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Phylogenetic relationships and intraspecific diversity of a North Patagonian Fescue: evidence of differentiation and interspecific introgression at peripheral populations

A. S. López · M. M. Azpilicueta · D. R. López · G. L. Siffredi · P. Marchelli

Abstract Peripheral sites usually offer suboptimal conditions for species with wide distributions, where expression of phenotypic variability and potential interspecific hybridization might be enhanced. The Patagonian steppe, the largest and southernmost dryland ecosystem in South America, is characterized by natural rangelands dominated by grasses. *Festuca pallescens* is a keystone species with a wide distribution in Patagonia over diverse environments reaching the extreme arid zones in the Somuncura plateau, a biogeographical island. Our aim is to study the phylogenetic relationships among *Festuca pallescens* populations as well as between this species and the sympatric *F. argentina* in North Patagonia. We analysed fourteen populations along a west-east transect of about 500 km in North Patagonia with three types of molecular markers: ITS, chloroplast *trn*L-F and eight nuclear microsatellites. Bayesian inferences, maximum parsimony and maximum likelihood analyses with *trn*L-F and ITS showed that *F. pallescens* is related to the Patagonian clade within the Festuca phylogeny. However, the easternmost populations of *F. pallescens* at Somuncura plateau were highly differentiated from the other populations and clustered with *F. argentina* (a sympatric species of the Asian-American clade). Principal coordinates analyses and Bayesian clustering performed with nuclear microsatellites as well as morphoanatomical traits, showed an intermediate position of one of these easternmost populations with respect to the two species, suggesting admixture. The high genetic variability observed in these peripheral populations highlight their relevance for conservation and might be indicating the existence of evolutionary processes triggering events of speciation in the Patagonian fescues.

Keywords *Festuca pallescens* · *Festuca argentina* · Patagonian fescues · ITS region · *trn*L-F · Microsatellites · Hybridization · Marginal populations

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Introduction

Drylands (arid, semi-arid and dry-sub humid ecosystems) are suitable environments to study adaptations to extreme climatic conditions. These ecosystems are of paramount importance because of their multi-

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functionality (Maestre et al. 2012) and high biodiversity (Davies et al. 2012) related to the presence of endemisms (Millennium Ecosystem Assessment Panel 2005; Davies et al. 2012). Drylands constitute the largest terrestrial biomes with high sensitivity to climate change and desertification (Sala et al. 2000; Reynolds et al. 2007) and are also known for a noteworthy environmental heterogeneity that might promote the expression of phenotypic variability (Hoffmann and Hercus 2000) and hybridization processes (Thompson et al. 2010). In addition, over 70% of the worlds' drylands are currently used for grazing of livestock in native vegetation (Asner et al. 2004). Therefore, understanding how environmental drivers influence genetic diversity in these vulnerable ecosystems becomes of high relevance.

In Argentina, the Patagonian steppe constitutes the largest and southernmost dryland ecosystem of South America (Palazzesi et al. 2014). Natural rangelands are strongly affected by desertification and overgrazing (Aguiar and León 1985; Bertiller 1990; Paruelo et al. 1993) becoming highly vulnerable to the fluctuations imposed by climate change. Generally, these ecosystems are dominated by perennial grasses with low capacity to cope with rapid environmental change (Chapin 1993). In addition, a strong precipitation gradient characterize the region (from above 900 mm in the west to less than 200 mm of annual precipitation in the east) generating a spatial distribution of changing vegetation, soil and forage resources (León et al. 1998). Consequently, the decline of abundance and diversity of native species towards peripheral driest eastern locations resulted in the formation of patches of bare soil with a recolonization by shrubs and unpalatable grasses (Aguiar and León 1985; Bertiller et al. 1993; Golluscio et al. 1998; Busso and Bonvissuto 2009).

Patagonia is characterized by a large environmental heterogeneity and a complex geomorphologic history. Pliocene and Pleistocene glaciations, Miocene marine transgressions and volcanism shaped the current topology and probably left an imprint in the vegetation. In North Patagonia (above 42° S), glaciers had a lesser extent (Flint and Fidalgo 1964), being confined to the foothills of the Andes and neighbouring zones, but with a continuous permafrost throughout the region (Rabassa et al. 2011). Towards the east occurs the Somuncura plateau, a biogeographically isolated and elevated landscape formed during the Tertiary. Layers of basaltic Cenozoic lavas formed this complex, a stable rigid block, during the Andean orogeny (Malumián and

Náñez 2011). The Miocene marine transgressions affected lowlands that surrounded the massif, but did not reach the plateau (Malumián and Náñez 2011). Instead, it remained volcanically active until the early Pliocene (Rabassa 2008). Therefore, this Precambrian plateau formed an insular structure surrounded by water (Burkart et al. 1999), enclosing particular microhabitats. This has favoured the presence of numerous endemisms, such as five plants species, at least fourteen invertebrates and ten vertebrates (Cei 1969; Cei and Scolaro 1981; León et al. 1998; Chebez 2005; Andrade and Monjeau 2014; Breitman et al. 2015). Furthermore, it constitutes a notable geomorphological feature located in the extreme of the distribution for many species of the Patagonian steppe (León et al. 1998; Andrade and Monjeau 2014). Although Somuncura plateau was declared a Protected Area (Río Negro Province, Argentina), only a few works reported on genetic and morphological variation of its biota (Cei and Scolaro 1981; Chebez 2005; Muzón et al. 2005; Andrade and Monjeau 2014; Breitman et al. 2015).

Peripheral sites usually offer suboptimal conditions for species with wide and environmentally diverse distributions, leading to progressively smaller populations prone to isolation and extinction (Eckert et al. 2008). In the case of the Somuncura plateau, apart from the geographic isolation there are also environmental constrains. Compared to western and more central localities, Somuncura has much lower precipitations (150 mm/year vs 800 mm/year), a higher mean diurnal range of temperature (14.4 vs 11.5°C) and a higher temperature annual range (29 vs 22.7°C; Data from WorldClim, Hijmans et al. 2005). Although genetic differences between geographically central and peripheral populations are rarely very large (Eckert et al. 2008), many dryland species showed high levels of genetic differentiation between extreme populations (Martínez-Palacios et al. 1999). In addition, the repeated exposure to stressful conditions of border populations could enhance the expression of phenotypic variability (Hoffmann and Hercus 2000) or local adaptations (Hoffmann and Blows 1994). These environments are also prone to the occurrence of natural interspecific hybridization and introgression (Thompson et al. 2010) that may increase genetic variation producing new gene combinations better adapted to novel environments (Johansen-Morris and Latta 2008). In any case, these responses contribute to a

higher flexibility and survival of a population in a context of changing environmental conditions.

Festuca pallescens (St. Yves) Parodi is a keystone species with a wide distribution over diverse environments, type of soils and ecological areas of Patagonia (Bertiller 1990; Nicora 1978; Catalán and Müller 2012). It is a highly palatable and preferred forage resource, and therefore it was intensively affected by overgrazing (Bertiller et al. 1993). In northern Patagonia, density of *F. pallescens* populations' declines to the east, where the species is increasingly fragmented forming spaced patches related to a reduction of wet meadows and to the lower topographic elevation (León et al. 1998). This allohexaploid species shows a wide morphological and anatomical variation (Oliva et al. 1993) as well as differences in phenology associated to air temperature (Bertiller et al. 1990), but its genetic diversity was never studied. The ability of *F. pallescens* of growing in different environments might be exclusively plastic, adaptive, or a combination of both. We hypothesized that at marginal locations, like the Somuncura plateau, where the species is isolated and growing in suboptimal conditions, environmental pressure promotes phenotypic and genetic variation. Moreover, hybridization and speciation processes could be also favoured.

Festuca is the largest genus among the Loliinae, with around 500 species (Inda et al. 2008). Polyploidization, past and recent hybridizations, and intergeneric crosses with other Loliinae are frequent (Inda et al. 2008 and references within). In spite of the well reported phylogenies of the genus *Festuca* (Torrecilla and Catalán 2002; Torrecilla et al. 2003; Catalán et al. 2004; Inda et al. 2008; Minaya et al. 2017) little is known about the Patagonian fescues, being so far only few species included in these studies (i.e. only *F. argentina* (Speg.) Parodi, *F. subantarctica* Parodi, *F. purpurascens* Banks & Sol. ex Hook. f., *F. gracillima* Hook. f*., F. magellanica* Lam. and *F. pyrogea* Speg.). Therefore, the position of *F. pallescens* within the infrageneric phylogeny, as well as the relation with sympatric species is unknown.

Our aim is to study the phylogenetic relationships among *F. pallescens* populations from North Patagonia. This is the first step towards the study of its genetic diversity and, in particular, to disentangle differentiation between populations and/or hybridization processes associated to its wide distribution. Accordingly, we analysed fourteen populations along a west-east transect that represent the diverse environments where this dryland species occurs. In particular, due to the considerable number of endemic species at the Somuncura plateau, we will analyse if the isolated populations at this easternmost site are genetically different from western and continuous populations. We intend to contribute to the scarce existing knowledge about the phylogenetic placement of the species, and to provide information of relevance for conservation planning of the Somuncura Protected Area.

