Latitudinal variation of genecological traits in native grasses of Patagonian rangelands

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Abstract. Geographical variation in genetically based traits helps to elucidate the effect of distinct ecological and evolutionary processes on widespread plants. Whereas abundant information exists on genetic patterns of woody species in western humid Andes, such information is scarce for the neighbouring dry Patagonian steppe. We examined genecological traits of two native forage species vulnerable to overgrazing (*Bromus pictus* and *Poa ligularis*) in dry Occidental Phytogeographical District. We compared within-population genetic diversity and among-population ($n = 6$) divergence by using isozyme electrophoresis. We also cultivated plants under common garden to compare genetically based morphology (plant height, number of tillers by plant and weight per tiller). Analysis showed that 8 and 13 loci were polymorphic of 9 and 19 resolved loci in at least one population for *Bromus* and *Poa*, respectively. In general, plant traits decreased from north to south in both species. Genetic and quantitative results (F_{ST}/Q_{ST} index) showed evidence of local adaptation in populations of both species. Genetic divergence among populations was significant. We detected two different geographical groups divided at the same latitude (42–43°S) in both species, supporting the hypothesis of a past vicariance event. Sustainable management of these forage species to cope with land-use and climate change will be enriched by the inclusion of genecological knowledge.

Introduction

Appraisal of latitudinal genecological variation provides hypotheses of historical and ecological processes shaping such geographic patterns. Spatial organisation at different scales is a key component of biodiversity of populations that must be considered for management planning (Noss 1990). The least inclusive dimension in biodiversity management is genetic variation, which may be used to delineate management units. These are populations that differ in genetic traits and maintain greater gene flow among them than they do with other such groups (Moritz 1994). Neutral genetic variation using molecular markers has been commonly applied to show intraspecific patterns. But to be used in management and conservation recommendations, this information needs to be combined with quantitative variation (Crandall *et al*. 2000).

From a historical perspective, population divergence of widespread species at the continental level has been largely explained by vicariance (i.e. the sepatarion or division of a group) caused by geographical or past genetic barriers (Inda *et al*. 2008). Although paleoecological records offer key information for evaluating species' biogeographic history, macro and microfossils of some plant groups such as grasses are notoriously sparse (Strömberg 2011). Alternatively, genetic polymorphisms may reveal legacies from an ancient past (Premoli *et al*. 2012).

Besides evolutionary history, many other contemporary factors affect the genetic structure of plant species. The extension of geographic range, breeding systems (Hamrick and Godt 1989) and disturbance (Premoli and Kitzberger 2005) are major factors influencing the genetic structure of natural plant populations. Comparing species that differ among these traits can help identify hierarchies among these drivers. For example, species with broader distributions have more genetic variability (Ellstrand and Elam 1993; Linhart and Premoli 1993; Premoli 1997). These commonly consist of large and continuous populations, and are therefore less affected by genetic drift and restricted gene flow, unlike small populations (Hamrick and Godt 1989). Since breeding system determines gene flow, outcrossing species are more genetically diverse than are selfers (Loveless and Hamrick 1984). Moreover, genetic diversity can be strongly modified by overexploitation of ecosystems by changing population size through processes such as fragmentation (Noss 1990; Schemske *et al*. 1994).

The grass family is of particular interest to humans. Globally, many people rely on grasses for their diet or that of their domestic animals (Kellogg 2001). However, most genetic studies using

grasses are mainly concerned with improving productivity, quality, and/or resistance to pests and diseases (e.g. Hoisington *et al.* 1999). However, genetic studies of natural grass populations have been largely overlooked and Patagonian grasses are not an exception. A recent phylogeographic study on *Hordeum species* proposed that climatic conditions during the last ice age did not constraint local survivorship (Jakob *et al.* 2009). Similar results were obtained in herbaceous and shrub populations from the Patagonian steppe (Cosacov *et al.* 2010; Sede *et al.* 2012) and also for trees on the eastern Andes (Premoli *et al.* 2000; Sérsic *et al.* 2011). Molecular evidence for long-lasting persistence of cold-tolerant trees through glacial periods on the drier slopes of the Andes were confirmed by ecological niche modelling (Premoli *et al.* 2010). In addition, genetic structure of distinct taxa inhabiting Patagonia follows a clear latitudinal pattern that supports the multiple-refugia hypothesis (*sensu* Premoli 1998; e.g. Marchelli and Gallo 2006; Vidal-Russell *et al.* 2011; Sérsic *et al.* 2011). In particular, major genetic discontinuities found in woody species at mid-latitudes of Patagonia (Mathiasen and Premoli 2010; Quiroga and Premoli 2010) suggest an ancient evolutionary history (Premoli *et al.* 2012). Therefore, regional-scale genetic studies are required to understand the biogeographic history of steppe vegetation inhabiting dry Patagonia. Besides historical factors affecting plant populations, abrupt physical gradients occur in Patagonia such as temperature and rainfall. However, few studies on tree species have analysed adaptive trait variation (e.g. Premoli and Brewer 2007; Premoli *et al.* 2007; Souto *et al.* 2009; Pastorino *et al.* 2010), which may also explain divergence patterns along latitudinal gradients.

