

Pleistocene climatic oscillations rather than recent human disturbance influence genetic diversity in one of the world's highest treeline species¹

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PREMISE OF THE STUDY: Biological responses to climatic change usually leave imprints on the genetic diversity and structure of plants. Information on the current genetic diversity and structure of dominant tree species has facilitated our general understanding of phylogeographical patterns.

METHODS: Using amplified fragment length polymorphism (AFLPs), we compared genetic diversity and structure of 384 adults of *P. tarapacana* with those of 384 seedlings across 32 forest sites spanning a latitudinal gradient of 600 km occurring between 4100 m and 5000 m a.s.l. in *Polylepis tarapacana* (Rosaceae), one of the world's highest treeline species endemic to the central Andes.

KEY RESULTS: Moderate to high levels of genetic diversity and low genetic differentiation were detected in both adults and seedlings, with levels of genetic diversity and differentiation being almost identical. Four slightly genetically divergent clusters were identified that accorded to differing geographical regions. Genetic diversity decreased from south to north and with increasing precipitation for adults and seedlings, but there was no relationship to elevation.

CONCLUSIONS: Our study shows that, unlike the case for other Andean treeline species, recent human activities have not affected the genetic structure of *P. tarapacana*, possibly because its inhospitable habitat is unsuitable for agriculture. The current genetic pattern of *P. tarapacana* points to a historically more widespread distribution at lower altitudes, which allowed considerable gene flow possibly during the glacial periods of the Pleistocene epoch, and also suggests that the northern Argentinean Andes may have served as a refugium for historical populations.

KEY WORDS AFLP; central Andes; elevational gradient; latitudinal gradient; phylogeography; *Polylepis tarapacana*; post-glacial migration

Biological responses to climatic change vary greatly in space and time (Loarie et al., 2009); such responses usually leave imprints on the genetic diversity and structure of plant populations (Hewitt,

2000). Information on the current distributions of the genetic diversity and structure of dominant tree species has facilitated our general understanding of phylogeographical patterns, including postglacial migration events, from which fossil evidence is lacking. In Europe and North America, genetic diversity of tree species has often been found to decline toward the pole because of postglacial migration from southern refugia and successive founder events (Hewitt, 2000; Petit et al., 2003).

In South America, the still limited knowledge on tree migration patterns and genetic structure of tropical high-mountain species points to complex scenarios (Quiroga and Premoli, 2007; Pautasso, 2009; Hensen et al., 2011, 2012). In some species, such as *Podocarpus parlatorei*, *Podocarpus nubigena*, and *Polylepis australis*, genetic diversity declines with increasing elevation and decreasing latitude (toward the equator; Quiroga and Premoli, 2007, 2010; Hensen et al., 2011). This decline indicates that these Andean high-mountain tree species migrated toward the equator following

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historic climate change and expanded to lower elevations during cooler periods while being restricted to higher elevations during warmer periods. According to this scenario, species may exhibit low population divergence and weak phylogeographic structures due to the relatively short (interglacial, ca. 15 000 yr) periods of isolation and long (glacial, ca. 100 000 yr) periods of expansion (Stewart et al., 2010). For several tree species, the southern distributional areas are assumed to function as long-term refugia (Quiroga and Premoli, 2007). For other species, the results of genetic studies support a scenario of multiple glacial forest refugia in mountain areas, facilitated by the heterogeneous mountain topographies (Premoli et al., 2000; Mathiasen and Premoli, 2010). In such cases, species may have survived in situ, resulting in pronounced genetic differentiation between populations (Oppennoorth et al., 2010).

The genus *Polylepis* (Rosaceae) includes about 30 wind-pollinated tree and shrub species endemic to the Andean mountain chain from Argentina and Chile to Venezuela (Kessler and Schmidt-Lebuhn, 2006). As a result of mainly human impacts, *Polylepis* forests represent one of the most endangered ecosystems in the world (IUCN, 2014). Pollen evidence indicates that the current distribution of *Polylepis* forests has been affected by Pleistocene glacial-interglacial cycles (Gosling et al., 2009). The impacts of both Pleistocene climatic changes and more recent human disturbance on the genetic diversity of several *Polylepis* species has been shown by previous genetic studies (Hensen et al., 2011, 2012; Gareca et al., 2013).