Material and methods

Sample sites and plant material

We collected leaf tissue of *Festuca pallescens* at five locations situated along a west-east oriented transect of about 500 km in North Patagonia (Argentina) during January of 2014 (Fig. 1). The material was identified in the field using exomorphological characters described for this species (including leaves with glaucous colour, sheaths with pulvini, and lemmas ending in an awn, e.g. Nicora 1978). At each location, between one to four populations from high steppes and wet-meadows were sampled, gathering 14 populations (Table 1). The average annual rainfall (mm/y) and its coefficient of variation (cv) vary notably between sampling sites being of 831.6 mm/y; cv 0.2 in the west and 170.8 mm/a; c.v. of 0.4 in the east. In addition, we sampled the sympatric species *F. argentina* at one location (Pilcaniyeu, coinciding with populations 7 and 8) and two species of Patagonian grasses (*Poa ligularis* Nees ex Steud. var. ligularis, and *Pappostipa speciosa* var. *speciosa* Trin. et Rupr.) as outgroups.

DNA extraction and PCR amplification

Young leaf tissue from tussocks were collected, frozen in liquid nitrogen and grounded to fine powder using an automatic mixer mill (Resch, Germany). DNA was extracted following a modified protocol of Doyle and Doyle (1987) according to Gonzalo-Turpin and Hazard (2009). We used three different DNA markers to study the phylogenetic relationships: the internal transcribed spacer (ITS), a chloroplast DNA region and eight nuclear microsatellites (SSRs). The complete ITS region (ITS1–5.8S-ITS2) was amplified with primers cy1-cy3 (Wright et al. 2006). Due to the highly conserved DNA sequences of the ITS nuclear region within species, one
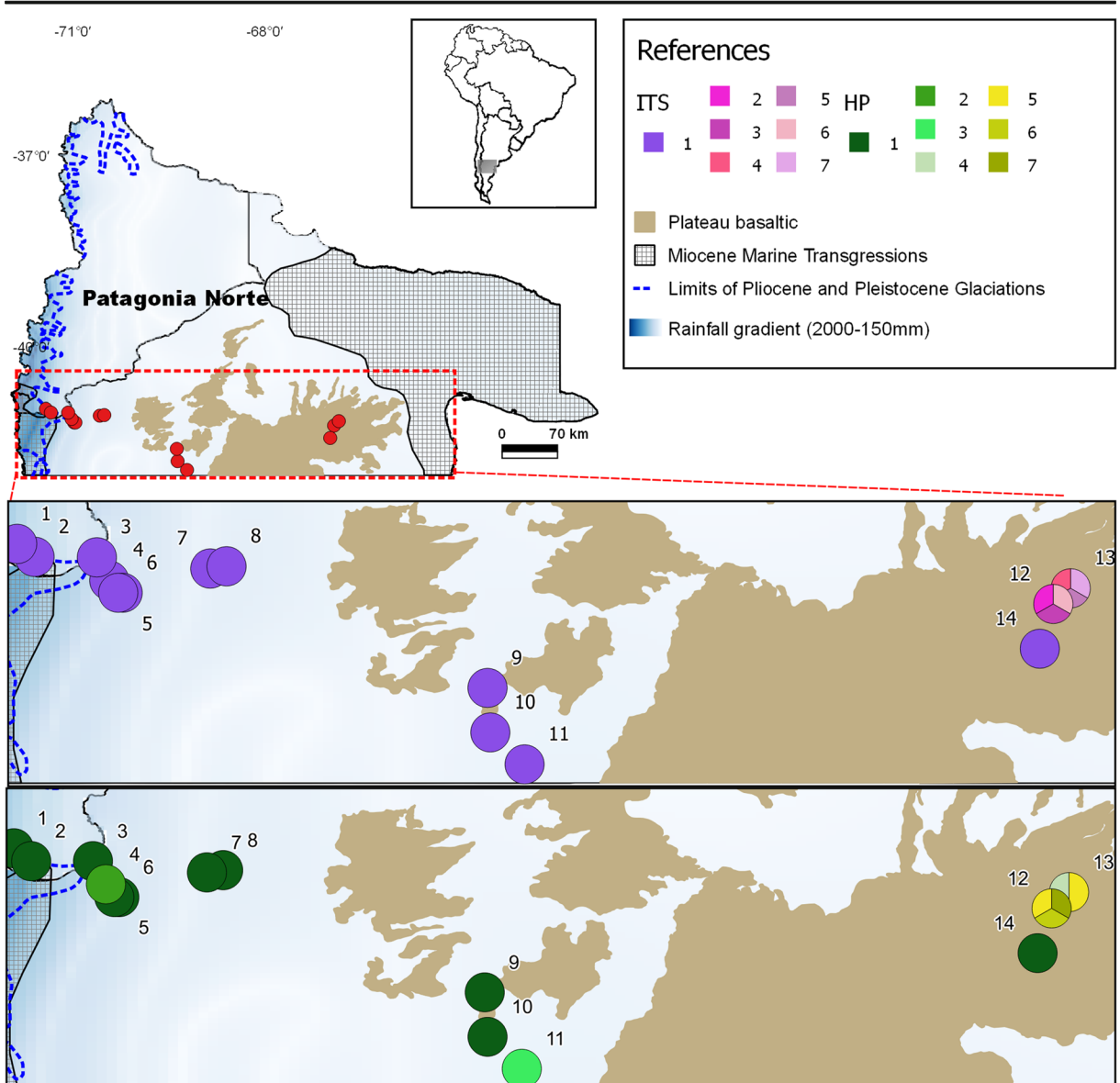
Fig. 1 Distribution of the nuclear (ITS) and plastid (HP) (*trn*L-F) genetic variants among the sampled populations. Numbers follow the description in Table 1. Violet (ITS) and green (cpDNA) colour palettes depict different variants for the two markers. Noteworthy geomorphological features are also shown in the figure: Oligocene-Miocene marine transgressions and limits of Pleistocene-Pliocene glaciations were modified from (Premoli et al. 2012) and (Pastorino and Gallo 2002), respectively. The west-east decreasing rainfall is also shown.

individual from each population was sequenced. However, given the higher heterogeneity and phenotypic variation observed in the field at the Somuncura plateau, 12 individuals from the three sampled populations were analysed. For the amplification of the ITS region, we used 40 ng of DNA as template, 0.6 units of Go-Taq DNA polymerase (Promega, Madison,WI, USA) with

5X Green GoTaq® reaction buffer (Promega), 1.5 mM $MgCl_2$, 250 µM of dNTPs, and 0.3µM of each primer in a total volume of 30 µl. PCR reactions were carried out with the following programme: 5 min at 94°C, 30 cycles of 30s at 94°C, 1 min at 56°C and 2 min at 72°C and a final cycle of 10 min at 72°C. The chloroplast *trn*L-F region was amplified using the universal primers c and f

Table 1 Characteristics of *Festuca pallescens* populations sampled. Floristic physiognomic types were characterized following.

Population	Sample site	Precipitation [mm]	Latitude	Longitude	Elevation [m a.s.l.]	Floristic physiognomic type	Plant cover	Dominat species
1	Península Huemul Ranch, Neuquén, Argentina	831	40°57′	71°25′	1,220	Shrub-grass steppe	20–30% grass, 40–50% shrubs	*Festuca pallescens; Pappostipa sp.; Poa ligularis; Adesmia boroniodes; Berberis sp; Mulinum spinosum*
2			41°1′	71°20′	845	Shrub-grass steppe	30–40% grass, 20–30% shrubs	*Festuca pallescens; Pappostipa speciosa vr. major; Mulinum spinosum; Berberis sp.*
3	San Ramón Ranch, Río Negro, Argentina	584	41°10′	70°59′	1,140	Exposed Shrub-grass steppe[1]	5–10% grass, 10–20% shrubs	*Festuca pallescens; Pappostipa sp; Acaena splendens; Mulinum spinosum*
4			41°10′	70°57′	1,100	Meadow	> 60% grass	*Festuca pallescens; Juncus sp.; Carex subantarctica*
5			41°7′	71°1′	902	Meadow	60% grass < 2 0% herbs	*Festuca pallescens;Poa pratensis; Taraxacum officinale; Juncus sp.; Carex subantarctica*
6			41°1′	71°4′	1,139	Grass steppe	50–60% grass, 5–10% shrubs	*Festuca pallescens; Festuca argentina; Pappostipa speciosa; Mulinum spinosum; Senecio sp.*
7	Pilcaniyeu Experimental Field, Río Negro, Argentina	264	41°4′	70°34′	1,260	Shrub-grass steppe	30–40% grass, 30–40% shrubs	*Festuca pallescens; Festuca argentina; Pappostipa sp.; Mulinum spinosum; Senecio sp.*
8			41°3′	70°30′	970	Meadow	80% grass	*Festuca pallescens; Juncus sp.; Hordeum sp.*
9	Ingeniero Jacobacci, Río Negro, Argentina	170	41°55′	69°12′	1,400	Grass steppe	> 60% grass, < 5% shrubs	*Festuca pallescens; Poa pratensis; Mulinum spinosum; Senecio sp.*
10			41°46′	69°21′	970	Salty Meadow	50-60% grass, 5–10% shrubs	*Festuca pallescens; Juncus sp.; Distichlis sp.; Mulinum spinosum; Senecio sp.*
11			41°35′	69°22′	1,135	Salty meadow	> 60% grass, < 5% shrubs	*Festuca pallescens; Juncus sp.; Distichlis sp.; Azorella trifurcata*
12	Somuncura plateau, Río Negro, Argentina	150	41°13′	66°54′	1,100	Shrub-grass steppe	30–40% grass, 5–10% shrubs	*Festuca sp.; Pappostipa sp.; Jaraba humilis; Mulinum spinosum; Adesmia campestris*
13			41°9′	66°50′	925	Shrub-grass steppe	20–30% grass, 5–10 shrubs	*Festuca sp; Pappostipa sp; Mulinum spinosum*
14			41°25′	66°58′	1,430	Meadow	50–60% grass, 5–10% shrubs	*Festuca pallescens; Poa ligularis; Senecio sp.*