We compared distribution patterns of genetic polymorphisms and adaptive trait variation following a common garden experiment of two conspicuous grass species native to the Patagonia steppe. These co-occurring, unrelated species were *Bromus pictus* and *Poa ligularis*, which have been subjected to

overgrazing by sheep within the study area for the last 70 years. We selected morphological traits associated with survival in both species in this ecosystem. We restricted our study area to a latitudinal gradient in the Occidental Phytogeographical District where these species dominate. We hypothetised that while similar historical processes have affected neutral genetic structure in rangeland grass species, quantitative trait differentiation may follow contemporary variation patterns of the physical environment. We selected six study locations at distinct latitudes where both species occur in overlapping geographical areas (i.e. sympatric). The research presented is part of a larger series of experiments. In the present paper, we aim to answer the following questions: (1) how much genetic diversity is present within populations of species with distinct breeding systems as *Bromus pictus* and *Poa ligularis*; (2) are these populations genetically structured; (3) does genetically based quantitative variation follow geographic patterns; and (4) is genetic divergence estimated by neutral marker (F_{ST}) similar to that by quantitative traits (Q_{ST})?

Materials and methods

Species and study area

We studied two unrelated C3 grass native species, *Bromus pictus* Hook var. *pictus* and *Poa ligularis* Nees ex Steud (hereafter, *Bromus* and *Poa*, respectively), distributed across the Patagonian arid steppes. Both grasses tolerate water stress but differ in growth rate and herbivore preference (*Bromus* > *Poa*) (Leva *et al.* 2009). We studied populations that occur within the grass–shrub steppe of the Occidental District of Patagonia, so as to control for environmental variability (mostly soils and climate; Fig. 1). We also used the phytogeographical district as a control for all large-scale biogeographic processes. These species differ in several aspects. *Bromus* has a more restricted distribution and
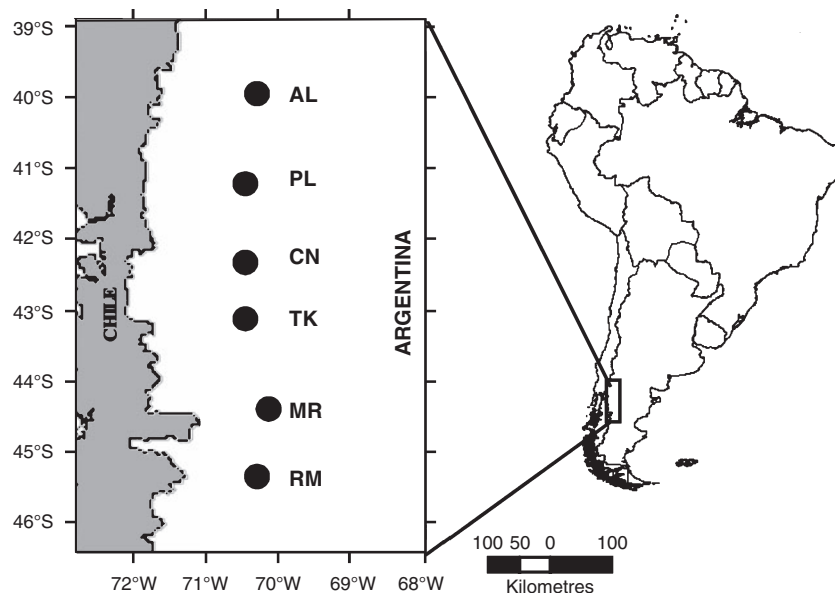

Fig. 1. Geographical location of sampled populations of *Bromus pictus* and *Poa ligularis* along the Patagonian latitudinal gradient on Occidental District in South America. Sampled populations are from north to south, as follows: Alicura (AL), Pilcaniyeu (PL), Cancha (CN), Tecka (TK), Moreno (MR) and Río Mayo (RM).

is found exclusively in Patagonia, ranging from 40° to 48°S. In contrast, the geographical distribution of *Poa* is larger, extending northward to 32°S. The species also have different reproductive systems. *Bromus* is self-pollinated (García *et al.* 2009), whereas *Poa* has male and female plants (Fernández Pepi *et al.* 2008) and, therefore, is cross-fertilised. In addition, *Bromus* is a decaploid with $2n = 10x = 70$ (Naranjo *et al.* 1990; García *et al.* 2009), and *Poa* is a tetraploid with $2n = 4x = 28$ (Hunziker 1978). *Poa* and *Bromus* are the most important grasses with respect to grazing being valuable forage species for domestic herbivores (Cipriotti and Aguiar 2005).

The Occidental District of the Patagonian steppe is characterised by a homogeneous floristic and physiognomic area along narrow latitudinal gradient. The vegetation corresponds to the dominant community, co-dominated by perennial grasses and shrubs, which contribute to >96% of the total plant cover and plant biomass (Oñatibia *et al.* 2010). Aridity is an important driver of Patagonian vegetation, with precipitation mainly concentrated in winter, with a strong water deficit in summer (Paruelo *et al.* 1998). Soils are coarsely textured aridisols, with high gravel content and low soil-water holding capacity along the region. Sheep grazing has been identified as one of the main causes of ecosystems degradation, a process that hampers sustainability in the region (Ares 2007).

Population sampling

We selected six sites where the two species co-occur within the study region, covering ~600 km from north to south (Table 1, Fig. 1). Six sample sites were chosen to obtain a good representation of the study area and to provide a manageable number of populations for all the experiments in our research program. Not all of these studies are reported in the present paper. All sites were grazed, but showed no signs of degradation (i.e. unaltered community richness, no weed species, no signs of soil erosion, low density of faecal pellets, preferred species were vital with flower culms). We performed molecular studies on all six populations using neutral isozyme markers and evaluated quantitative traits under common-garden trials. For molecular studies, we collected three tillers from 30 individuals randomly selected from each population. Individuals were separated by at least 10 m and interspersed across 1 ha (spring 2007). These fresh samples were individually labelled, kept cool and transported to the laboratory for protein extraction and isozyme electrophoresis. In January 2007, mature seed of many *Bromus* and *Poa* individuals from each population were randomly hand-collected. Seed were germinated in Petri dishes and seedlings were transplanted into pots of 1 L filled with sand, which were located in a greenhouse at the Institute for Agricultural Plant Physiology and Ecology (IFEVA, 34°35′S, 58°29′W). We used these plants to evaluate quantitative traits of the six populations for each species under homogeneous environmental settings (i.e. common garden). Plants were watered weekly.