The patchy distribution of many *Polylepis* forest stands can be partly explained by the effect of human activities, either directly by timber extraction or indirectly by cattle grazing and associated grassland burning (Kessler, 2002; Renison et al., 2006). Due to recurrent, partly anthropogenic fires, tropical *Polylepis* forests failed to recolonize high-elevation sites after the Last Glacial Maximum (Di Pasquale et al., 2008; Bush et al., 2015). Additional recent fragmentation through human activity (e.g., timber extraction) might be reflected in the higher genetic diversity observed in adults that germinated before fragmentation and lower diversity in seedlings that developed after fragmentation. This pattern was found for *Polylepis incana* in Ecuador (Hensen et al., 2012), while genetic diversity of *Polylepis subtusalbida* in Bolivia appears not to have been greatly affected by recent human activities (Gareca et al., 2013).

Here, we present a survey of amplified fragment length polymorphism (AFLP) variation within and between populations of *Polylepis tarapacana*, a tetraploid species (Schmidt-Lebuhn et al., 2009) distributed in scattered stands at high elevations in the border regions of Bolivia, Peru, Argentina, and Chile. In Bolivia and Argentina, extensive *P. tarapacana* forests have been harvested as a source of charcoal for mining activities since the Spanish conquest. Recent human impact has mainly resulted in habitat degradation, brought about particularly by livestock grazing but also by wood harvesting. The species is listed as *lower risk* or *near threatened* (IUCN, 2014), as such, understanding the genetic consequences of the recent history of the species is of interest to its conservation and sustainable management. Given that the patchy distribution of several *Polylepis* species is the result of human influence (Kessler, 2002; Renison et al., 2015), we expected to find lower genetic diversity in seedlings (<5 yr old) than in older adults (>100 yr old). We additionally wanted to explore whether the species survived in situ or shifted its elevational and latitudinal range during Pleistocene interglacial-glacial cycles and suspected that the genetic diversity of this tree species would decrease with increasing elevation and

decreasing latitude (toward the equator), as reported already for other Andean tree species (Quiroga and Premoli, 2007, 2010; Hensen et al., 2011).

MATERIALS AND METHODS

Study species—*Polylepis tarapacana* Philippi (Rosaceae) is an evergreen tree species with a mean height of about 3 m, which inhabits the semiarid Andean highlands from southernmost Peru across western Bolivia to northern Chile and Argentina. The species is one of the world's highest treeline species. It is normally distributed above 3900 m a.s.l. and can exceptionally reach about 5000 m a.s.l. on the Sajama Vulcano in Bolivia (IUCN, 2014). The species is characterized by twisted trunks and branches, compound leaves with leaflets no greater than 1 cm wide, and silvery trichomes on the lower surface (Kessler, 1995). Its flowers are apetalous and wind-pollinated, and fruits are one-seeded, gravity-dispersed nutlets with a low-dispersal capacity (Cierjacks et al., 2008).