[1] High proportion of bare soil

[2] Organic matter

(Taberlet et al. 1991) with 90 ng of DNA, 1 unit of Go-Taq DNA polymerase (Promega, Madison,WI, USA) with 5X Green GoTaq® reaction buffer (Promega), 2 mM MgCl₂, 200 μM of dNTPs, and 0.2 μM of each primer in a total volume of 50 μl. The amplification programme was: 1 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C and a final cycle of 7 min at 72°C. Finally, a set of eight microsatellite loci (SSR) developed for *Lolium* perenne, Festuca-*Lolium* complex of grasses and *Festuca* spp (Lauvergeat et al. 2005; Fu et al. 2006; Jensen et al. 2007), were successfully transferred being polymorphic in Patagonian fescues (López et al. 2015). PCR amplifications were carried out according to Lauvergeat et al. (2005).

PCR products for the three markers were checked for positive amplification in 1% agarose gels, stained with Syber Safe (Invitrogen, Eugene, OR, USA), and visualized with a blue light trans illuminator. The amplified ITS and *trn*L-F regions rendered a band of approximately 700 bp and 1,000 bp, respectively, that was purified using the Wizard Genomic DNA Purification Kit (Pomega) and then sequenced in a capillary sequencer (ABI 3700, Genomics Unit, Biotechnology Institute of INTA, Hurlingham, Argentina). Microsatellite primers were labelled with different fluorophores and then pooled in three groups for analysis in the ABI 3700 sequencer. Fragments visualization and allele scoring was performed with Genemarker Version 1.97 (SoftGenetics, State College, PA, USA).

Morphoanatomical analyses

Due to the observed phenotypic variation at the Somuncura plateau, an analysis of the main morphological characters characterizing *F. pallescens* (Catalán and Müller 2012) was done. We analysed field samples of typical *F. pallescens* and of the sympatric species *F. argentina* from Pilcaniyeu, Argentina (i.e., from central populations of both these species), and of *F. pallescens* from the sampled Somuncura marginal populations. In addition, cross-sections of leaves were used for morphoanatomical analyses, to determine position and extension of sclerenchymatic tissue within leaf blade. This feature has been described as important for species identification of Patagonian species of *Festuca* (e.g. Dubcovsky and Martínez 1988; Catalán and Müller 2012). Transverse sections were obtained from middle portions of mature leaves that were cut

approximately 12 cm away from the base of the lamina (i.e. from the distal end of the leaf sheath) with a razor blade. Sections were observed at 10x with a Leica SGE microscope.

Phylogenetic analyses

Chromatograms from forward and reverse sequences were corrected with 'seqtrace' 0.9.0 (Stucky 2012) and consensus were created using BioEdit v7.2.5 (Hall 1999). All the ITS sequences from Somuncura populations and one sample of *F. argentina* (F.arg2) presented a heterozygous indel in a single position. Therefore, we used the software OLFinder (Dixon 2010) to resolve the shifts in phase between the forward and reverse strand sequence. All the species of *Festuca* distributed in South America that were available in Genebank (Catalán et al. 2004; Inda et al. 2008) were used for the phylogenetic analysis (15 species; Table S1). In addition, species of Festuca from different parts of the world retrieved from Genebank were also included: 18 species for ITS and 19 species for *trn*L-F (Table S1). *Pappostipa speciosa* var. *speciosa* and *Poa ligularis* are two species of Poaceae generally found in *F. pallescens* rangelands. These species are phylogenetically very distant from *F. pallescens* and were used as outgroups. ITS and *trn*L-F sequences of *F. pallescens, F. argentina*, *Poa ligularis* and *Pappostipa speciosa* var. *speciosa* were deposited in Genebank (Accession numbers are provided in Table S1; supplementary materials). Sequences were aligned using Muscle with manual adjustments when necessary in AliView 1.18 (Larsson 2014). The boundaries of each sequence were established by the alignment with the species of *Festuca* obtained from Genebank (Torrecilla and Catalán 2002; Torrecilla et al. 2003; Catalán et al. 2004).The presence of the heterozygous indel was coded as a binary data and incorporated in the sequence data matrix. Gaps were treated as missing data.

To evaluate the phylogenetic relationships between populations we run Bayesian inferences (BI), maximum parsimony (MP) and maximum likelihood (ML) analyses with ITS and *trn*L-F data matrices both separately and combined. The BI, MP and ML analyses were implemented with Mr. Bayes 3.2 (Ronquist et al. 2012), Paup*4.0 beta10 (Swofford 2003) and on line software PhyML (Guindon et al. 2010), respectively. To run BI and ML, we estimated the optimal nucleotide substitution model for the ITS

dataset (GTR+G with 4 gamma rate categories), the *trn*L-F dataset (TPM1uf + G) and the concatenated dataset (GTR+G with gamma rate categories), after testing 56 nucleotide substitution models based on the Akaike information criterion (AIC) implemented in 'jmodeltest' 2.0 (Guindon and Gascuel 2003; Posada 2008; Darriba et al. 2012). Bayesian analyses were carried out by running twice the same parameters to both independent datasets (1 million generations initiated from different random trees, sampling every 100th generations model parameters such as nucleotide substitution rates, gamma shape, proportion of invariable sites, nucleotide frequency) estimated by MrBayes. After discarding 0.25 of the total sampling ('burn-in'), parameters were sampled when reaching stationary. The ITS and *trn*L-F 50% majority rule consensus trees, obtained from 15,000 trees, were supported by values branches from the posterior probability (PP). Clades with more than 95% PP values were considered well supported. Parsimony analysis was carried out on each independent data matrix. Following Torrecilla and Catalán (2002), Torrecilla et al. (2003) and Inda et al. (2008), data matrices were subjected to heuristic analysis to find all equally parsimonious trees. First, we run 10,000 random-order-entry trees, MULPARS ON, with TBR branch swapping and saving no more than 10 trees of length equal or shorter than 10 per replicate to compute a consensus tree that was used afterwards as a negative constraint for a second search. Then, the second heuristic search consisted on 5,000 random-order-entry trees, MULPARS ON, with TBR branch swapping and saving no more than 5 trees of length equal or shorter than 5 per replicate. All parsimonious trees were used to compute their respective strict and 50% majority rule consensus tree. Bootstrap support for branches of the parsimonious trees was calculated through heuristic searches of 1000 replicates. Most clades presented bootstrap values between 90–100%, so they were considered well supported.

Pairwise genetic distances both for ITS and cpDNA (*trn*L-F) were calculated in R using 'dist.dna' ('ape' package) (Paradis et al. 2004). This function computes a matrix of pairwise distances from DNA sequences using 11 DNA substitution models and the raw distance. Finally, we used a median-joining network algorithm (Bandelt et al. 1999) to reconstruct the relationships between haplotypes using Network 5.0.0.0 (www.

fluxus-engineering.com). A posterior post-processing option (MP calculation) was used to eliminate unnecessary median vectors and links (Polzin and Daneschmand 2003).

Microsatellite loci were analysed in the same set of individuals as for the phylogenies using Bruvo's distance (Bruvo et al. 2004), which is suitable for polyploid organisms. It is based on a measure of genetic distance similar to the band-sharing indices used with dominant data, but taking into account mutational distances between alleles (Clark and Jasieniuk 2011). The advantage of this measure is that it dispenses with knowledge of ploidy and allele dosage, which is difficult to determine in polyploids. Using the estimated Bruvo's distance we calculated a principal coordinate analyses (PCoA) with the 'polysat' package (Clark and Jasieniuk 2011) in R (R Core Team 2013) and a Neighbour-joining tree estimation with 'ape' package (Paradis et al. 2004) in R (R Core Team 2013). On the other hand, to determine the number of clusters and evaluate signals of admixture, we used STRUCTURE ver. 2.3.4. (Pritchard et al. 2000). The different levels of ploidy were coded as recommended by the documentation of Structure for loci with copy number ambiguity, i.e. coding the missing alleles as recessive but not ambiguous and using the RECESSIVE ALLELES model. The ambiguous loci (i.e. when there is uncertainty about the number of copies of each allele) were coded repeating one allele as described by Pritchard et al. (2000). The iterations were run using the admixture model with correlated allele frequencies because it is more conservative and provides greater power to detect populations that are closely related (Porras-Hurtado et al. 2013). We run Structure 16 times for each K, varying the range of K from 1 to 9, using a length of burn-in period of 200,000 iterations and 10^6 MCMC replications. The optimal number of clusters was determined following (Evanno et al. 2005) in the online platform Harvester (Earl and vonHoldt 2012). We used Clumpak (Kopelman et al. 2015) to combine files from the16 replicates of the best K and prepare the graphical representation.