Isozyme electrophoresis

We estimated genetic variability of these six populations of *Bromus* and *Poa* by using isozymes. No other co-dominant marker was available for the two species, requiring that isozymes be used. An advantage of isozymes is that they

Table 1.   Geographic location of sampled populations of *Bromus pictus* and *Poa ligularis* and within-population genetic-variation indices for each population

Percentage of polymorphic loci *sensu stricto* (P_{SS}), total number of alleles (A_T), mean number of alleles per locus (A), mean effective number of alleles (A_E), low-frequency alleles relative to the total number of alleles ($A_{<0.1} : A_T$), the observed and expected heterozygosity (H_O and H_E), respectively, and within-population inbreeding (F_{IS}). Population means are shown at the bottom and standard errors are in parentheses. Populations are ordered from north to south

Species	Population	Identification code	Location	Altitude (m a.s.l.)	P_{SS} (%)	A_T	A	A_E	$A_{<0.1} : A_T$	H_O	H_E	F_{IS}
Bromus pictus	Alicura	AL	40°24′S, 70°31′W	1166	77	19	2.11	1.51	0.21	0.227	0.275	0.160
	Pilcaniyeu	PL	41°7′S, 70°40′W	993	66	17	1.89	1.53	0.06	0.215	0.26	0.157
	Cancha	CN	42°47′S, 70°57′W	784	77	20	2.22	1.47	0.30	0.189	0.26	0.259
	Tecka	TK	43°31′S, 70°45′W	890	77	20	2.22	1.49	0.35	0.159	0.265	0.388
	Moreno	MR	44°55′S, 70°12′W	549	77	22	2.44	1.48	0.36	0.277	0.276	−0.022
	Río Mayo	RM	45°42′S, 70°20′W	527	77	19	2.11	1.45	0.37	0.162	0.255	0.353
Population mean					76	19.5 (0.67)	2.16 (0.07)	1.49 (0.01)	0.28 (0.05)	0.206 (0.018)	0.263 (0.004)	0.216 (0.061)
Poa ligularis	Alicura	AL	40°24′S, 70°31′W	1166	58	42	2.21	1.60	0.12	0.254	0.286	0.092
	Pilcaniyeu	PL	41°7′S, 70°40′W	993	68	42	2.21	1.53	0.17	0.242	0.283	0.126
	Cancha	CN	42°47′S, 70°57′W	784	63	40	2.11	1.44	0.20	0.229	0.249	0.064
	Tecka	TK	43°31′S, 70°45′W	890	53	39	2.05	1.52	0.23	0.199	0.251	0.189
	Moreno	MR	44°55′S, 70°12′W	549	63	40	2.11	1.47	0.23	0.167	0.244	0.300
	Río Mayo	RM	45°42′S, 70°20′W	527	68	45	2.37	1.66	0.27	0.236	0.299	0.193
Population mean					62	41.33 (0.88)	2.18 (0.05)	1.54 (0.03)	0.20 (0.02)	0.221 (0.013)	0.269 (0.010)	0.161 (0.035)

show patterns of chromosomal segregation in polyploids. Autotetraploids display polysomic segregation patters where homologous chromosomes are equally likely to pair with each other during meiosis and occasionally forming multivalents. Instead, allotetraploids show a disomic segregation pattern, with the two chromosomes originating from the same parent preferentially pairing together during meiosis, forming only bivalents (Soltis and Rieseberg 1986). Fixed heterozygosity would be indicative of allopoliploidy.

Enzyme extracts were prepared by crushing samples in liquid nitrogen with the extraction buffer of Mitton *et al.* (1979). Homogenates were centrifuged and stored at $-80°C$ until electrophoresis. Isozyme electrophoresis was performed on 12% starch gels (Starch Art Corporation, Smithville, Texas, USA) to resolve 9 and 19 putative loci for *Bromus* and *Poa*, respectively (Table 2). Gels were run for 5 h, using the following combination of gel and electro-buffers: morpholine-citrate @ 25 mA (Ranker *et al.* 1989) and histidine-citrate @ 35 mA (King and Dancik 1983). For each enzyme, alleles were numbered sequentially, from the most anodal to the most cathodal. Because isozyme markers are co-dominant, we could distinguish homozygous and heterozygous genotypes at each locus. Thus, we could calculate gene frequencies for each locus and parameters of within-population diversity and among-population divergence for each species.

Genetic diversity, population and species level

For each species, levels of within-population isozyme variation were described by standard measures, by using GeneAlEx 6.1 software (Peakall and Smouse 2006). These were the percentage of polymorphic loci using *sensu stricto* criterion (P_{SS}), the total number of alleles (A_T), the mean number of alleles per locus, including both monomorphic and polymorphic loci (A), the mean effective number of alleles (A_E), the number of low-frequency alleles ($A_{<0.1}$), observed and expected heterozygosity under Hardy–Weinberg equilibrium (H_O and H_E, respectively), and inbreeding due to non-random mating within population (F_{IS}). Simple regression was performed to explore the relationship

between the latitude of origin of each population and these genetic parameters.