Species sampling—We sampled 384 adults (>2 m high) and 384 seedlings (<20 cm high) from a total of 32 forest plots (approx. 200 × 50 m, 12 adults and 12 seedlings per plot). The plots were located within 18 forest remnants covering most of the elevational distribution of the species (4100 to ~5000 m) across a latitudinal distance of about 600 km (Table 1, Fig. 1). In northern Bolivia and Chile, population sizes exceeded 10 000 adults, while in southern Bolivia and Argentina population sizes were smaller (less than 5000 individuals). In seven of the forests, we sampled transects comprising two to four plots over elevational ranges of up to 600 m (Table 1). Sampled individuals were separated by at least 10 m to minimize the chance of sampling closely related individuals. Leaves were stored in bags with silica gel. The distribution of the samples was divided into four geographic regions according to geographic distances and mountain barriers (separating Chile from Bolivia and Argentina): northern Bolivia (National Park Sajama; 144 adults and 144 seedlings from 12 plots distributed across four forests); southern Bolivia (Salar de Uyuni; 84 adults and 84 seedlings; seven plots from three forests); northern Argentina (108 adults and 108 seedlings, from nine plots in seven forests); and northern Chile (48 adults and 48 seedlings, from four plots in four forests; Table 1). Climate in the four regions is characterized by a relatively wet, warm season during summer and a dry, cold season during winter. In northern Bolivia, the mean annual precipitation is at about 330 mm (Hoch and Körner, 2005) with a steep decline toward the west into the Atacama Desert of Chile and a less pronounced reduction in precipitation toward the south into northern Argentina, where mean annual precipitation is about 100 mm (Carilla et al., 2013). For each population, mean annual temperature (°C) and precipitation (mm) levels were based on Worldclim 30 s resolution data (Hijmans et al., 2005). We used the ArcGIS 10 “extract multivalued to points” tool in the Spatial Analyst Extension.

DNA extraction and AFLP analysis—AFLP markers have been successfully used in studies of phylogeographic structures of other *Polylepis* species (Hensen et al., 2011, 2012; Gareca et al., 2013). The AFLP method followed Hensen et al. (2012). We chose five primer pairs that amplified reliably and showed polymorphism in pretests: *EcoRI* AGC/*MseI* CTG, *EcoRI* AGC/*MseI* CAT, *EcoRI* ACT/*MseI* CTG, *EcoRI* AAG/*MseI* CAT, and *EcoRI* AAG/*MseI* CTG. To test