Results

The easternmost populations of *F. pallescens* at Somuncura plateau (populations 12 and 13) showed distinctiveness from the rest of the populations sampled along the west-east transect (Fig. 1). There was a high

level of diversity at both nuclear and plastid markers, with the presence of six different ITS sequences and four chloroplast haplotypes. In contrast, all the other populations showed no polymorphism for the ITS region and only minor variations for the chloroplast (Fig. 1). Population 14, also at Somuncura plateau, holds the same genetic constitution than the rest of the *F. pallescens* populations. Therefore, from now on we will refer to *Somuncura populations* as solely referring to populations 12 and 13.

Morphoanatomical analyses

Differences between typical, central populations of *F. pallescens* and the marginal individuals sampled at Somuncura were also evident for several of the morphological characters analysed (Table 2). We also compared the Somuncura populations with the sympatric *F. argentina* and found some traits similar and others intermediate between them. For example, leaf colour at Somuncura populations was glaucous like in *F. pallescens*. On the contrary, the ligule at the apex of the leaf-sheath and the presence of adaxial to abaxial girders of sclerenchyma of the Somuncura populations resembled those of *F. argentina*. Finally, some traits were clearly intermediate between *F. pallescens* and *F. argentina*: size of pulvinus at the base of the lamina, length of the awn at lemma apex, and presence of a continuous band of sclerenchyma under the abaxial epidermis in leaf cross-section. In addition, we detected differences between the two Somuncura populations:

population 13 had a higher proportion of sclerenchyma than population 12 (Table 2).

ITS region

All the analysed individuals of *F. pallescens* showed the same ITS sequence; except for Somuncura populations where six different ITS sequences were found (Fig. 1). The individuals from population 14, also at the Somuncura plateau, were identical in sequence to the rest of the *F. pallescens* samples. *Festuca argentina* also showed high polymorphism since each individual sampled had a different ITS sequence. All sequences from populations 12 and 13 presented shifts in phase between the forward and reverse strand due to the presence of a heterozygous indel at the same position, whereas in only one sample of *F. argentina* a heterozygous indel was detected, but in a different position. Neither the other samples of *F. pallescens* nor the other species showed heterozygous indels. The ITS dataset included 47 sequences belonging to 38 taxa and 621 aligned nucleotide positions, where 107 (17.23%) resulted parsimony informative.

The three methods showed similar tree topologies; therefore, we present the Bayesian results (Maximum Parsimony and Likelihood analyses can be accessed from supplementary materials, Fig S1). *Festuca pallescens* and samples from Somuncura belong to different clades. In fact, Somuncura formed a strongly supported clade with *F. argentina* (Fig. 2). *Festuca pallescens* is sister to the Patagonian species

Table 2 Morphoanatomical traits comparing *Festuca argentina*, *F. pallescens* and Somuncura populations.

| Morphoanatomical traits	*Festuca argentina*	*Festuca pallescens*	Somuncura 12	Somuncura 13
Leaf colour	bright green	glaucous	glaucous	glaucous
Ligule at apex of leaf-sheath interior	clearly visible	hardly visible	visible (similar to *F. argentina*)	visible (similar to *F. argentina*)
Pulvinus at base of lamina	absent	present, relatively large and noticeable	present, small and less distinguishable than in *F. pallescens*	present, small and less distinguishable than in *F. pallescens*
Awn at lemma apex	absent	present and >1 mm	Present and aprox. 0.2 mm	Present and < 0.2 mm
Adaxial to abaxial girders of sclerenchyma of vascular bundles in leaf cross-section	present	absent	present	present
Continuous band of sclerenchyma under abaxial epidermis in leaf cross-section	absent	present (sometimes interrupted in parts)	absent (but with a higher proportion of schlerenchyma than in *F. argentina*)	absent (but with a higher proportion of schlerenchyma than in Somuncura 12)
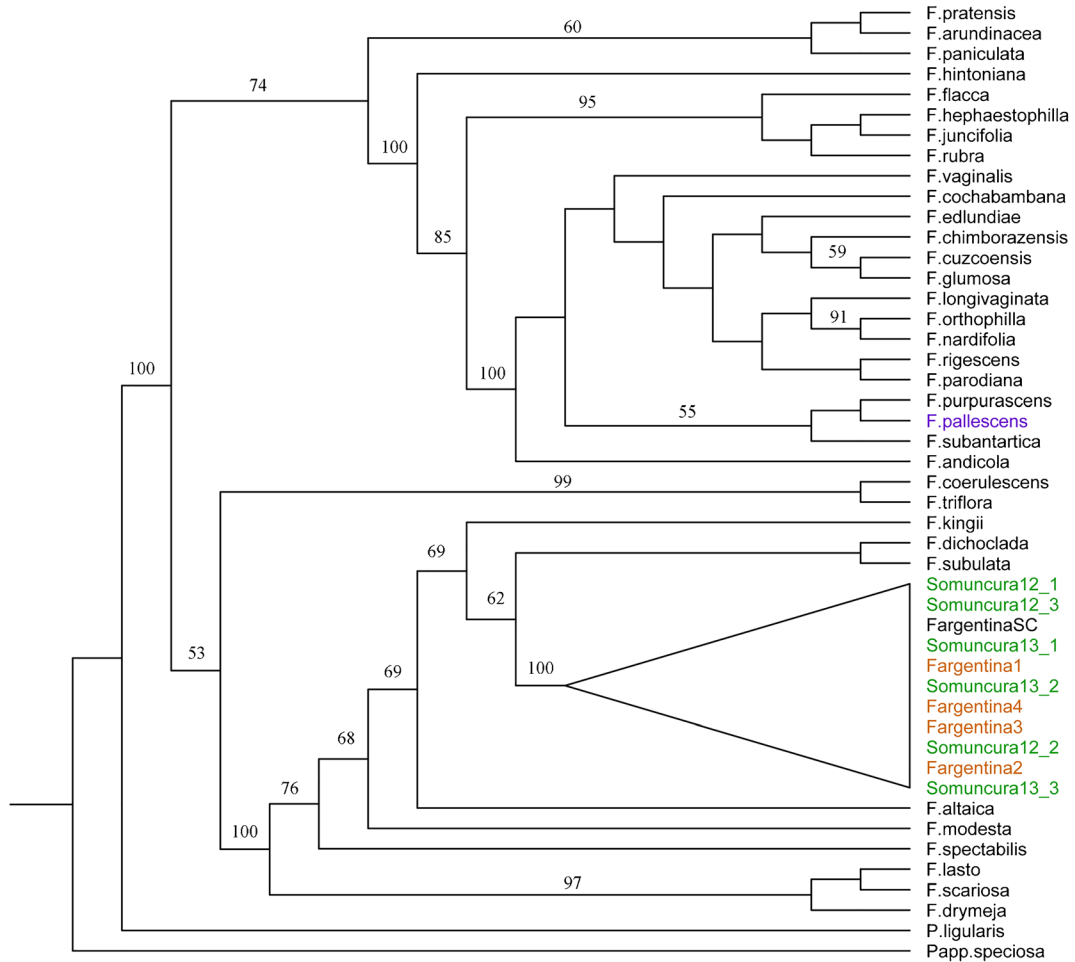
Fig. 2 Bayesian tree based on nuclear (ITS) marker. Studied taxa are dyed with violet (*F. pallescens*), green (Somuncura) and orange (*F. argentina*). Numbers above the branches indicate posterior probability (PP); posterior probability support values lower than 50% are not shown. *Pappostipa speciosa* var. *speciosa* and *Poa ligularis* were used as outgroups.

F. purpurascens and then it is sister to *F. subantartica*, but the relationships show low support. This clade is closely related to the South-American species (*F. chimborazensis, F. rigenscens, F.vaginalis, F. parodiana, F. glumosa, F. andicola, F. cochabambana, F. nardifolia, F. longivaginata, F. orthophilla* and *F. cuzcoensis*) and to a North-American species (*F. edlundiae*; Fig. 2 and Fig. S1b). Maximum Parsimony majority rule consensus tree showed a polytomy that included the three Patagonian species (*F. pallescens, F. subantartica* and *F. purpurascens*) with the South-American species mentioned before and one North-American species (Fig. S1a). Samples from Somuncura and *F. argentina* were represented as a polytomy closely related to *F. dichoclada, F. subulata, F. kingii* and *F. altaica* in all topologies (Fig. 2 and Fig. S1). Pairwise

genetic distances were small between *F. argentina* and Somuncura (ranging from 0 to 0.7%) and considerably larger between Somuncura and *F. pallescens* (between 7.3 and 8.1%).

Notably, even though Somuncura showed most polymorphic sites shared with *F. argentina*, some sites were shared with *F. pallescens,* some were exclusive, and most were heterozygous positions between the two species suggesting introgression (Table S3).

