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Genetic Diversity and Structure in *Austrocedrus chilensis* Populations: Implications for Dryland Forest Restoration

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Abstract

In South America, 94% of dry-temperate lands present some degree of environmental degradation, highlighting the need for ecological restoration. We analyzed geographic patterns of genetic variation in *Austrocedrus chilensis*, a dominant conifer of the steppe-forest ecotone in the eastern Andes, to examine its potential for restoration. We sampled 67 locations in Argentina and estimated genetic parameters to determine the effects of historical factors affecting diversity, together with inbreeding and gene flow, using 12 allozyme loci. Genetic diversity decreased southwards in eastern populations, which are marginal for the range of the species and patchily distributed, while high genetic admixture was detected in continuous western populations, possibly reflecting postglacial migrations from northern and eastern sources. Higher inbreeding ($F_{IS} > 0.14$) was recorded in northern compared with southern populations, attributed to the impact of recent bottlenecks resulting from anthropogenic fires. Gene flow was found to be moderate overall ($F_{ST} = 0.12$). The implications of these results for restoration actions focusing on *Austrocedrus* were explored. Relatively small, inbred yet genetically diverse northern populations should be the subject of passive restoration efforts, while experimental common gardens should be established toward the south, to support active restoration approaches. This illustrates how ahead of time information on patterns of genetic variation can support restoration efforts for dryland tree species.

Key words: beyond-range restoration, fire, glaciations, passive restoration, Patagonia.

Introduction

Understanding patterns of genetic variation of individuals and populations provides an important basis for ecological restoration actions (Väli et al. 2008). Therefore, evaluations of within-population diversity, inbreeding, and the degree of genetic divergence among populations can be of value to restoration projects if the goal is to promote the establishment of self-sustaining populations (Falk et al. 2006). Such a goal may include retaining local gene pools (Rice & Emery 2003), maintaining sufficient adaptive genetic variation (Hufford & Mazer 2003), and ameliorating the deleterious effects of inbreeding in small or marginal populations (Frankel & Soulé 1981; Lande 1988; Reed et al. 2003; Frankham 2005).

Drylands comprise 30% of the earth's surface. In South America, 94% of these dry-temperate lands have seen some degree of degradation or desertification (UNDP 2004), indicating the widespread need for ecological restoration. Such restoration could potentially involve re-establishment of native forest cover. One of the most widespread and drought-tolerant trees of the southern Andes is *Austrocedrus chilensis* (D. Don) Florin & Boutelje, a monotypic genus of the Cuppresaceae. Under the higher precipitation regimes of the western Andes, this species forms mixed continuous forests, whereas toward the drier eastern edge of its distribution, it occurs in sparse patches in which it is often monodominant. Areas along the west-east forest-steppe ecotone in southern Argentina have traditionally been viewed by foresters and land managers as barren lands unable to support native forest. As a result, they have been used for extensive sheep and cattle ranching, or the establishment of exotic (mostly pine) plantations, which have had negative impacts on native dry forests (Veblen et al. 2008). This may be compounded by climate change, while models forecast a mean temperature increase of 2–4°C by 2100 for all of South America, large summer precipitation reductions (25–40%) are predicted in the southern Patagonian Andes (IPCC 2007). *Austrocedrus* would be a candidate species for use in attempts to re-vegetate areas threatened by such

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anthropogenic processes, particularly under expected scenarios of climate change.

This investigation focuses on the patterns of genetic variation in the context of developing plans for restoring *Austrocedrus* forests. Previous research has failed to identify a consistent pattern of genetic variation in *Austrocedrus*. Little genetic structure was documented in a previous study employing isozymes ($F_{ST} = 0.060$) (Pastorino & Gallo 2009). Although, relatively high phenotypic plasticity was suggested for morphological traits (Gyenge et al. 2005; Pastorino et al. 2010), and genetically controlled differences driven by temperature were found in cuticular lipids (Dodd & Afzal-Rafii 2000). At the level of individual populations, relatively high isozyme and microsatellite diversity was detected in eastern *Austrocedrus* populations, which was interpreted as survival in local refugia during ice ages (Pastorino & Gallo 2009; Arana et al. 2010). However, a biogeographic study of the species' entire range, provided evidence of elevated genetic diversity and the presence of unique alleles, suggesting long-lasting persistence in ice-free areas, on western slopes of the Andes. In addition, suggested that high genetic diversity in eastern-most populations maybe explained as the survival of *Austrocedrus* in fire refugia during late Holocene and postglacial colonization routes (Premoli et al. 2011).