TABLE 1. Geographic data, genetic data and climatic data for the collection sites of adults and seedlings of *Polylepis tarapacana* in the Central Andes.

Pop	F	Lat (°S)	Long (°W)	Elev (m a.s.l.)	Mtemp (°C)	Mpre (mm)	Adults			Seedlings		
							n	PPB	H _e	n	PPB	H _e
Bolivia North												
1	a	18.111	68.962	5000	4.1	342	12	64.2	0.187	12	64.4	0.185
2	a	18.107	68.948	4800	2.2	345	12	64.9	0.182	12	65.6	0.189
3	a	18.105	68.952	4600	2.9	342	12	63.9	0.181	12	62.2	0.189
4	a	18.102	68.958	4400	4	342	12	62.2	0.18	12	63.9	0.187
5	b	18.099	69.032	4415	3.4	339	12	65.2	0.185	12	65.6	0.187
6	c	17.998	68.934	4800	1.8	360	12	65.2	0.192	12	60.9	0.177
7	c	18.007	68.953	4600	2.7	357	12	64.1	0.182	12	64.3	0.183
8	c	18.012	68.935	4400	3.2	353	12	66.9	0.197	12	66	0.183
9	d	18.155	68.863	4920	1.1	342	12	67.7	0.193	12	63.7	0.178
10	d	18.157	68.866	4800	1.1	342	12	69	0.192	12	66	0.194
11	d	18.166	68.872	4600	1.9	338	12	58.7	0.177	12	64.5	0.19
12	d	18.182	68.877	4400	3.5	335	12	58.1	0.189	12	63.7	0.175
											66.7	0.19
Bolivia South												
13	e	20.654	67.700	4557	4	94	12	65	0.198	12	65.3	0.198
14	e	20.656	67.706	4345	4.5	95	12	64.5	0.202	12	71.4	0.201
15	f	19.599	67.647	4130	4.6	181	12	68.6	0.204	12	54.6	0.193
16	f	19.584	67.665	4554	4.9	185	12	63.9	0.203	12	59.1	0.202
17	f	19.596	67.666	4351	5.5	188	12	66.7	0.188	12	64.9	0.212
18	g	19.832	67.655	4769	3.1	155	12	62.2	0.193	12	64.3	0.194
19	g	19.812	67.647	4578	3.8	158	12	69.5	0.199	12	68.4	0.188
								59.6	0.2	12	74.2	0.194
Argentina												
20	h	22.534	66.273	4583	4.7	126	12	67.3	0.194	12	67.7	0.196
21	i	23.575	66.273	4466	5.4	116	12	67.1	0.194	12	70.1	0.197
22	j	22.301	66.635	4398	5.4	98	12	63.9	0.198	12	62.4	0.197
23	k	22.478	66.632	4302	6.1	101	12	68.2	0.192	12	69.7	0.195
24	l	22.536	66.571	4588	4.1	104	12	70.5	0.206	12	72.7	0.202
25	l	22.550	66.571	4794	2.6	105	12	70.1	0.201	12	66.9	0.189
26	l	22.548	66.562	4942	2.5	106	12	69	0.196	12	70.1	0.191
27	m	22.871	66.355	4433	5.1	117	12	63.2	0.178	12	61.7	0.19
28	n	22.976	66.304	4644	3.7	120	12	63.4	0.183	12	66.9	0.194
								69.9	0.198	12	69	0.207
Chile												
29	o	18.250	69.167	4545	2.1	312	12	59.4	0.185	12	62.4	0.186
30	p	18.935	69.001	4550	1.8	246	12	59.8	0.176	12	63.2	0.183
31	q	19.197	68.817	4209	4	210	12	44.7	0.173	12	63.9	0.19
32	r	20.751	68.567	4250	4.1	81	12	66.5	0.187	12	63	0.187
Total								64.6	0.191		65.3	0.191