Chloroplast DNA (trnL-F) region

Three haplotypes were identified among the *F. pallescens* populations (HP1, HP2 and HP3), HP1 being the most frequent. Among the Somuncura

individuals, four variants were detected (HP4, HP5, HP6 and HP7; Fig. 1), HP5 being shared with *F. argentina*. The cpDNA (*trn*L-F) dataset included 43 sequences (39 taxa) with 907 aligned positions; 63 (6.94%) variable sites were parsimony informative.

Organelle trees in all analyses showed in general the same topology as the ITS trees. The Bayesian tree showed that haplotypes 5 and 9 of *F. pallescens* were linked and haplotype 1 (the most common haplotype found in *F. pallescens*) was associated to *F. purpurascens* (Patagonia). These taxa were closely related to *F. chimborazensis* (Ecuador), *F. edlundiae* (Canada) and *F. glumosa* (Colombia). In addition, *F. subantartica* presented the same haplotype as the most common haplotype in *F. pallescens* (see legend

in Fig. 3). The private haplotype of population 13 from Somuncura and the other two haplotypes found in population 12 also from Somuncura were close to *F. amplisssima* (México). This clade was sister to *F. argentina,* and then to a clade composed of *F. breviglumis* (México) and *F. dichoclada* (Perú), see Fig. 3. However, PP support values were very low (less than 50%). Pairwise genetic distances were low between *F. argentina* and Somuncura (between 0 and 0.24%) and considerably larger between Somuncura and *F. pallescens* (between 1.9 and 2.03%). The network shows that nineteen mutated positions separate *F. pallescens*5 (HP2) from Somuncura 12_2 (HP4), while haplotypes of *F. pallescens* and Somuncura differed by only one or two mutations to each other,
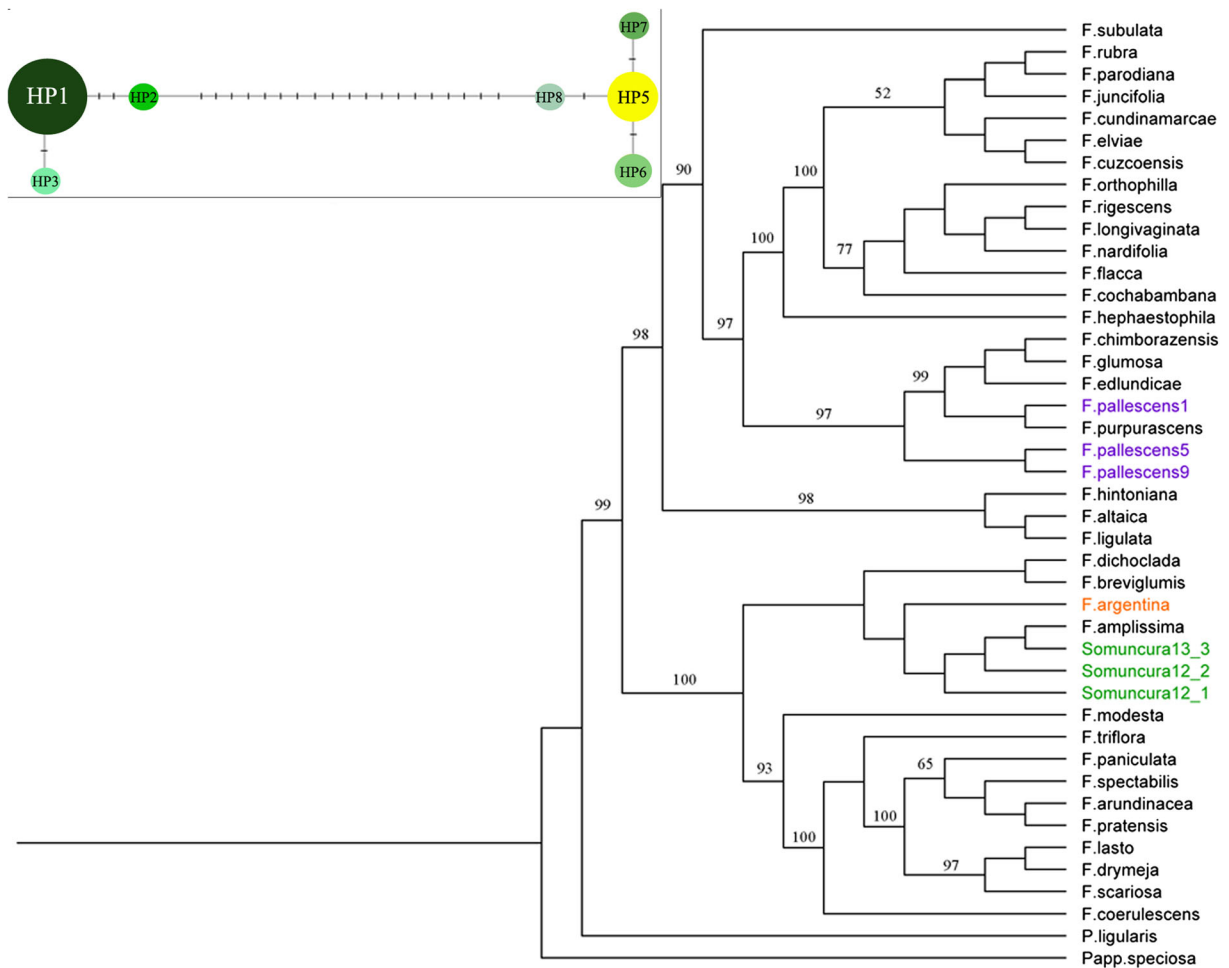

Fig. 3 Bayesian tree based on plastid (*trn*L-F) marker. The taxa under study are coloured violet (*F. pallescens*), green (Somuncura) and orange (*F. argentina*). Support values (posterior probability) are indicated above branches. Posterior probability support values lower that 50% are not shown. * *Festuca subantartica* has the same haplotype as *F. pallescens 1*. The **top-left inset** shows the haplotype network.

respectively (inset in Fig. 3). The combined data set (ITS_*trn*L-F concatenated data) showed a topology similar to the organelle tree (Fig. S3).

Microsatellite loci

All the analyses performed with the microsatellite data set were consistent showing four well-defined groups. Individuals of *Festuca argentina* and *Festuca pallescens* were very different from each other in agreement with phylogenetic analyses. Interestingly, these markers allowed, in addition, the separation of the two populations of Somuncura. Individuals from population 12 were closer to *F. argentina* while individuals from population 13 were situated at an intermediate position between *F. argentina* and *F. pallescen*s in both the PCoA and the Neighbour joining tree (Fig. 4).

The clustering performed with Structure detected the best partition at $K = 2$ (according to the method by Evanno et al. 2005) (Fig. S4). However, meanLnK reaches the plateau at $K = 3$ and $K = 4$; therefore, we show the three clustering results (Fig. 4). Clearly the best partition identifies the two species, but also shows admixture in population 13 from Somuncura while population 12 was genetically identical to *F. argentina*. At $K = 3$, populations 13 of Somuncura formed an independent cluster
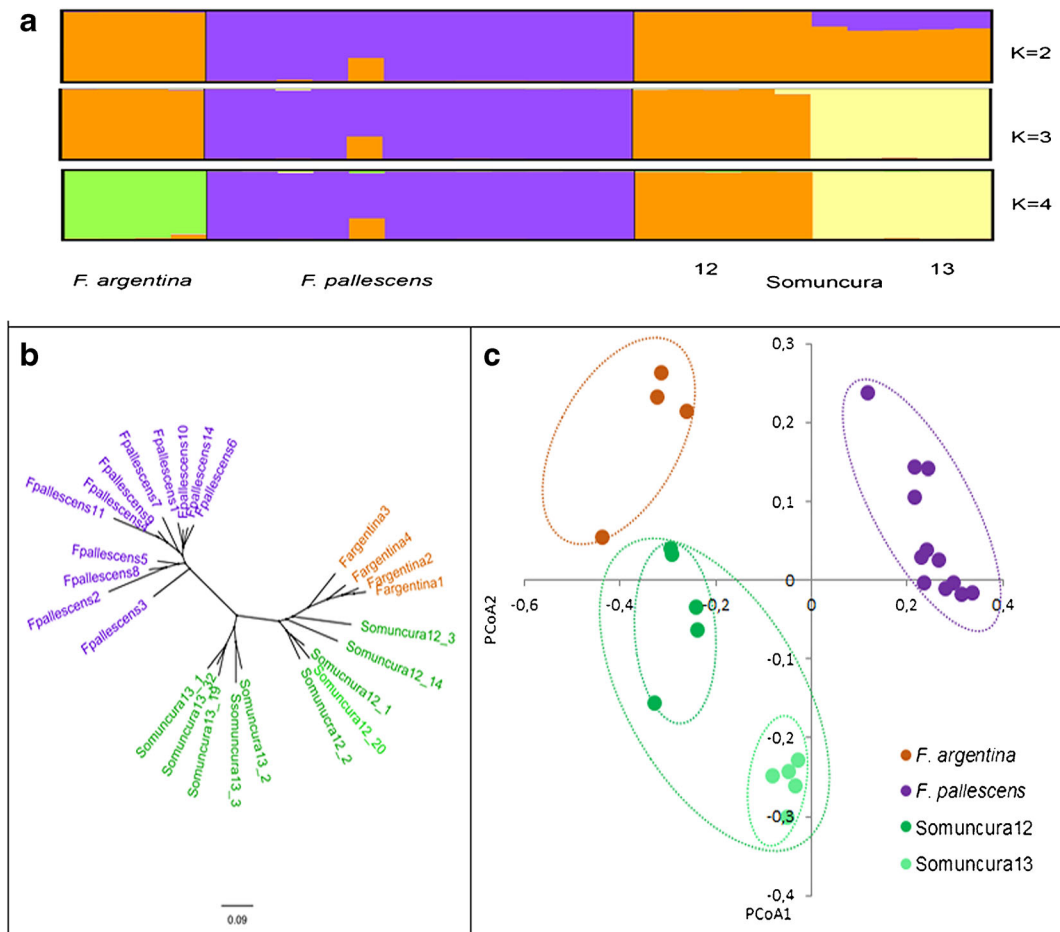