Among-population divergence

Heterogeneity of allele frequencies of all loci among populations within each species was evaluated by chi-square tests. To assess genetic similarity among populations within each species, we performed multivariate cluster analyses based on unweighted arithmetic mean (UPGMA) from a matrix of genetic distance (Nei 1978). Also, we performed Bayesian analyses to assess population structure by using the admixture and the frequency-dependent allele models with the software STRUCTURE v 2.3.1. We used a burn-in of 200 000 iterations to eliminate the effect of initial configuration, followed by 2 000 000 iterations in Markov Chain Monte Carlo (MCMC) to generate datasets for $K = 1–6$, replicated four times for each value of K (Pritchard *et al.* 2000). The optimal number of clusters was chosen using Evanno *et al.* (2005) ΔK statistic. In addition, we performed a Mantel test to estimate correlations between genetic and geographical distance (Mantel 1967) among all possible pairs of populations. We compared geographical distances (km) among collection sites and genetic-distance matrix. Statistical significance testing was performed by 999 permutations of genetic-distance matrices using the Mantel test of program GeneAlEx 6.1 (Peakall and Smouse 2006).

Total inbreeding (F_{IT}) was partitioned into that observed within (F_{IS}) and among (F_{ST}) populations using Wright's (1965) F-statistics. These coefficients were estimated using FSTAT v 2.9.1 software (Goudet 2001), which also provides confidence intervals (CI 99%) using the permuted method of Weir and Cockerham (1984). Gene flow (Nm) was estimated from F_{ST} as $Nm = 1 / 4 (1 / (F_{ST} − 1))$, where Nm represents the proportion of migrants per generation.

Quantitative traits under common garden

Plants were grown in the greenhouse for 5 and 3.5 months for *Bromus* and *Poa*, respectively. At the conclusion of the growth period, plant height, number of tillers per plant and weight per

Table 2.   Buffer systems and loci used for isozyme electrophoresis

Two buffer systems were used to resolve 9 and 19 putative loci for *Bromus pictus* and *Poa ligularis*, respectively

Species	Buffer	Locus
Bromus pictus	Morpholine-citrate	Isocitrate dehydrogenase (*Idh*-1)
		Peroxidase (*Per*-1, *Per*-2)
		Phosphoglucoisomerase (*Pgi*-1, *Pgi*-2)
	Histidine-citrate	Glycerol deshidogenasa (*G2d*-1)
		Malate dehydrogenase (*Mdh*-2, *Mdh*-3)
		Shikimate dehydrogenase (*Skdh*)
Poa ligularis	Morpholine-citrate	Alcohol dehydrogenase (*Adh*-1, *Adh*-2)
		Aldolase (*Ald*)
		Isocitrate dehydrogenase (*Idh*-1, *Idh*-2)
		Malic enzyme (*Me*-1, *Me*-2)
		Peroxidase (*Per*-1, *Per*-2)
		Phosphoglucoisomerase (*Pgi*-1, *Pgi*-2)
	Histidine-citrate	Glycerate-2-dehydrogenase (*G2d*-1, *G2d*-2)
		Malate dehydrogenase (*Mdh*-1, *Mdh*-2, *Mdh*-3)
		Shikimate dehydrogenase (*Skdh*)
		Sorbitol dehydrogenase (*Sdh*-1, *Sdh*-2)

tiller were measured. Two fully expanded leaves from 10 individuals from each population were collected, scanned to determine the area and weighed. We estimated specific leaf area (SLA) for each sample as area (cm^2) per weight (g). SLA is positively related to relative growth rate which is an ecological relevant trait. It has been found that SLA is negatively associated with detrimental biotic and abiotic conditions. Plants with a lower SLA are expected in cold and dry environments or under high herbivory (Poorter and Remkes 1990). Each plant was harvested and dry shoot and root matter were weighed. Populations were compared by one-way ANOVA for each response variable. Data were checked for ANOVA assumptions and variables were transformed to stabilise variances. *Post hoc* comparison was performed with a Bonferroni test. A Pearson correlation among those quantitative traits and latitude was performed for both species. Analyses were performed using the statistical software InfoStat (2008). Data from these seven variables were also used to estimate the degree of among-population divergence in quantitative traits Q_{ST}, following Merilä and Crnokrak (2001) to compare with F_{ST}.

Results

Genetic diversity within populations and species

Isozyme analysis showed that 8 and 13 loci were polymorphic from 9 and 19 loci resolved for *Bromus* and *Poa*, respectively, for at least one of six populations. In *Bromus*, the percentage of polymorphic loci *sensu stricto* for the nine loci was 77.8% for most populations, except for Pilcaniyeu, which had the lowest value (66.7%, Table 1). This population also had the lowest A, the highest A_E, and only one allele with frequency less than 10% (Fig. 2). Most populations had four or more alleles with frequency <10%, up to a maximum of eight alleles in Moreno population.

In *Poa*, polymorphism *sensu stricto* for the 19 loci studied was 62.3%. Pilcaniyeu and Río Mayo were the most polymorphic populations (68.4%), followed by Cancha and Moreno (63.2%), and then by Alicura and Tecka (57.9% and 52.6%, respectively) (Table 1). The southernmost population had the highest A, A_E, and a larger number of $A_{<0.1}$. Northern populations Alicura and Pilcaniyeu also had high values of A_E (Table 1).

Observed heterozygosity (H_O) was lower than H_E, although no clear latitudinal trend was observed in any species (Table 1). Similarly, average within-population inbreeding (among individuals in relation to populations) was positive and significantly different from zero in both species, with $F_{IS} = 0.217$ and 0.175, in *Bromus* and *Poa*, respectively,

suggesting a significant average reduction of heterozygosis (Table 3).

No association ($P > 0.05$) was found between most genetic-diversity parameters (A, A_T, P_{SS}, A_P, A_E, H_O, and H_E) and latitude. However, the number of $A_{<0.1}$ increased in a southerly manner in *Poa* ($r^2 = 0.92$, $P = 0.0025$) (Fig. 2). This relationship was weak ($r^2 = 0.52$) for *Bromus* and the slope was not significantly different from zero ($P = 0.105$; Pilcaniyeu population showed a significantly lower number of alleles than did the rest). The same trend was observed when comparing the percentage of $A_{<0.1}$ with the total number of alleles by population for each species ($A_{<0.1} : A_T$; Table 1).