Notes: Pop, populations; F, forest remnant (a-r); n, the number of individuals; Lat, latitude; Long, longitude; Elev, elevation; Mtemp, mean annual temperature; Mpre, mean annual precipitation; PPB, percentage of polymorphism bands; He, heterozygosity.

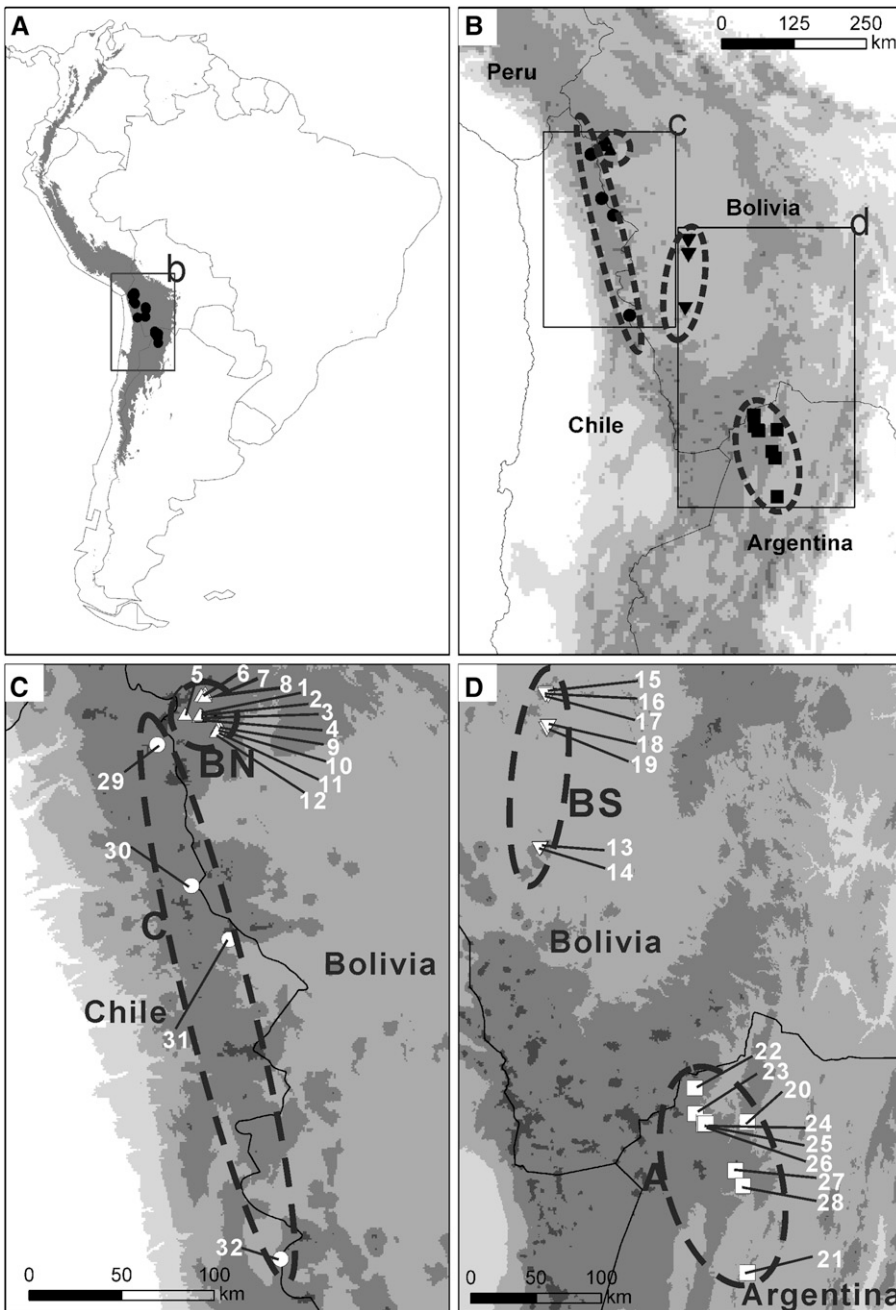


FIGURE 1 Location of the sampled populations of *Polylepis tarapacana* in the central Andes. Population numbers refer to Table 1.

for reproducibility, we used >20% replicated individuals (Appendix S1, see online Supplemental Data) and followed the protocol of Ley and Hardy (2013). We generated output files for automatic scoring on the Fragment Profile of the MegaBACE package (Applied Biosystems), which converted peak data into a binary allelic matrix. The output file was prepared for the program SPAGeDi v1.4 (Hardy and Vekemans, 2002) to test for the reproducibility of peaks using broad sense heritability (H^2) and its significance, calculated as F_{ST} . Peaks with an $H^2 > 0.25$ and $P < 0.05$ were considered heritable for this study (Appendix S1). Among the 768 individuals, readable fingerprints could not be obtained with primer pairs *EcoRI AGC/MseI*

CTG, *EcoRI AGC/MseI* CAT, *EcoRI ACT/MseI* CTG, *EcoRI AAG/MseI* CAT, and *EcoRI AAG/MseI* CTG in 22, 14, 16, 31 and 23 individuals, respectively. These individuals were coded at respective markers as missing data. The AFLPdat R package (Ehrich, 2006) was used to transfer data between the different software packages used.

Data analyses—Given the polyploid nature of our study species, it was not possible to unambiguously estimate allele frequencies. In accordance with Bonin et al. (2007), we analyzed our AFLP data based on both the band-based and fragment-frequency-based approaches.

Genetic diversity at the population level was assessed as the percentage of polymorphic bands (PPB) and as Nei’s expected heterozygosity (H_e ; Nei, 1987) using a Bayesian method with uniform prior distribution (Zhivotovsky, 1999) in the software package AFLPSURV 1.0 (Vekemans et al., 2002). We detected whether genetic diversity (PPB and H_e) differs significantly between adults and seedlings with paired t tests with permutations in R 3.1.2 (R Core Team, 2014; R package broman, Broman and Broman, 2014). The data set was analyzed assuming Hardy–Weinberg equilibrium. Pairwise F_{ST} values were calculated for each population pair using the program Arlequin 3.5 (Excoffier and Lischer, 2010). Significance was evaluated through 1000 permutations.