Fig. 4 Analyses with microsatellite loci. **a** – Assignment of the individuals to each of the different clusters ($k = 2$ to $k = 4$) obtained with STRUCTURE; **b** – Radial neighbour-joining tree based in Bruvo distance calculated with the 'polysat' package in R; **c** – Principal coordinates analysis based on Bruvo distance for microsatellite loci. Four groups can be distinguished: Individuals of *F. pallescens* are coloured in violet, *F. argentina* in orange, and Somuncura in dark green (population 12) and light-green (population 13).

whereas population 12 clustered with *F. argentina*. Finally, at $K = 4$ the same four groups as in the PCoA are recovered.

The closeness of population 13 to *F. pallescens* is ought to the presence of a larger proportion of shared alleles (Fig. S4). In general, individuals of Somuncura at some microsatellite loci have at least one allele that was predominant in *F. pallescens* and another that was characteristic of *F. argentina*, indicating possible events of past hybridization between these species. In addition, private alleles were found in both populations of Somuncura.

Discussion

Genetic diversity within peripheral populations

We detected high genetic variation with the three analysed markers within two of the populations sampled at the marginal sites in Somuncura plateau. Peripheral populations usually hold lower levels of genetic diversity (Eckert et al. 2008) because their isolation leads to increasing possibilities of inbreeding and genetic drift. In our study, the conserved ITS region and the plastid marker depicted high levels of variation at these locations, and private alleles were detected with the SSRs. Although concerted evolution is believed to reduce intragenomic variation among copies within the ITS tandem arrays (Liao 1999), some variation still might exist. Therefore, the high level of polymorphism detected among *F. pallescens* from marginal Somuncura populations is outstanding.

Festuca pallescens and *F. argentina* have distinct morphological traits that allow their identification (Catalán and Müller 2012), but they hold large phenotypic variation (Parodi 1953; Nicora 1978; Bertiller et al. 1990; Oliva et al. 1993) that could make them difficult to distinguish. Besides, distinct ecological features characterize each species. *Festuca pallescens* forms large rangelands in Patagonia (e.g. high steppes or grasslands in meadows), reaching high altitudes (0 to 1,800 m asl; Catalán and Müller 2012), while *F. argentina* is generally found in patches within several types of steppes (e.g. shrub-grassy steppes of *Mulinum spinosum* and *Poa ligularis,* or grassy steppes of *F. pallescens* – Bran et al. 2000), usually at altitudes between 270 and 700 m asl (Catalán and Müller 2012). Therefore, the smaller population sizes could contribute to a stronger genetic

drift effect and a higher genetic differentiation in *F. argentina*. In addition, towards the east of the west-east oriented transect, *F. pallescens* populations occur less frequently due to the decline of meadows, being found at high altitudes, where moist conditions prevail (Bertiller et al. 1990; Gaitán et al. 2010). Accordingly, Somuncura populations formed small grass-steppes in these drylands, with scattered individuals within the community that were isolated from other grass steppes.

Phylogenetic relationships and genetic distances indicate that Somuncura populations are closer to the sympatric species *F. argentina* than to other *Festuca pallescens* populations. However, microsatellites allowed a clear distinction from each of the two species as well as between the two populations. Moreover, a considerable degree of admixture was detected in one of the populations, suggesting hybridization (Fig. 4). In addition, morphoanatomical results showed some intermediate traits in the populations of Somuncura together with characters that resemble one of each of the two sympatric species. Furthermore, *F. pallescens* populations from North Patagonia are genetically uniform since a single identical ITS sequence was observed in all samples. On the other hand, Somuncura population are highly variable, showing six ITS sequences among 10 individuals. We also detected a high level of polymorphism in *F. argentina*: Each of the four sampled individuals had a different sequence and we did not find shared chloroplast haplotypes between *F. pallescens* and Somuncura.

In view of the level of differentiation detected in the populations of Somuncura, we might propose three possible alternatives: (a) Somuncura populations are in fact *F. argentina,* (b) they are an ecotype of *F. argentina,* or (c) they are hybrids between *F. pallescens* and *F. argentina* and might be in the process of speciation. Supporting our first hypothesis is the close genetic relationship: all the phylogenetic analyses clustered Somuncura together with *F. argentina* or constituting a polytomy. In addition, the PCoA based on microsatellite data grouped population 12 very closely to *F. argentina* and individuals of Somuncura shared several morphoanatomical traits with *F. argentina*. However, not all the morphoanatomical traits were similar (in fact some resulted intermediate with *F. pallescens*), and we found four haplotypes and a large genetic variation in the nuclear marker among Somuncura individuals. An outstanding difference is the morphology of the lemma: *Festuca argentina* differs from other species of the

genus because of its muticous lemmas (Dubcovsky and Martínez 1988; Catalán and Müller 2012), and Somuncura lemmas had a diminutive awn at lemma apex. This led us to propose our second hypothesis that Somuncura could actually be an ecotype of *F. argentina*, genetically differentiated and morphologically similar to *F. pallescens*. Supporting this alternative hypothesis is the consistent clustering obtained with STRUCTURE, PCoA and Neighbour Joining using the microsatellite loci (Fig. 4) that clearly separated Somuncura populations. Geographically isolated areas lacking competitors could promote the production of adaptive radiations (Simpson 1953, Straud and Losos 2016). In fact, the biogeographic isolation of Somuncura plateau promoted the evolution of different taxa (León et al. 1998; Chebez 2005; Andrade and Monjeau 2014). Therefore, Somuncura populations might be a novel ecotype of *F. argentina* developed in this isolated environment. In order to radiate, a clade should develop 'key innovations' (Straud and Losos 2016) that provide the ability to adapt taking advantage of the available resources. As an example, Somuncura populations presented some morphoanatomical traits, such as pulvinus at the base of the lamina and a higher proportion of sclerenchyma under abaxial epidermis than *F. argentina*. Sclerenchyma is a specialized tissue that provides strength and support to leaves, especially in arid conditions with periods of strong water deficit that produce loss of cell turgence, such as the dry, windy environments of Somuncura.

Finally, the presence of shared polymorphic sites between Somuncura and *F. pallescen*s, the heterozygous positions between *F. pallescens* and *F. argentina*, the shared alleles at microsatellite loci and the intermediated morphoanatomical traits, suggests a possible hybridization process. The closeness of Somuncura populations to *F. argentina* could be due to a long-lasting backcrossing towards this species. Somuncura populations occur in steppes at 900–1,100 m asl where *F. pallescens* is abundant (Andrade and Monjeau 2014). On the contrary, *F. argentina* is a dominant species in shallow soil steppes at lower elevations (e.g. slopes of the plateau; León et al. 1998; D. Bran, personal communication). Therefore, this difference in altitudinal zonation among the predominant niches of each species might currently create a barrier to reproduction, but in the past their distributions could have overlapped, promoting hybridization and backcrosses between the hybrid and *F. argentina*. In spite of being outcrossing

species, the differences in ploidy levels (*F. pallescens* 6x; *F. argentina* 4x – Dubcovsky and Martínez 1991, 1992) might limit their successful reproduction. Interspecific hybridization between species with different ploidy levels is uncommon, but possible and it was reported in this genus. For example, *F. arundinacea* (allohexaploid) is the result of the cross between the diploid *F. pratensis* and the allotetraploid *F. fenas* (Inda et al. 2014). In addition, the Lolium-Festuca complex, obtained from more distantly related ryegrass and fescue species, proved to be a useful combination of traits and resulted in stable and successful commercial varieties (Thomas and Humphreys 1991). Another example is the pentaploid *Paspalum durifolium*, which shares intermediate morphological and reproductive characters with its tetraploid and hexaploid progenitors, but produces a small amount of seeds, mainly due to genetic instability after irregular meiotic behaviour (Quarín and Caponio 1995).