The objectives of this investigation were (1) to determine the genetic patterns of within-population diversity, inbreeding and among-population divergence throughout the entire natural range of *Austrocedrus* in the eastern Andes, and (2) to identify whether any population bottlenecks may have occurred in the past. Eastern *Austrocedrus* populations are relatively small and isolated, and are subjected to higher fire frequency than those situated further west, which are larger and more continuous populations, suggesting that the former may be of particularly high priority for conservation and restoration actions. As *Austrocedrus* is endemic to the region and is considered globally threatened (IUCN 2010) and given that climate change may alter its range and/or population sizes, it will likely be a future focus of restoration activities. This study also explores how knowledge of genetic structure, past history, and susceptibility to disturbance can contribute to the design of appropriate restoration strategies.

Methods

Species and Habitat

Austrocedrus chilensis is a native dioecious conifer of a monotypic genus, with wind-dispersed pollen and winged seeds. Occurring on the eastern slopes of the Andes in southern Argentina, it is distributed discontinuously in the north of its range (from 36°30′ to 39°30′S) and more continuously and extensively southward (from 39°30′ to 43°35′S) (Seibert 1982). Over the natural range of the species, most of the precipitation occurs during autumn and winter, with the drier period being during the summer, although there is a N–S gradient of increasing precipitation. At the center of *Austrocedrus*' range, the rain shadow effect of the Andes is associated with a W–E natural fragmentation gradient. Thus, mean annual precipitation declines from c. 2,500 mm at the continental divide to less than 500 mm only 100 km to the east in the steppe (Barros et al. 1983). As precipitation declines eastwards, aridity increases, and the plant density and stature reduce. At xeric sites (<1,000 mm/yr of precipitation), natural regeneration is highly restricted, both temporally and spatially.

Austrocedrus chilensis is of international conservation concern and is listed as Vulnerable, which means that is facing a high risk of extinction in the wild as a result in a decline in its area of distribution (IUCN 2010). The species has been negatively affected by land use patterns, including logging, use of fire, and livestock grazing. Especially at the eastern edge of its range, introduced herbivores such as European hares, rabbits, and exotic deer are also negatively affecting *Austrocedrus* establishment and survival (Veblen et al. 1999).

Sampling

This study was conducted in the forest-steppe ecotone on the eastern slopes of the Patagonian Andes, Argentina, between 37° and 43°S, covering an area of more than 5° in latitude and 1° in longitude. To identify the broad-scale trends in genetic differentiation throughout dryland forest of *Austrocedrus*, sampled populations were combined into three arbitrary regions (north, center, and south hereafter N, C, and S), according to their geographical location. Also, we subdivided each region into continuous and marginal stands to consider the natural precipitation gradient and forest patch size, which decrease in eastern marginal populations. Fresh leaf tissue was collected from 20 to 30 randomly selected adult trees in each of 67 natural populations, giving a total of 1,853 individuals (Fig. S1 and Appendix S1, Supporting Information). Samples consisted of 20-cm long terminal twigs of fresh leaf tissue collected from each tree, which were kept in a portable cooler until arrival to the laboratory.

Laboratory Analysis

Enzymes were extracted with the buffer of Mitton et al. (1979). Homogenates were stored at −80°C until they were absorbed onto Whatman No. 3 paper wicks that were loaded into 12% w/v starch gels. Protocols developed for *Austrocedrus* yielded only five loci (Pastorino & Gallo 2009). Therefore, we increased the number of polymorphic loci. Horizontal electrophoresis was conducted using three systems: two morpholine-citrate gel and electrobuffer: one at pH 7.5 (Ranker et al. 1989), and the other at pH 6.1 (Clayton & Treliak 1972), both running for 6 hour at 30 mA, and a tris–citrate gel and electrobuffer pH 6.2 by Adams and Joly (1980). Anodal, and in the case of one enzyme cathodal, slices were cut horizontally and stained for enzyme activity using the agarose-staining methods of Mitton et al. (1979) and Soltis et al. (1983). Eight enzyme systems resolved 12 loci. These were glycerate 2 dehydrogenase (G2D), isocitrate dehydrogenase (Idh), malate dehydrogenase (Mdh1, Mdh2), malic enzyme (Me1, Me2), cathodal peroxidase (Percat), phosphoglucoisomerase (Pgi1,

Pgi2), 6-phosphogluconate dehydrogenase (6Pgd1, 6Pgd2), and shikimate dehydrogenase (Skdh). The scoring of isozyme genotypes consisted of assigning consecutive numbers so that the most anodal locus and/or allele were designated with the lowest numeral. Loci are considered putative as no genetic analysis was performed, although gel banding patterns and interpretation of results were similar to those obtained in other plant species (Murphy et al. 1996).