We analyzed the relationships between genetic diversity (H_e and PPB) and the environmental variables of elevation, latitude, mean annual temperature, and mean annual precipitation in interaction with life stage (i.e., seedling vs. adults) using linear mixed effects models with the package “lme4” (Bates et al., 2014) in R 3.1.2 (R Core Team, 2014). For each response variable, we fitted four different ANCOVA models, comprising one of the environmental variables in interaction with life stage, respectively. All models contained the nested random effects of region, forest, and population and were fitted with a maximum likelihood approach. The full models

were then simplified in a stepwise backward manner by removing terms that were not significantly based on likelihood ratio (χ^2) tests to obtain the minimal adequate models. To check which of the four minimal models explained variation in H_e and PPB best, we used the Akaike information criterion (AIC) (Akaike, 1973) adjusted for small sample sizes (AICc, Burnham and Anderson, 2004) in the R package AICcmodavg (Mazerolle, 2015). Model analytic plots (Crawley, 2012) confirmed normality of errors and homogeneity of variance for all models with untransformed variables. Moreover, we assessed correlations between pairwise of the four different environmental variables using Pearson’s correlation coefficient (r_s).

For the analysis of population structure, a Bayesian model-based approach was implemented in the program STRUCTURE 2.3.4 (Pritchard et al., 2000) for adults and juveniles, respectively. Data were coded as a dominant allele matrix. Due to the tetraploid nature of the species, we handled the genotypes with ambiguous allele copy numbers and analyzed the data set with the recessive allele option. We ran the analyses under the admixture model with correlated allele frequencies among populations. Ten runs were performed for each number of clusters $K = 1-12$ using a burn-in and a run length of 100 000 iterations each. The K that best described the data were determined following inspection of the mean values of $L(K)$, $L'(K)$, $L''(K)$, and ΔK (Evanno et al., 2005). The individual ancestry coefficients were calculated by the average pairwise similarity of individual assignments across runs with the program CLUMPP (Jakobsson and Rosenberg, 2007) using the FullSearch method and weighted by the number of individuals in each population; the program Distruct (Rosenberg, 2004) was used to plot the individual ancestry coefficients.

In addition, we employed a principal coordinate analysis (PCoA) to distinguish similar genetic groups of individuals with the package vegan (Oksanen et al., 2013) in R 3.1.2. We also used an AMOVA to describe genetic structure and to measure the amount of variation found within and between populations; F statistics were extracted and significance levels were tested with 1000 permutations for each analysis. AMOVA was performed with Arlequin 3.5 (Excoffier and Lischer, 2010).

Mantel tests (Mantel, 1967) were used to examine whether genetic distances (pairwise F_{ST} values) correlated with geographic distances using the vegan package (Oksanen et al., 2013). Mantel tests were performed on the entire data set, each of the four geographic groups, and on the different combination of the geographic groups.