Neutral among-population divergence

Allele frequencies differed significantly among populations of each species (Table 4). In *Bromus*, the allele frequency was significantly different ((χ^2-tests, $P < 0.001$) in seven of eight (87.5%) polymorphic loci analysed. In *Poa*, the frequency of 12 of 13 (92%) polymorphic loci was significantly different (χ^2-tests, $P < 0.001$). Therefore, the two species exhibit a significant genetic structure in allele frequencies for the most
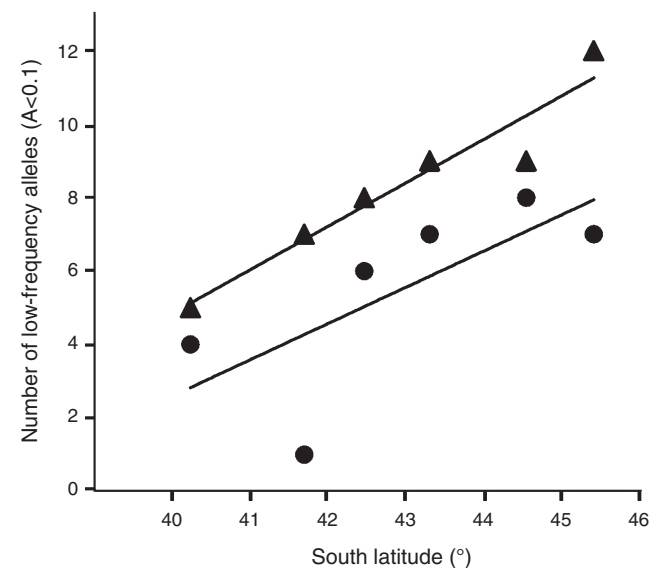

Fig. 2. Relationship between the number of low-frequency alleles ($A_{<0.1}$) and latitude for *Bromus pictus* ($y = 1.184x - 2.51$; $r^2 = 0.52$, $P = 0.10$; ●) and *Poa ligularis* ($y = 0.987x - 36.891$ ($r^2 = 0.92$, $P < 0.01$; ▲).

Table 3. *F*-statistics for 9 and 19 polymorphic loci of *Bromus pictus* and *Poa ligularis*
Total inbreeding (F_{IT}), within (F_{IS}) and among (F_{ST}) populations. Gene flow (Nm) was estimated from F_{ST}. Confidence intervals (99%) with lower and upper limits and standard errors in parentheses are showed for each *F*-statistics. If the interval does not include zero, *F*-values are significant

Parameter	*Bromus pictus*			*Poa ligularis*		
	F-statistic	CI 99%		*F*-statistic	CI 99%	
		Lower	Upper		Lower	Upper
F_{IT}	0.319 (0.105)	0.096	0.567	0.254 (0.074)	0.075	0.436
F_{IS}	0.217 (0.104)	0.032	0.510	0.175 (0.067)	0.024	0.352
F_{ST}	0.128 (0.037)	0.047	0.202	0.095 (0.024)	0.039	0.151
Nm	3.5			3.7		

loci analysed. Divergence among populations was moderate in *Bromus* ($F_{ST} = 0.128$) and *Poa* ($F_{ST} = 0.095$), yet significant. The estimated average gene flow (Nm) was 3.5 and 3.7 migrants per generation in *Bromus* and *Poa*, respectively (Table 3). These results suggest that each species has a heterogenous genetic makeup.

The UPGMA based on allele frequencies yielded similar population-clustering patterns in both species, mainly consisting of two latitudinally distinct groups (Fig. 3). Northern populations (Alicura and Pilcaniyeu) were genetically alike but distinct from the rest. Similarly, populations Tecka and Moreno were grouped

together with the southernmost Río Mayo population. These north–south groups were separated between 42°S and 43°S latitude. The Cancha population located at 42°47′S, however, clustered with the northern *Poa* populations and with the southern *Bromus* populations. STRUCTURE indicated that $K = 3$ best described both datasets (Fig. 4). As with the UPGMA, one of the STRUCTURE groups mostly consisted of individuals from northern Alicura and Pilcaniyeu populations, and southern populations Tecka and Moreno comprised the second group. The third *Bromus* cluster contained the southernmost Río Mayo population, whereas in *Poa*, it consisted of the mid-latitude Cancha population (Fig. 4).

Finally, Mantel tests indicated a high and significant correlation ($r = 0.72$, $P = 0.011$) between genetic and geographical distances for *Bromus*, whereas in *Poa*, this correlation was lower but still significant ($r = 0.49$, $P = 0.048$).

Quantitative traits under common garden

Six of a total of seven plant traits measured under common cultivation conditions varied latitudinally among populations of any one species. Within each species, four traits measured decreased significantly ($P < 0.05$) from north to south (Table 5). In particular, most characteristics related to biomass acquisition diminished with increasing latitude, suggesting genetic controls on such traits in both species.

Populations of both species had significant differences in the height, number of tillers per plant and weight of tillers (Fig. 5). In *Bromus*, plants from the three northern populations were taller (~30 cm) than those from the southern populations (~25 cm; $F = 7.1$, $P < 0.01$; Fig. 5a), whereas the southernmost Río Mayo population had fewer tillers per plant (mean = 14, $F = 3.8$, $P < 0.01$). The remaining populations had a mean of 18–20 tillers per plant (Fig. 5c). Additionally, tillers were heavier in northern populations ($F = 15.8$, $P < 0.01$) (Fig. 5d).