Phylogenetic analyses do not fully support that *F. pallescens* and *F. argentina* would be the parental species of the putative hybrid, and we cannot discard the existence of other non-sampled potential parental species in Somuncura. Besides, even though *F. pallescens* and *F. argentina* coexists at some locations along their natural distribution ranges, to the best of our knowledge, natural hybridization has never been reported. Cytogenetic studies are necessary to further evaluating the possible hybridization and introgression between the two fescues. Therefore, with the evidence of our data, we suggest that Somuncura populations could be an ecotype of *F. argentina* originated by an ancient hybridization and subsequent introgression, that generally differs exomorphologically in its glaucous leaves, lemmas with short awns and presence of pulvinus in leaf base, in which characters it is similar to *F. pallescens*.

Festuca pallescens and Somuncura Populations within the *Festuca* sp. phylogeny

The genus *Festuca* has been studied for a long time (Torrecilla and Catalán 2002; Torrecilla et al. 2003; Catalán et al. 2004; Inda et al. 2008; Minaya et al. 2017), but knowledge about phylogenetic relationships of Patagonian fescues is scarce (Oliva et al. 1993), and particularly *F. pallescens* was never included in the published phylogenies. Most studies in Patagonian fescues focused on cytogenetics (Dubcovsky and Martínez 1988, 1991, 1992) and physiology (Bertiller et al. 1990;

Bertiller and Defossé 1990). Therefore, our results provide novel information about the genetic relationships of *F. pallescens* with the rest of the studied South American fescues.

The state-of-the-art phylogeny of *Festuca* recognizes 23 clades (Inda et al. 2014). The Patagonian species *F. subantartica* and *F. purpurascens* belong to America I clade (Inda et al. 2008) as part of the *fine leaved* clade (Catalán et al. 2004). Our results showed that *F. pallescens* is strongly related to these two species as they formed a sister clade in the nuclear Bayesian topology and constituted a polytomy (i.e. high genetic similarity) in MP topology. However, these results are not concordant with phenetic and cytological studies (Dubcovsky and Martínez 1988, 1991) which do not relate *F. pallescens* to *F. purpurascens*. Moreover, the three species have different but overlapping distribution areas: *F. subantartica* was described in Lago Argentino, Santa Cruz (Inda et al. 2008), *F. purpurascens* is found in the Andes, from Neuquén to Santa Cruz, and *F. pallescens* occurs from Neuquén to Coyle River in Santa Cruz (Nicora 1978). Increasing sampling of Patagonian fescues could help to resolve the polytomy.

On the other hand, the Somuncura-*F. argentina* clade is closely related to the Asian-American clade, specially to North-American fescues, and very distant from currently studied Patagonian and Andean fescues (Inda et al. 2008). Also, Somuncura and *F. argentina* formed a polytomy, which could be due to the genetic similarities between the sequences, the existence of hybridization that triggers adaptive radiation (Seehausen 2004) or the lack of concerted evolution (Bailey et al. 2003). Somuncura and *F. argentina* are more closely related to some North-American fescues (*F. subulata*, *F. dichoclada*, *F. kingii* and *F. altaica*; Fig. 2). They are also associated with *F. breviglumis* and *F. amplissima* (both from Mexico) as well as to *F. modesta* (an Asian fescue; Fig. 3). Moreover, *F. argentina* has been classified as a *broad-leaved* fescue (see Inda et al. 2008 and references within). However, Somuncura and *F. argentina* were also closed to *F. subulata* and *F. altaica*. These fescues were described as *broad-to-fine-leaved* fescues representing a transition between the two main groups. The broad-leaved trait seems to be a plesiomorphic character (Catalán et al. 2007) suggesting either an ancient origin of *Festuca*

argentina or a recent species with an ancestral character. Broad-leaved species undergo multiple radiations from the center of origin that involved differentiation from its ancestors due to climatic changes that occurred 12 My ago (Inda et al. 2008). Arid and semi-arid regions of Argentina are susceptible to climatic fluctuation (Zárate and Tripaldi 2012), and the environmental constrains in Somuncura plateau could have promoted the high genetic variation detected within those populations and triggered a speciation process in the Somuncura-*F. argentina* clade. On the other hand, hybridization and polyploidization events allowed the colonization of newly arid areas during the glacials and interglacials periods of the Pleistocene in the South American hemisphere (Dubcovsky and Martínez 1992; Hewitt 1996). Although, sampling of this work is restricted to North-Patagonia, it could be possible that *F. pallescens* had originated during that geological period in Patagonia, as it has been proposed for the polyploid American clades I and II (Inda et al. 2008).

Conclusion

The high genetic variability observed in two populations within the protected area at Somuncura plateau might be indicating the existence of evolutionary processes that could trigger events of speciation in the Patagonian fescues. In spite of having intermediate morphoanatomical traits and shared alleles with *F. pallescens,* most evidences indicate that Somuncura populations do not correspond to *F. pallescens*. Although more studies are needed to unravel the phylogenetic relationships among the Patagonian fescues, Somuncura populations might be an ancient introgression that lead to a morphologically and genetically differentiated ecotype of *F. argentina* (i.e. a narrow endemism; Kruckeberg and Rabinowitz 1985). Our results also suggest that an incipient speciation process within the genus could be occurring in this isolated biogeographical island. The correct identification of field specimens is clearly mandatory for conservation and management purposes, especially for forage species that are an invaluable resource in natural rangelands of Patagonia. Here we provide a tool to help in the molecular identification of the key species *F. pallescens*.

References

Aguiar MR, León RJ (1985) Sheep grazing causes important disturbances in western Patagonian semi-arid grasslands. *Phytocoenologia* 13:181–196

Andrade A, Monjeau A (2014) Patterns in community assemblage and species richness of small mammals across an altitudinal gradient in semi-arid Patagonia, Argentina. *J Arid Environm* 106:18–26

Asner GP, Elmore AJ, Olander LP, Martin RE, Harris AT (2004) Grazing systems, ecosystem responses, and global change. *Annual Rev Environm Res* 29:261–299

Bailey CD, Carr TG, Harris SA, Hughes CE (2003) Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molec Phylogen Evol* 29:435–455

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molec Biol Evol* 16:37–48

Bertiller MB (1990) Grazing effects on sustainable semiarid rangeiands in Patagonia: the state and dynamics of the soil seed bank. *Environm Managem* 20:123–132

Bertiller MB, Defossé GE (1990) Grazing and plant growth interactions in a semiarid *Festuca pallescens* grassland (Patagonia). *J Range Managem* 43:300–303

Bertiller MB, Elissalde NO, Rostagno CM, Defossé GE (1993) Environmental patterns and plant distribution along a precipitation gradient in western Patagonia. *J Arid Environm* 29:85–97

Bertiller MB, Irisarri MP, Ares JO (1990) Phenology of *Festuca pallescens* in Relation to Topography in North-Western Patagonia. *J Veg Sci* 1:579–584

Bran D, Ayesa J, López C (2000) *Regiones ecológicas de Río Negro*. Comunicación Técnica Nº 59 EEA INTA San Carlos de Bariloche – Río Negro, Argentina

Breitman MF, Martinez RJN, Avila LJ, Sites JW, Morando M (2015) Phylogeography and morphological variation of the northernmost distributed species of the *Liolaemus lineomaculatus* section (Liolaemini) from Patagonia. *Amphibia-Reptilia* 36:373–387

Bruvo R, Michiels NK, D'Souza TG, Schulenburg H (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molec Ecol* 13:2101–2106

Busso CA, Bonvissuto GL (2009) Soil seed bank in and between vegetation patches in arid Patagonia, Argentina. *Environm Exp Bot* 67:188–195

Burkart R, Bárbaro NO, Sánchez RO, Gómez DA (1999) Ecoregiones de la Argentina. *Administración de Parques Nacionales*, Argentina. 43pp

Catalán P, Müller J (2012) *Festuca* L. In Zuloaga FO, Rugolo ZE, Anton AMR (eds) *Flora de Argentina*, vol 3, (II). CONICET, Buenos Aires, Argentina, pp 219–250

Catalán P, Torrecilla P, Lopez-Rodriguez JA, Muller J, Stace CA (2007) A systematic approach to subtribe Loliinae (Poaceae: Pooideae) based on phylogenetic evidence. *Aliso: J Syst Evol Bot* 23:380–405

Catalán P, Torrecilla P, Rodríguez JÁL, Olmstead RG (2004) Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and trnL–F sequences. *Molec Phylogenet Evol* 31:517–541

Cei, J. M. (1969) The patagonian telmatobiid fauna of the volcanic Somuncura Plateau of Argentina. *J Herpetol* 3:1–18

Cei JM, Scolaro JA (1981) A new northern subspecies of *Liolaemus kingi* in Argentina. *J Herpetol* 15:207–210

Chapin III FS (1993) Functional role of growth forms in ecosystem and global processes. *Scaling Physiol. Process Leaf Globe* 287–312

Chebez JC (2005) Guia de las reservas Patagonia Norte I, Ed. Albatros

Clark LV, Jasieniuk M (2011) Polysat: an R package for polyploid microsatellite analysis. *Molec Ecol Resources* 11:562–566

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature, Meth* 9:772–772

Davies J, Poulsen L, Schulte-Herbrüggen B, Mackinnon K, Crawhall N, Henwood WD, et al (2012) Conserving Dryland Biodiversity. pp 84