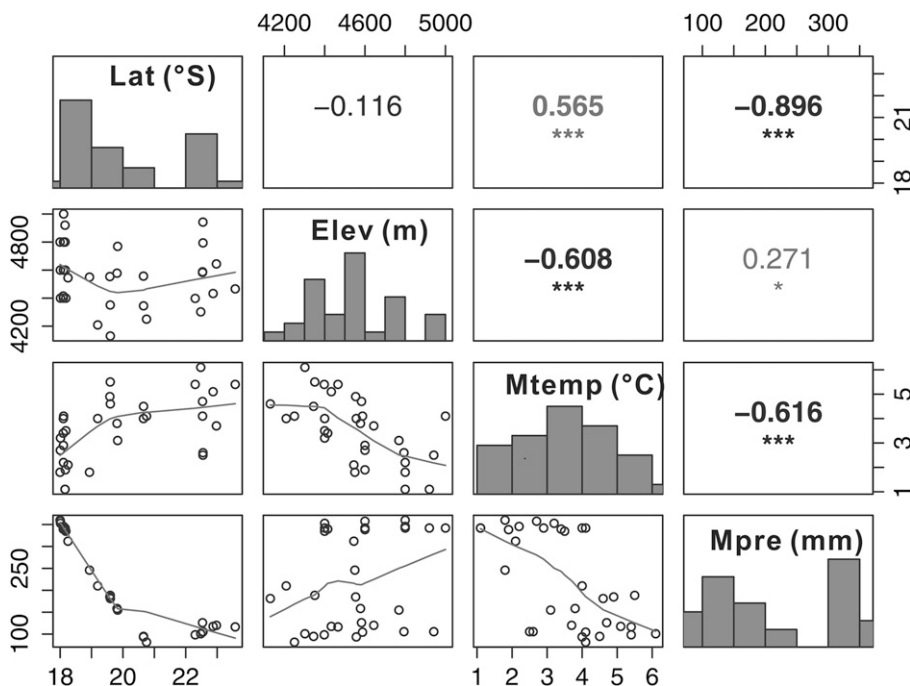


FIGURE 2 Pearson's correlation coefficient between pairwise factors. *Abbreviations:* Lat, Latitude; Elev, Elevation; Mtemp, Mean annual temperature; Mpre, Mean annual precipitation.

RESULTS

A total of 465 polymorphic markers were obtained from the five primer combinations (Appendix S1). Measures of within-plot diversity yielded high values (Table 1). The average proportion of polymorphic bands (PPB) ranged between 44.7 and 70.5% for *P. tarapacana* adults and between 54.6 and 74.2% for juveniles. Values of average gene diversity (H_e) ranged between 0.17 and 0.21 for both adults and seedlings (Table 1). Adults and seedlings showed no significant difference in genetic diversity PPB (paired permutation t test: $P = 0.57$) and H_e (paired permutation t test: $P = 0.90$).

According to the ANCOVA, we found that H_e was significantly positively related to latitude ($\chi^2 = 5.98$, $df = 1$, $P < 0.05$) and mean annual temperature ($\chi^2 = 3.85$, $df = 1$, $P < 0.05$) and significantly negatively related to mean annual precipitation ($\chi^2 = 8.07$, $df = 1$, $P < 0.01$), while elevation had no significant effect. None of the environmental variables had a significant effect on PPB. In all cases, neither the interaction nor the main effect of life stage was significant. For H_e , mean annual precipitation explained the data best (AICc = -438.0), followed by latitude (AICc = -435.9), mean annual temperature (AICc = -433.8), and elevation (AICc = -432.1). In addition, based on Pearson's correlation coefficient (Fig. 2), we found elevation and latitude to be uncorrelated, but they were both significantly correlated to mean annual temperature (elevation: $r_s = -0.61$, $P < 0.001$; latitude: $r_s = 0.57$, $P < 0.001$) and mean annual precipitation (elevation: $r_s = 0.27$, $P < 0.05$; latitude: $r_s = -0.90$, $P < 0.001$). The two climatic variables, in turn, were negatively correlated ($r_s = -0.61$, $P < 0.001$).

The results of the Bayesian structure analysis (Fig. 3) revealed peak values of $K = 2$ and 4 clusters for *P. tarapacana* adults and $K = 2$ and 4 clusters for the seedlings (Appendix S2, see online Supplemental Data). PCoA (Fig. 4) also showed four main clusters. Clusters of adult plants corresponded to Bolivia North, Bolivia South, Argentina, and Chile, and seedlings produced the same clusters. The overall fixation indices (F_{ST} values) of 0.102 for *P. tarapacana* adults and 0.100 for the seedlings provided evidence of weak spatial isolation (Table 2). The pairwise F_{ST} values were highly significant and ranged between 0.055 (Bolivia North and Argentina) and 0.132 (Bolivia North and Chile) for adults and 0.054 (Bolivia North and Argentina) and 0.126 (Bolivia North and Chile) for seedlings (Table 3).

The AMOVA found that molecular variance was highest within plots of *P. tarapacana* (adults 89.75%, seedlings 89.96%, Table 2). Variance among four geographic regions and among forest remnants yielded lower values, which were again similar between adults and seedlings (7.11% and 3.14% vs. 6.6% and 3.44%, respectively). The Mantel test indicated isolation by distance across all forest plots for adults ($r_M = 0.342$) and seedlings ($r_M = 0.364$; Table 4) and for the Argentinean cluster, but no isolation by distance was detected for the other three clusters.