In *Poa*, plants at Alicura were the tallest and shortest at Moreno and Pilcaniyeu, respectively ($F = 5.5$, $P < 0.01$; Fig. 5e). Nonetheless, the latter populations had more tillers per plant ($F = 4.24$, $P < 0.01$; Fig. 5g). Tiller biomass was higher in Alicura and Tecka plants, intermediate at Pilcaniyeu, Cancha and Río Mayo, and lowest in Moreno population ($F = 5.6$, $P < 0.01$; Fig. 5h).

Table 4. Heterogeneity of allele frequencies at each polymorphic locus across populations of *Bromus pictus* and *Poa ligularis*

Asterisks indicate the level of statistical χ^2 significance per locus (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Monomorphic loci (i.e. those having one allele at all populations) were *Pgi*-1 in *Bromus pictus*, and *Adh*-2, *G2d*-1 *Idh*-2, *Me*-1, *Pgi*-1, *Sdh*-2 in *Poa ligularis*

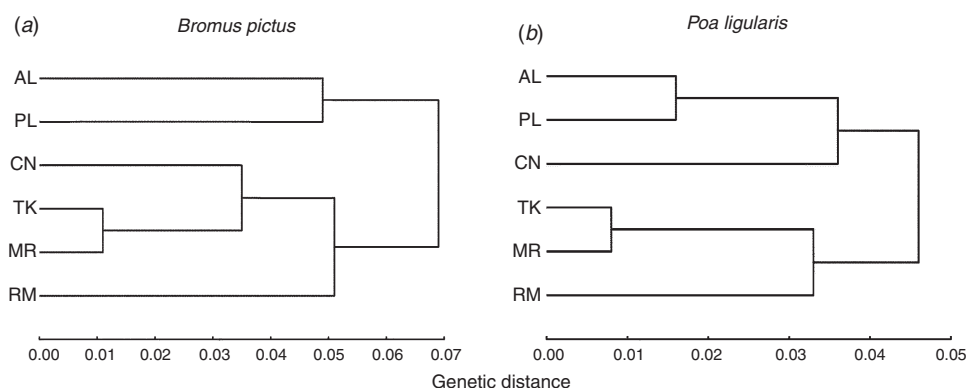| Species	Locus	No. of alleles	χ^2	Degrees of freedom	P-value
Bromus pictus					
	G2d-1	3	161.77	10	***
	Idh-1	3	96.81	10	***
	Mdh-2	3	99.66	10	***
	Mdh-3	3	23.87	10	***
	Per-1	2	8.39	5	ns
	Per-2	2	20.32	5	***
	Pgi-2	5	70.05	20	***
	Skdh	3	50.77	10	***
Poa ligularis					
	Adh-1	3	64.23	10	***
	Ald	3	35.33	10	***
	G2d-1	3	6.86	10	ns
	Idh-1	3	23.87	10	***
	Mdh-1	3	21.23	10	**
	Mdh-2	3	27.75	10	***
	Mdh-3	4	83.8	15	***
	Me-2	3	34.64	10	***
	Per-1	4	138.25	15	***
	Per-2	3	129.21	10	***
	Pgi-2	5	55.59	20	***
	Sdh-1	3	43.8	10	***
	Skdh	4	63.08	15	***

Fig. 3. Cluster analysis based on unweighted pairgroup method with arithmetic mean (UPGMA) of Nei's unbiased genetic distance (Nei 1978) for populations of (a) *Bromus pictus* and (b) *Poa ligularis*.
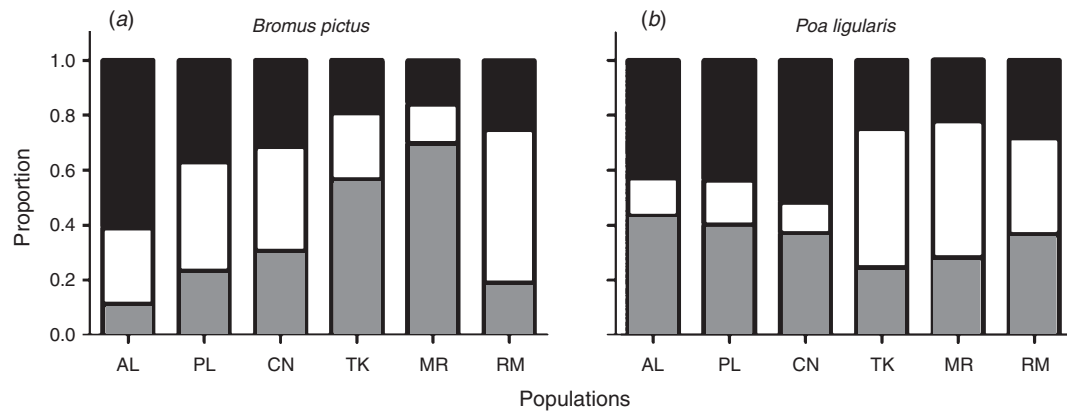
Fig. 4. Proportion of individuals of each population that were assigned to each of the three groups (*K*) detected by STRUCTURE in (*a*) *Bromus pictus* and (*b*) *Poa ligularis*. Coloured segments represent the individual's estimated membership fractions in *K* clusters.