Data Analysis

Genetic diversity was analyzed at the population and region level. The N region consists of 23 populations including 619 individuals located between 37 and 39°S latitude characterized by dry climatic conditions and low vegetation cover. C includes 25 populations and 710 sampled individuals from 40 to 41°S, where the gradient of continuous to fragmented forest at the eastern margin is more evident. S comprises 19 populations totaling 524 individuals located between 41 and 43°S, where precipitation and size of forest patches are relatively high and the mean tree age is relatively low (K. Heinemann 2010, Universidad Nacional del Comahue, Argentina, unpublished data).

Genetic Variation. The extent of isozyme variation at the population level was described by standard gene diversity measures using POPGENE v.1.31 (Yeh et al. 1999). These were the mean number of alleles per locus (N_A), mean effective number of alleles (A_E), percentage of polymorphic loci using no criteria ($P_{\%}$), observed (H_O), and expected (H_E) heterozygosity. FSTAT v. 2.9.1 (Goudet 2000) computes allelic richness (r), as average sample size was 28 individuals per population, with sample sizes of most populations exceeding 20 diploid individuals (i.e. $g = 40$ gene copies). We therefore chose to compare allelic richness after rarefaction with a common sample size of $g = 40$.

We assessed the statistical relationships between latitude and genetic diversity parameters by means of linear regressions. Mean and standard error of each genetic diversity parameter were calculated within each region (N, C, S). Differences in these parameters between regions were analyzed by performing separate univariate AMOVA's using GenAlEx with a 999 permutations jackknifing procedure, to determine pairwise significant differences ($p < 0.05$) among regions (Peakall & Smouse 2006).

Genetic Inbreeding and Divergence. We estimated within-population inbreeding (F_{IS}) and then the within region inbreeding by averaging F_{IS} values for the populations within each region. The among-population divergence within and between regions was calculated using F_{ST} (Wright 1965), as the fraction of total genetic variance attributable to differences among populations using FSTAT v. 2.9.1 (Goudet 2000). The mean and 95% confidence intervals (CI) were calculated by jackknifing and bootstrapping over polymorphic loci, respectively.

Bottlenecks. Northern and southern *Austrocedrus* populations differ in the number of individuals. We used the program BOTTLENECK version 1.2.02 (Piry et al. 1999) to test for recent population bottlenecks. This program compares single population H_E with the predicted value for the observed number of alleles under the assumption of the mutation-drift equilibrium model (Ewens 1979), generating a distribution through simulating the coalescent process under the infinite allele model. We used a mode-shift indicator that allows the identification of populations that suffered recent bottlenecks from allele frequency data (Luikart et al. 1998). A one-tailed Wilcoxon signed-rank test was used to identify heterozygosity excess, which has been suggested as the best method to analyze fewer than 20 loci (Piry et al. 1999).

Admixture Analysis. We tested for the presence of genetically homogenous groups of *Austrocedrus* individuals using a Bayesian approach implemented in Structure version 2.1 (Pritchard et al. 2000). The clustering schedule follows a model where each population consists of individuals with distinct allele frequencies. Therefore, the program estimates population allele frequencies and assigns individuals to populations on the basis of their genotypes. A total of 1,853 *Austrocedrus* genotypes were assigned to different number of populations (K) ranging from 1 to 30. For each K value we ran 20 Markov chain Monte Carlo replicates with a 10,000 burn-in period and a run length of 100,000 iterations. We used the admixture ancestry model and the assumption of correlated allele frequencies among samples as suggested in Falush et al. (2003). The number of potential clusters (K) was determined heuristically by the *ad hoc* statistic ΔK, which calculates the rate of change of the log-likelihood of the present data set between consecutive K values, following Evanno et al. (2005). We ran the program Distruct (Rosenberg 2004) to graphically display the results from the Structure analysis.