Dixon CJ (2010) OLFinder - a program which disentangles DNA sequences containing heterozygous indels. *Molec Ecol Resources* 10:335–340

Doyle J, Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15

Dubcovsky J, Martínez A (1988) Phenetic relationships in the *Festuca* spp. from Patagonia. *Canad J Bot* 66:468–478

Dubcovsky J, Martínez AJ (1991) Chromosome complement and nucleoli in the *Festuca pallescens* alliance from South America. *Canad J Bot* 69:2756–2761

Dubcovsky J, Martínez AJ (1992) Cytotaxonomy of the *Festuca* spp. from Patagonia. *Canad J Bot* 70:1134–1140

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resources* 4:359–361

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molec Ecol* 17:1170–1188

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molec Ecol* 14:2611–2620

Flint RF, Fidalgo F (1964) Glacial geology of the East flank of the Argentine Andes between Latitude 39°10′S. and Latitude 41°20′S. *Bull Geol Soc Amer* 75:335–352

Fu YB, Qiu J, Peterson GW, Willms WD, Wilmshurst JF (2006) Characterization of microsatellite markers for rough fescue species (*Festuca* spp.). *Molec Ecol Notes* 6:894–896

Gaitán JJ, López CR, Bran DE (2010) Vegetation composition and its relationship with the environment in mallines of north Patagonia, Argentina. *Wetlands Ecol Managem* 19:121–130

Golluscio RA, Deregibus VA, Paruelo JM (1998) Sustainability and range management in the Patagonian steppes. *Ecol Austral* 8:265–284

Gonzalo-Turpin H, Hazard L (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *J Ecol* 97:742–751

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series. pp 95–98

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol J Linn Soc* 58:247–276

Hijmans RJ, Cameron SE, Parra JL, Jones PG and Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978

Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives. *Trends Ecol Evol* 9:223–227

Hoffmann AA, Hercus MJ (2000) Environmental stress as an evolutionary force. *BioScience* 50:217

Inda LA, Sanmartín I, Buerki S, Catalán P (2014) Mediterranean origin and Miocene-Holocene Old World diversification of meadow fescues and ryegrasses (*Festuca* subgenus *Schedonorus* and *Lolium*). *J Biogeogr* 41:600–614

Inda LA, Segarra-Moragues JG, Müller J, Peterson PM, Catalán P (2008) Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. *Molec Phylogen Evol* 46:932–957

Jensen LB, Holm PB, Lübberstedt T (2007) Cross-species amplification of 105 *Lolium perenne* SSR loci in 23 species within the Poaceae. *Molec Ecol Notes* 7:1155–1161

Johansen-Morris AD, Latta RG (2008) Genotype by environment interactions for fitness in hybrid genotypes of *Avena barbata*. Evol. *Int J Organic Evol* 62:573–585

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molec Ecol Resources* 15:1179–1191

Kruckeberg AR, Rabinowitz D (1985) Biological aspects of endemism in higher plants. *Annual Rev Ecol Syst* 16:447–479

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30:3276–3278

Lauvergeat V, Barre P, Bonnet M, Ghesquiere M (2005) Sixty simple sequence repeat markers for use in the *Festuca–Lolium* complex of grasses. *Molec Ecol Notes* 5:401–405

León RJ, Bran D, Collantes M, Paruelo JM, Soriano A (1998) Grandes unidades de vegetación de la Patagonia extra andina. *Ecol Austral* 8:125–144

Liao D (1999) Concerted evolution: molecular mechanism and biological implications. *Amer J Human Genet* 64:24–30

López A, Siffredi GL, Becker G, López DR, Marchelli P (2015) Assessing genetic diversity in *Festuca pallescens* along a rainfall gradient in Nord Patagonia. In *5th International Congress of Forage Breeding*. October 2015. Buenos Aires, Argentina

Maestre FT, Salguero-Gómez R, Quero JL (2012) It is getting hotter in here: determining and projecting the impacts of global environmental change on drylands. *Philos Trans, Ser B* 367:3062–3075

Malumián N, Náñez C (2011) The Late Cretaceous-Cenozoic transgressions in Patagonia and the Fuegian Andes: foraminifera, palaeoecology, and palaeogeography. *Biol J Linn Soc* 103:269–288

Martínez-Palacios A, Eguiarte LE, Furnier GR (1999) Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan Desert. *Amer J Bot* 86:1093–1098

Millennium Ecosystem Assessment Panel (2005) *Ecosystems and human well-being*. Washington DC

Minaya M, Hackel J, Namaganda M, Brochmann C, Vorontsova MS, Besnard G, Catalán P (2017) Contrasting dispersal histories of broad- and fine-leaved temperate Loliinae grasses: range expansion, founder events, and the roles of distance and barriers *J Biogeogr* 4:1980–1993

Muzón J, Spinelli GR, Pessacq P, Von Ellenrieder N, Estevez AL, Marino PI, Pérez Goodwyn PJ, Angrisano EB, Díaz F, Fernández LA (2005) Insectos acuáticos de la meseta del Somuncurá, Patagonia, Argentina. Inventario preliminar. *Rev Soc Entomol Argent* 64:47–68

Nicora, E G (1978) Gramineae In Correa MN (ed.) *Flora patagónica 3*. Colección Científica del INTA, Instituto Nacional de Tecnología Agropecuaria. ed. Buenos Aires, Argentina

Oliva G, Martínez A, Collantes M, Dubcovsky J (1993) Phenotypic plasticity and contrasting habitat colonization in *Festuca pallescens*. *Canad J Bot* 71:970–977

Palazzesi L, Barreda VD, Cuitiño JI, Guler MV, Tellería MC, Ventura Santos R (2014) Fossil pollen records indicate that Patagonian desertification was not solely a consequence of Andean uplift. *Nature, Commun* 5

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290

Parodi, L.R. 1953. Las especies de Festuca de la Patagonia. *Revista Argent Agron* 20:177–229

Paruelo J, Bertiller M, Schlichter T, Coronato F (1993) *Secuencias de deterioro en distintos ambientes patagónicos: Su caracterización mediante el modelo de estados y transiciones*. San Carlos de Bariloche: INTA-GTZ Proyecto LUDEPA-SME

Pastorino MJ, Gallo LA (2002) Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean-Patagonian forest. *J Biogeogr* 29:1167–1178

Polzin T, Daneschmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operat Res Lett* 31:12–20

Porras-Hurtado L, Ruiz Y, Santos C, Phillips C, Carracedo A, Lareu M (2013) An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in Genetics*, 4(98). doi:10.3389/fgene.2013.00098

Posada D (2008) jModelTest: Phylogenetic Model Averaging. *Molec Biol Evol* 25:1253–1256

Premoli AC, Mathiasen P, Acosta MC, Ramos VA (2012) Phylogeographically concordant chloroplast DNA divergence in sympatric *Nothofagus* s.s. How deep can it be? *New Phytol* 193:261–275

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155: 945-959

Quarín CL, Caponio I (1995) Cytogenetics and reproduction of *Paspalum dasypleurum* and its hybrids with *P. urvillei* and *P. dilatatum* ssp. *flavescens*. *Int J Pl Sci* 156:232–235

Rabassa J (2008) Late Cenozoic Glaciations in Patagonia and Tierra del Fuego. In J. Rabassa (Ed.), *Developments in Quaternary Sciences*, 11: 151-204

Rabassa J, Coronato A, Martinez O (2011) Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. *Biol J Linn Soc* 103:316–335

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org

Reynolds JF, Smith DMS, Lambin EF, Turner BL, Mortimore M, Batterbury SPJ, et al. (2007) Global desertification: building a science for dryland development. *Science* 316:847–851

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542

Sala OE, Stuart Chapin F, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson R B, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff N, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global Biodiversity Scenarios for the Year 2100. *Science*, 287 (5459): 1770-1774. doi:10.1126/science.287.5459.1770

Seehausen O (2004) Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198–207

Simpson GG (1953) *The major features of evolution*. New York: Columbia Univ. Press

Straud JT and Losos JB (2016) Ecological opportunity and adaptive radiation. *Annual Rev Ecol Evol Syst* 47:507–32

Stucky BJ (2012) SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *J Biomolec Technol* 23: 90–93

Swofford DL (2003) PAUP. Phylogenetic analysis using parsimony (and other methods). Version 4. Sinauer Associates

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl Molec Biol* 17:1105–1109

Thomas H, Humphreys MO (1991) Progress and potential of interspecific hybrids of *Lolium* and *Festuca*. *J Agric Sci* 117:1–8

Thompson JD, Gaudeul M, Debussche M (2010) Conservation value of sites of hybridization in peripheral populations of rare plant species. *Conservation Biol* 24:236–245

Torrecilla P, Catalán P (2002) Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. *Syst Bot* 27:241–251

Torrecilla P, Rodríguez JAL, Stančík D, Catalán P (2003) Systematics of *Festuca*. *Pl Syst Evol* 239:113–139

Zárate MA, Tripaldi A (2012) The aeolian system of central Argentina. *Aeolian Res* 3:401–417

Wright S, Keeling J, Gillman L (2006) The road from Santa Rosalia: a faster tempo of evolution in tropical climates *Proc Natl Acad Sci USA* 103:7718–7722