Table 5. Pearson correlation among seven quantitative traits of *Bromus pictus* and *Poa ligularis* and latitude

Significant (*P* < 0.05) correlations are indicated with an asterisk

Trait	*Bromus pictus*		*Poa ligularis*	
	r	*P*-value	*r*	*P*-value
Specific leaf area (cm² g⁻¹)	0.09	0.85	−0.83	0.03*
Height (cm)	−0.85	0.03*	−0.46	0.35
Number of tillers plant⁻¹	−0.52	0.28	−0.21	0.68
Biomass per tiller	−0.88	0.017*	−0.68	0.13
Shoot biomass (g)	−0.77	0.07	−0.89	0.016*
Root biomass (g)	−0.89	0.018*	−0.91	0.013*
Total biomass (g)	−0.84	0.03*	−0.91	0.014*

Bromus had similar SLA among populations ($F = 0.68$, $P = 0.64$), with $190\ cm^2\ g^{-1}$ on average (range 175–200; Fig. 5*b*), whereas *Poa* exhibited significant differences among populations ($F = 3.2$, $P < 0.01$), with lower SLA in the southernmost populations ($131\ cm^2\ g^{-1}$; Fig. 5*f*).

Mean divergence in quantitative traits (Q_{ST}) was higher than F_{ST} in both species. In *Bromus*, $Q_{ST} = 0.442$, and in *Poa* $Q_{ST} = 0.446$. The difference between Q_{ST} and F_{ST} was 0.31 and 0.35 in *Bromus* and *Poa*, respectively.

Discussion

In the present paper, genetic variability of natural populations of native grasses of the Patagonian steppe was evaluated for the first time, on the basis of both neutral markers and plant traits of ecological significance. Neutral markers indicated that the polyploids *Bromus* and *Poa* had high genetic diversity within populations. All populations showed higher values of polymorphism (between 58% and 78%) than did those found in a previous meta-analysis including grasses (40%, Godt and Hamrick 1998). Both species also showed significant genetic structure associated with geographic location. Positive correlations between genetic and geographical distances among populations suggested that isolation by distance is acting along this studied gradient. Allele-frequency distributions also detected similarities among populations located closer to one another according to cluster analysis in both species. Bayesian analysis confirmed this latitudinal subdivision.

Abiotic and biotic environmental stress is a common driver of plant evolution. Higher genetic diversity in polyploids may provide greater tolerance to stressful environmental conditions (Levin 1983; Chen 2007). This has probably allowed *Bromus* and *Poa* to be successful in a xeric environment such as the Patagonian steppe.

Plant biomass under greenhouse conditions decreased in populations towards the south in both species. We suggest that this change has an adaptive value because the clinal patterns of phenotypes can provide strong evidence of differentiation by natural selection (Jonas and Geber 1999). Specific leaf area (SLA), a trait positively related to relative growth rate (RGR), was similar among populations of *Bromus*, but decreased in southern populations of *Poa*. In general, thicker leaves, and therefore lower SLA, occur in stressful environments (Knight and Ackerly 2003). Possibly, this trait showed variation only in *Poa* populations because this species has a wider distribution range than does *Bromus*, and the southern *Poa* populations are located at the marginal edge of this range. Other plant traits differed among populations but not in a clinal manner. With our current knowledge, it is difficult and risky to elaborate on direct causes for these patterns. However, biomass and SLA pattern point to more stressful conditions to the south, such as lower temperatures and greater water stress. In addition, as latitude increases, daylength during the growing season increases (+1 h between the extremes of this gradient). Therefore, plants are exposed to a longer period of water demand in the south than in the north. Correlatively, decreased night length in the south results in shorter recovery time to re-establish water potential in aboveground tissues. Given that the differences in quantitative traits for these species were measured under common garden conditions, it is likely that these are genetically based. However, in the present experiment, plants were grown under near-optimal conditions and comparisons between common garden and natural sites are required to obtain more information on the adaptive significance of these traits. In particular, reciprocal transplant experiments may help elucidate the relative contribution of the genetic and environmental variance on phenotypic expression.

The comparison of genetic differentiation at neutral markers (F_{ST}) and at quantitative traits (Q_{ST}) is a commonly used method
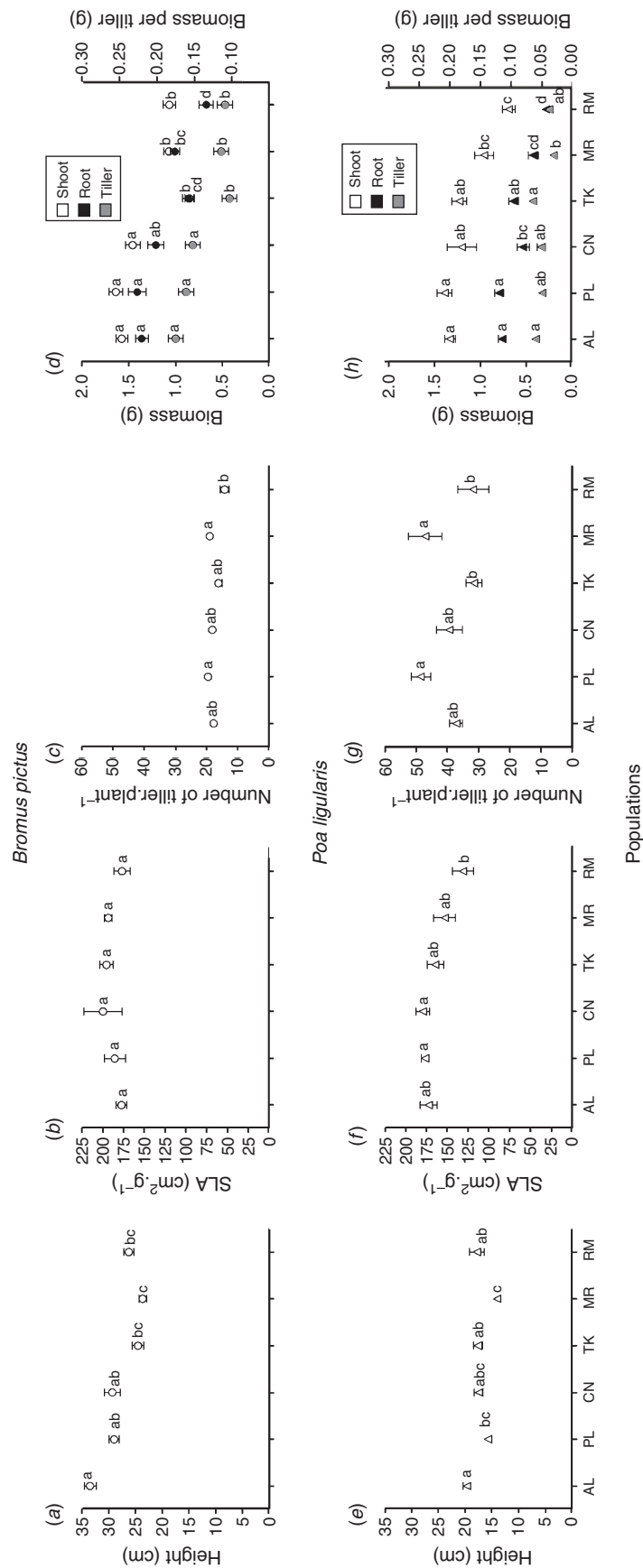
Fig. 5. Plant traits of each population of *Bromus pictus* (circles) and *Poa ligularis* (triangles) from common garden. (*a, e*) Height, (*b, f*) specific leaf area (SLA), (*c, g*) number of tiller per plant, (*d, h*) shoot, root and tiller biomass. Data are means ± s.e.