Isolation By Distance. A Mantel (1967) test was used to evaluate the isolation by distance model and its significance was determined with 9,999 permutations using the program GenAlEx 6.2 (Peakall & Smouse 2006). The pairwise populations linear geographical distances were estimated using the same program.

Results

Genetic Variation

All 12 analyzed loci were polymorphic *sensu stricto*, and four out of a total of 48 alleles were private alleles, that is, those present in just one population (Appendix S1). Within-population genetic diversity parameters decreased southward ($r = -0.479$, -0.486, and -0.312; $p < 0.05$ for the effective number of alleles A_E, gene diversity H_E, and allelic richness r, respectively). This significant trend was more evident for marginal sites (i.e. eastern populations; $r = -0.624$, -0.541, and -0.402; $p < 0.05$ for A_E, H_E, and r, respectively), while genetic diversity of continuous western populations

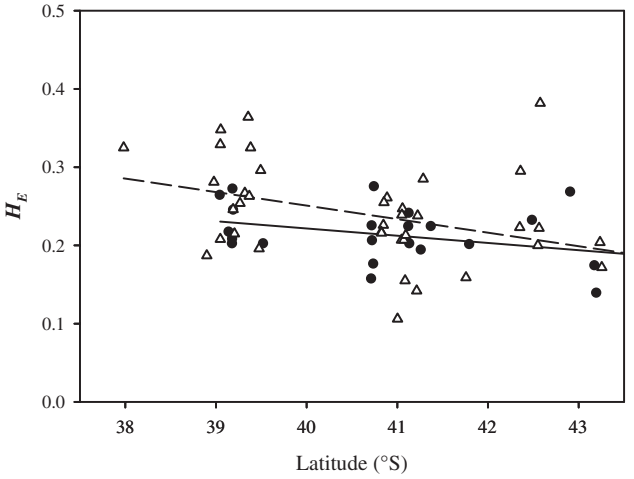

Figure 1. Correlation between population expected heterozygosity (H_E) and latitude in 67 sampled sites of *Austrocedrus chilensis* in Patagonia Argentina. Black circles-solid line indicates continuous populations, and empty triangles-dotted line indicate marginal ones.

showed no such latitudinal association (Fig. 1). Latitudinal regions (N, C, S) showed statistically significant differences in mean number of alleles per locus (N_A), the effective number of alleles (A_E), total genetic diversity (H_E), and allelic richness (r) ($PhiPT_{(1,999)} = 0.08$, 0.136, 0.116, 0.09; $p = 0.020$, 0.001, 0.002, and 0.007, respectively). Populations from N always attained higher values than populations from S (Table 1).

Genetic Inbreeding and Divergence

Average inbreeding within all N populations was high and positive ($F_{IS} > 0.14$), as were eastern populations in C, where more than 80% of all possible tests departed significantly from zero (Appendix S1). Southern and western populations at the center of the range displayed a lower degree of inbreeding ($F_{IS} < 0.06$) (Appendix S1). Overall genetic divergence among populations was significant but moderate $F_{ST} = 0.116$ (CI = 0.086–0.148) and similar for the different areas. Within regions pairwise F_{ST} values ranged from less than 0.091 in S continuous populations to 0.158 within the central marginal group of populations (Table 1).

Population Bottlenecks

The analysis performed to infer bottlenecks differentiated N populations (with 30% of the significant signed tests, $p < 0.05$) from C and S (with <5% of significant tests; Bn St p values in Appendix S1).

Genotypic Admixture Analysis

The Structure analysis indicated that the overall genetic profile of the 1,853 samples could be described with two or

Table 1. Summary of mean genetic parameters, AMOVA results and F statistics, as mean divergence among and average inbreeding within populations, over 12 variable loci, in 67 populations of *Austrocedrus chilensis* in three regions (North, Center, South), in turn subdivided in marginal (M) and continuous (CO) populations.

