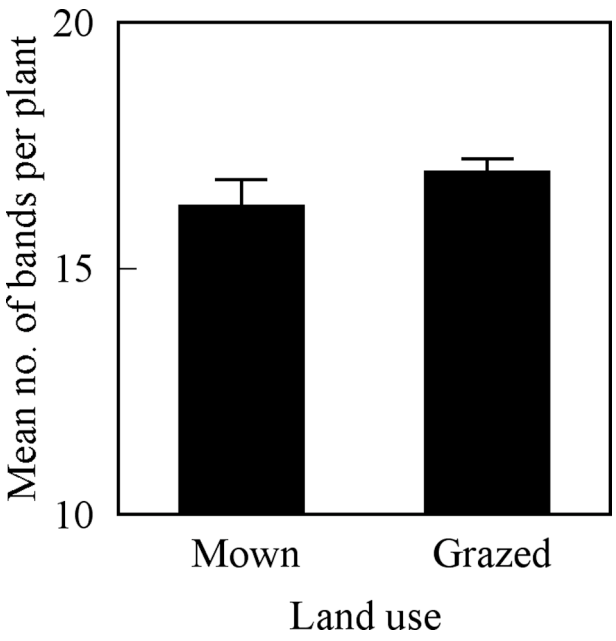

TABLE 1. *Summary of analysis of molecular variance (AMOVA) of microsatellite phenotypes of plants of* Poa alpina *from 54 agriculturally used parcels and from 20 natural sites grouped in 12 villages.*

Source of variation	df	Variance component		
		Absolute	%	*P*
54 populations from agriculturally used parcels				
Among villages	11	0.7981	6.76	< 0.001
Among parcels within villages	42	2.1790	18.39	< 0.001
Within parcels	361	8.8422	74.86	< 0.001
Total	414	11.8122	100.00	
20 populations from natural sites				
Among villages	10	1.0184	8.42	< 0.001
Among parcels within villages	9	2.2502	18.61	< 0.001
Within parcels	134	8.8203	72.96	< 0.001
Total	153	12.0889	100.00	

AMOVA was based on the matrix of pairwise Euclidean distance between individuals in the presence of microsatellite bands. % = Percentage of total variation

TABLE 2**.** *Population genetic measures for 54 populations of* Poa alpina *from agriculturally used parcels of grassland in the Swiss Alps.*

Microsatellite locus	Mean number of bands per plant and population	$r'_{S(6)}$	$r'_{T(6)}$	$\rho_{ST(6)}$	$\rho_{ST(6)}$ among meadows	$\rho_{ST(6)}$ among pastures
Poa CA1D4	2.88	7.19	9.03	0.20	0.29	0.18
Poa GAC1	4.44	11.62	16.35	0.29	0.36	0.27
Poa GA1C3	2.23	5.12	6.75	0.24	0.34	0.21
Poa CA1F4	3.87	8.39	10.57	0.21	0.25	0.19
Poa CAB12	3.37	7.29	10.33	0.29	0.34	0.28
Mean	**3.36**	**7.92**	**10.61**	**0.25**	**0.32**	**0.23**

$r'_{S(6)}$ = Band richness per population with a standardized sample size of six plants

$r'_{T(6)}$ = Band richness of the hypothetical total population with a sample size of six plants

$\rho_{ST(6)}$ = Differentiation for band richness among populations

$\rho_{ST(6)}$ among meadows = Differentiation for band richness among 13 meadows

$\rho_{ST(6)}$ among pastures = Differentiation for band richness among 41 pastures

TABLE 3. *Summary of analyses of the mean number of microsatellite bands per plant (MB) and band richness per parcel (BR) in 54 populations of* Poa alpina *from agriculturally used parcels of land in the Swiss Alps.*

Source of variation	df	SS_{MB}	F_{MB}	p_{MB}	SS_{BR}	F_{BR}	p_{BR}
Culture	2	4.34	0.24	n.s.	7.72	2.04	n.s.
Village[Culture]	9	81.38	7.54	p<0.01	17.01	1.12	n.s.
Altitude	1	3.94	3.28	p<0.1	9.60	5.67	p<0.05
Fertilisation	1	0.25	0.21	n.s.	0.05	0.03	n.s.
Land use	1	8.78	7.32	p<0.05	6.83	4.04	p<0.1
Fertilisation*land use	1	0.60	0.50	n.s.	0.55	0.33	n.s.
Culture*altitude	2	4.00	1.00	n.s.	1.45	0.31	n.s.
Village[Culture]*altitude	9	18.05	1.67	n.s.	20.80	1.37	n.s.
Culture*fertilisation	2	1.34	0.39	n.s.	3.90	1.16	n.s.
Culture*land use	2	5.47	1.16	n.s.	0.98	0.08	n.s.
Village[Culture]*fertilisation	7	12.12	1.44	n.s.	11.78	0.99	n.s.
Village[Culture]*land use	3	7.09	1.97	n.s.	18.10	3.57	p<0.1
Altitude*fertilisation	1	0.11	0.09	n.s.	0.00	0.00	n.s.
Altitude*land use	1	0.40	0.33	n.s.	6.26	3.70	p<0.1
Residuals	11	13.19			18.61		

Culture denotes the Romanic, Germanic, and Walser cultural traditions. Village denotes the 12 study villages. Fertilisation denotes the difference between unfertilised and fertilised parcels. Land use denotes differences between mown and grazed grasslands. In the sequential sums of squares ANCOVA, effects of culture were tested against remaining variation among villages. n.s. denotes values of $p > 0.1$