to estimate the relative impacts of genetic drift and natural selection (Martin *et al*. 2008). Our results showed that $Q_{ST} > F_{ST}$ in both species. Theory predicts, under this finding, that directional selection for different local optima will occur (Merilä and Crnokrak 2001). Therefore, morphological traits are being influenced by diversifying selection, whereas isozymes are mainly driven by neutral forces as genetic drift and barriers for gene flow. This is the first evidence that these processes are occurring in Patagonian steppes.

We found a difference in genetic variability along the latitudinal gradient, specifically in the number of rare alleles and in allele-frequency distributions in *Poa*. Rare alleles, i.e. those found at a frequency of <10%, followed a latitudinal increase from north to south, whereas no differences with latitude were obtained in *Bromus*. Theory holds that the loss of alleles is a consequence of bottlenecks and/or founder effects (Comps *et al*. 2001). When populations suffer bottlenecks and, therefore, reduce their size, low-frequency alleles are eliminated first because of genetic drift (Nei *et al*. 1975), whereas heterozygosity is a parameter less sensitive (Barrett and Kohn 1991). In addition, low-frequency alleles are not usually well represented in migrant populations when they colonise a new territory. The glacial hypothesis, based on fossil evidence, holds that during cold periods, plant species survived in more northerly (warm) latitudes, with recolonisation during the post-glacial being in a north–south direction (Villagrán and Hinojosa 2005). This hypothesis was not supported by increased genetic diversity of woody species towards the southernmost (cold) distribution and suggested the existence of refugia in areas as far south as Tierra del Fuego (Premoli *et al*. 2010). Genetically diverse populations in terms of heterozygosity and polymorphism along this latitudinal cline for both *Bromus* and *Poa* are consistent with the *in situ* survival hypothesis but not with the survival in the north (warm) and latitudinal migration towards the south (cold) after glacial retreat, as suggested for northern hemisphere taxa. Similar trends were suggested for other species of the Patagonian steppe (Jakob *et al*. 2009; Tremetsberger *et al*. 2009; Sede *et al*. 2012). This is possible because substantial areas remained free of ice during the glacial episodes (Rabassa 2008).

One important and exciting result in the present study is the presence of a 'genetic gap' in both species at the same geographical location, between 42° and 43° latitudes. This result concurs with earlier studies on three grass species (Jakob *et al*. 2009), one perennial herb (Cosacov *et al*. 2010), and rodents (Cañón *et al*. 2010; Lessa *et al*. 2010) of the Patagonian steppe, as well as for tree species from Andean habitats in Patagonia (Mathiasen and Premoli 2010; Quiroga and Premoli 2010). Sérsic and coworkers (2011) suggested that stepparian breaks probably result from Quaternary events such as glaciation cycles or the dynamism of river basins during glaciations–deglaciation periods. For woody species in the Andean Patagonian forest (Mathiasen and Premoli 2010; Quiroga and Premoli 2010; Premoli *et al*. 2012), it has been proposed that this ancient break is based on the paleogeographic features of Patagonia. These Quaternary or Pre-Quaternary explanatory hypotheses causing this allopatric divergence in plant species cannot be excluded until we have greater certainty about the origin and evolution of the grasses.

We have found marked genetic differences in neutral and adaptive traits between northern and southern populations along the latitudinal range of both studied species. Significant intraspecific patterns can guide the design of conservation strategies (Moritz 1994; Crandall *et al*. 2000) of these native forage species. Such genecological variation along latitudinal gradient is of paramount importance to respond to changing conditions under global warming as well as for conservation of the genetic pool under grazing intensification (Aguiar and Román 2007). *Bromus* and *Poa* are highly palatable to sheep (Graff *et al*. 2007). They are native grasses adapted to arid and cold environment and thus they are highly valuable components of Patagonian (and South American) biodiversity. Despite their importance, efforts to protect them are rare (Aguiar and Román 2007). The evidence presented here by means of neutral and adaptive traits shows that significant divergence by F_{ST} and Q_{ST}, respectively, exists among studied populations. This suggests that the combined effects of isolation and genetic drift, together with divergent selective regimes, have shaped *Bromus* and *Poa* gene pools, which should be considered in urgent restoration actions.

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