	N_A*	A_E*	H_O	H_E*	r*	$P_{\%}$	F_{IS}	F_{ST}
$North_M$	2.075	1.493	0.241	0.273	2.067	73.33	0.149**	0.115**
	(0.170)	(0.109)	(0.073)	(0.057)	(1.438)	(9.02)	(0.040–0.265)	(0.081–0.151)
$North_{CO}$	2.010	1.354	0.204	0.228	1.750	73.96	0.141**	0.099
	(0.151)	(0.062)	(0.042)	(0.028)	(0.707)	(9.38)	(0.044–0.250)	(0.046–0.176)
$North_{RM}$	2.010[a]	1.454[a]	0.204	0.258[a]	2.034[a]	73.96	0.136**	0.111**
	(0.158)	(0.113)	(0.064)	(0.052)	(0.160)	(8.74)	(0.041–0.242)	(0.080–0.149)
$Center_M$	2.000	1.346	0.180	0.214	2.733	69.45	0.175**	0.158**
	(0.258)	(0.098)	(0.052)	(0.048)	(2.658)	(13.97)	(0.059–0.302)	(0.097–0.207)
$Center_{CO}$	2.022	1.321	0.203	0.213	2.200	73.33	0.035	0.097**
	(0.183)	(0.068)	(0.038)	(0.033)	(1.549)	(7.66)	(−0.014–0.090)	(0.034–0.175)
$Center_{RM}$	2.022[a]	1.333[b]	0.189	0.214[b]	1.995[a]	71.00	0.114**	0.139**
	(0.222)	(0.085)	(0.048)	(0.042)	(0.204)	(11.81)	(0.043–0.206)	(0.081–0.191)
$South_M$	1.962	1.357	0.208	0.216	2.364	71.21	0.058	0.123**
	(0.320)	(0.156)	(0.083)	(0.068)	(1.804)	(10.78)	(−0.089–0.216)	(0.070–0.171)
$South_{CO}$	1.875	1.299	0.188	0.195	1.625	70.84	0.046	0.091**
	(0.231)	(0.094)	(0.054)	(0.044)	(2.066)	(9.96)	(−0.028–0.131)	(0.054–0.130)
$South_{RM}$	1.875[b]	1.332[b]	0.199	0.207[b]	1.822[b]	71.05	0.053	0.113**
	(0.231)	(0.134)	(0.071)	(0.058)	(0.038)	(10.16)	(−0.058–0.180)	(0.066–0.157)
All populations pooled	4.000	1.382	0.203	0.255	3.718	100	0.106**	0.116**
	(0.739)	(0.242)	(0.109)	(0.137)	(0.764)		(0.028–0.213)	(0.086–0.148)

NA, mean number of alleles per locus; AE, mean number of effective alleles; HO and HE, observed and expected heterozygosity, respectively; r, allelic richness; P%, percentage of polymorphic loci. Standard deviations in parentheses.

F statistics mean and 95% CI, in brackets, were calculated by jackknifing and bootstrapping over polymorphic loci, respectively. Only Center region shows significant differences in F_{IS} between marginal (M) and continuous (CO) populations.

* Statistically significant differences between regions; $p > 0.05$. Superscript letters represent pairwise AMOVA's homogeneous groups. RM subindex is regional mean genetic parameters calculated as mean values for the populations within a region.

** Significantly different from zero.

six genetic groupings. A peak of ΔK value was detected at $\Delta K = 2$, reflecting a difference between N and S populations [Ln P(2) = −20,048.5, $\Delta K(2) = 9.08$], but a secondary genetic structure for our data set was obtained at $K = 6$[LnP(6) = −19,231.3, $\Delta K(6) = 0.91$] (Fig. 2). Individuals were assigned to each of these six hypothetical groups or regions based on genetic similarities (Table 2; Fig. 2). The breakdown of the clusters by region were, cluster 1: 27 and 22% from N/CO and C/CO; cluster 2: 30, 25 and 24% from S/M, S/CO, and C/CO; cluster 3: 19, 21, and 29% from N/CO, C/CO and S/CO; cluster 4: 24% from C/M; cluster 5: 27% from N/M; cluster 6 consisted of a higher proportion of individuals from marginal than from continuous sources assigned into this group (Table 2). Most individuals were generally assigned with high probabilities; however, signals of admixture with several hypothetical clusters were detected in all regions, particularly within central populations.

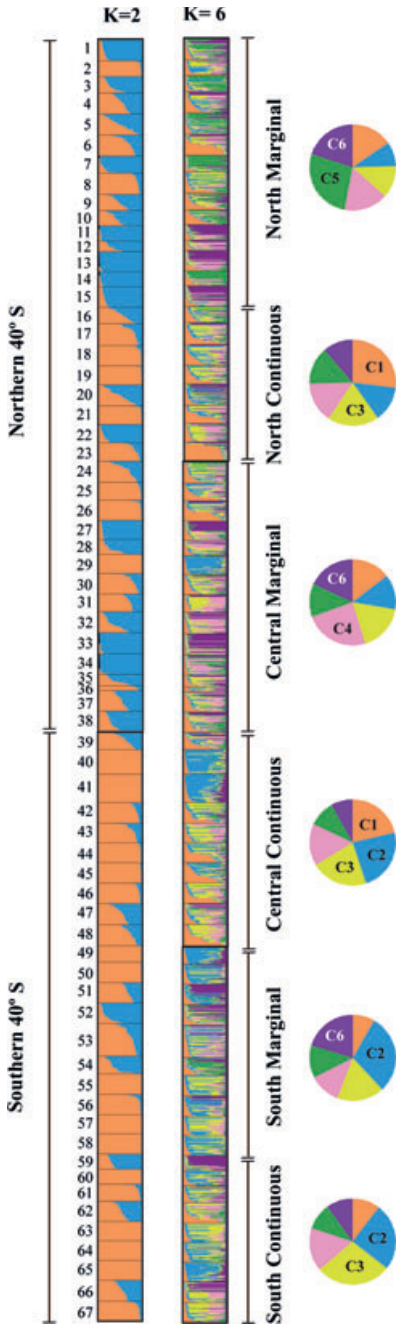

Figure 2. Structure results as proportion of individuals assigned to each cluster considering $K = 2$; and $K = 6$, in 67 sampled sites of *Austrocedrus chilensis* in Patagonia Argentina. Individuals were ordered according to their inferred ancestry calculated by means of Bayesian methods using allelic frequencies. Pie charts represent the average coefficients of ancestry (Q) obtained at $K = 6$.

Table 2. Average coefficients of ancestry (Q) obtained from Structure at $K = 6$.

	Cluster (K)					
Region$_{Type}$	*1*	*2*	*3*	*4*	*5*	*6*
North$_M$	0.153	0.090	0.122	0.166	**0.267**	*0.199*
North$_{CO}$	**0.269**	0.134	**0.190**	0.154	0.139	0.114
Center$_M$	0.145	0.133	0.174	**0.243**	0.123	*0.182*
Center$_{CO}$	**0.213**	**0.236**	**0.214**	0.156	0.100	0.080
South$_M$	0.083	**0.298**	0.177	0.116	0.122	*0.202*
South$_{CO}$	0.102	**0.252**	**0.285**	0.162	0.099	0.099

Regions grouped north, center, and south *Austrocedrus* stands, while type refers to marginal (M) or continuous (CO) populations. The highest values of each group of populations are indicated in bold. Italicized values are the highest within each region in cluster 6.

Isolation By Distance

No significant correlation was recorded between pairwise population matrix of Nei unbiased genetic distance and geographical distances across all pairs of samples (Mantel test, $r = 0.091$, $p = 0.053$, $n = 67$).

Discussion

Genetic diversity of *Austrocedrus* is geographically structured. Small and scattered populations located in the drier climates of the northeast, although more genetically diverse, were characterized by relatively high inbreeding and have been impacted by recent bottlenecks to a greater degree than southern populations. Also, central and southern-most populations located toward the wetter end of the rainfall gradient depicted a higher degree of admixture. Shared genetic variants in these populations are probably a product of common ancestry, likely from northern and eastern sources. Our results thus reflect the complex history of the southern Andes, including Pleistocene glaciations and Holocene human-driven disturbances such as fire. Both ice and fire combined with steep environmental gradients acting at different temporal scales are likely to account for the observed genetic patterns. Therefore, scenarios of "expansion from glacial refugia" or "decline due to fragmentation caused by Holocene fires" are difficult to unravel as the genetic characteristics of populations will be influenced by their size, the degree of isolation, and the length of time

elapsed since disturbance. Decreasing genetic diversity toward the south may reflect the effects of drift during postglacial colonization from northern sources (Kitzberger et al. 2010). This is in agreement with fossil pollen and charcoal records suggesting that *Austrocedrus* colonized central area 6,000 year BP, and southern latitudes more recently still (3,000 year BP) (Whitlock et al. 2006). Such cold-sensitive species most probably suffered major range shifts during ice ages. The occurrence of large southern populations with reduced inbreeding might suggest that *Austrocedrus* is undergoing population expansion toward suitable high-latitude areas, which may be favored under climatic warming. Nonetheless, the remarkably high genetic variation recorded in population 54 located in a southern marginal location is puzzling. Whether such elevated genetic diversity is the result of large populations and recent fire history in the south or in situ survival during ice ages is still an open question. Similarly high isozyme diversity was found toward the southern range of the monotypic Cupressaceae *Fitzroya cupressoides* (Premoli et al. 2000). More studies are needed, including analysis of macrofossils and ancient DNA to disentangle past events occurring at different timescales.

Historical disturbance regimes also affected genetic structure of *Austrocedrus*. Pre-European fire histories of *Austrocedrus* stands suggest that indigenous populations may have imposed a regime of frequent fires in the north (Kitzberger et al. 1997; Veblen et al. 1999). This long-lasting disturbance regime, together with the synergistic effects of drier climates, would have led to a small number of individuals surviving in fire-free areas, that is, fire refugia sensu Kitzberger et al. (2009), such as rocky outcrops. This would explain higher levels of inbreeding in the north. In contrast, southerly and relatively more humid westerly areas appear to have been affected by more recent fire history related to European colonization during the early 20th century, where extended fires were used to clear vegetation for agriculture and cattle ranching (Kitzberger et al. 1997; Veblen et al. 1999).

Austrocedrus-dominated dryland forests of northern Patagonia are reservoirs of genetic diversity and might therefore be relatively resilient to climate-influenced disturbances. Repetitive photography has documented natural regeneration from fire refugia immersed in a matrix of dryland steppe (Veblen & Lorenz 1988). Given that disturbance regimes interact with climate, forest restoration requires short- and long-term approaches (Millar et al. 2007). In the short-term, one strategy would be to allow *Austrocedrus* natural regeneration from remnant trees within fire refugia. Thus, restoration actions should include cattle exclosure from rocky outcrops (i.e. fire refugia) to facilitate passive restoration. If, on the other hand, active restoration activities were to be undertaken (e.g. in recently burned areas with few or nil remnant trees), the choice of seed sources would need careful consideration. In particular, local species/populations are better adapted to the local environment. Therefore, they are likely to demonstrate higher growth and survival, while maintaining the genetic integrity of the site, and preventing any potential pollution of the local gene pool (Harris et al. 2006). However, under current assumptions of changing climates, relaxing these guidelines may be appropriate (Millar et al. 2007). Expanding seed zone sizes and admixture of germplasm from adjacent zones might be considered, particularly in species such as *Austrocedrus* that demonstrate at least some degree of phenotypic plasticity (Gyenge et al. 2005; Pastorino et al. 2010).

Significant genetic structure was confirmed by average $F_{ST} = 0.12$ and admixture models which showed that northern populations are most genetically distinct from southern ones. This finding also has implications for ecological restoration. At least two "seed transfer zones" might be defined in *Austrocedrus*, within which plants can be moved with less consequences for population fitness. These include north with central-east populations and south with central-west populations. Nonetheless, the main challenge for land managers will be to obtain information on the genetic variation in adaptive traits, such as common garden experiments using distinct provenances in various regional conditions (Harris et al. 2006; Millar et al. 2007).

Implications for Practice

- Passive restoration actions are suggested for *Austrocedrus* populations showing evidence of significant inbreeding and recent bottlenecks, but still genetically diverse.
- The use of local germplasm for active restoration initiatives could increase population size, counteracting inbreeding effects.
- Predicted climate change gives the opportunity for restoration trials to be established beyond species' current ranges into new suitable areas.
- Expanding admixture of germplasm from adjacent zones might be considered for species that display high levels of phenotypic plasticity.
- It is inaccurate to assume that marginal, small isolated populations are genetically impoverished, and large continuous populations are highly genetically variable.
- Intraspecific genetic patterns can effectively inform restoration projects.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map depicting 67 sampled populations of *Austrocedrus chilensis* in Patagonia Argentina.

Appendix S1. Geographic location of 67 studied populations of *Austrocedrus chilensis* and summary of within-population genetic diversity statistics. R: relative location along the species range (N: north, C: central, and S: south); Type: type of forest (CO: Continuous and M: Marginal); N: number of sampled individuals; N_A: mean number of alleles per locus; A_E: mean number of effective alleles; H_O and H_E: observed and expected heterozygosity, respectively; r: allelic richness (na: not applied when the number of sampled individuals is less than 20); $P_{\%}$: percentage of polymorphic loci; F_{IS}: average fixation index; Bn St: p values of the sign test for evidence of a recent bottleneck under the Infinite Allele Model, in bold statistically significant values. Underlined populations hold private alleles.

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