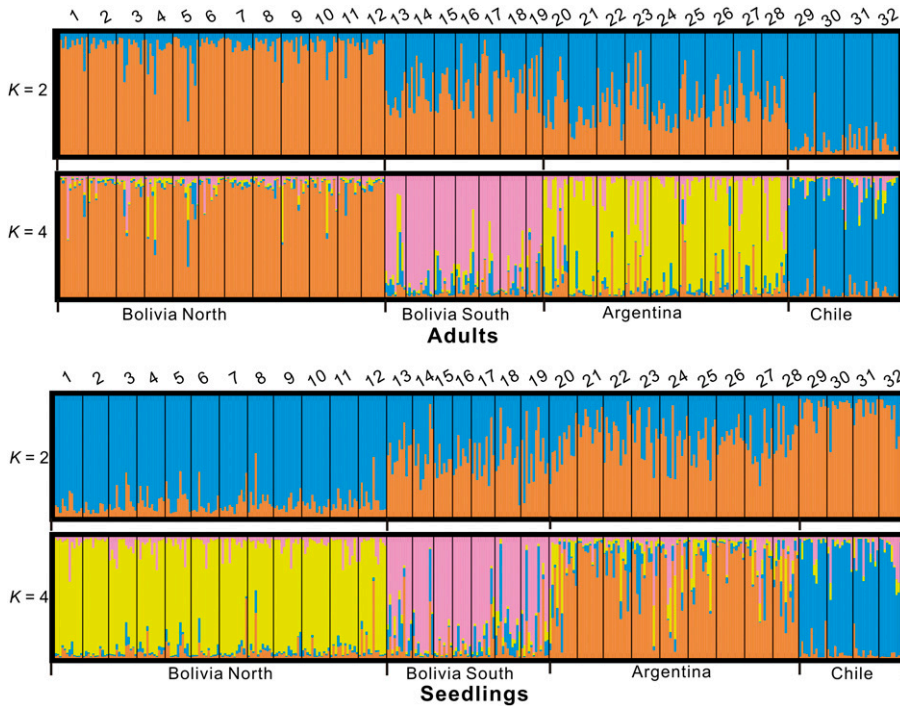


FIGURE 3 Bayesian structure analysis of *Polylepis tarapacana* with the STRUCTURE software. Probability of assignment for $K = 2$ and 4 for adults (above), and $K = 2$ and 4 for seedlings (below) in 32 populations, respectively. Each individual is represented by a single vertical line divided into K colored segments, where K is the number of genetic clusters. Black ticks separate the regions indicated below the figure. Labels above the plots indicate population information, and the labels below the plots provide region information.

DISCUSSION

We discovered genetic patterns of one of the world’s highest treeline species, *P. tarapacana*, using AFLP markers. First, we found moderate to high genetic diversity and low genetic differentiation, and no significant difference of genetic diversity between life stages (adults and seedlings). Second, we detected four geographical clusters for adults and seedlings with low genetic differentiation. The results indicate that the current genetic pattern of this species is

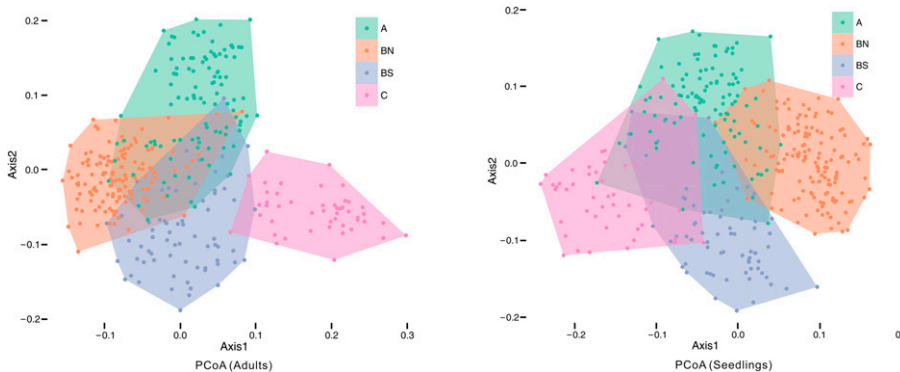


FIGURE 4 Principal coordinate analysis (PCoA) of AFLPs among the 384 adults (left) and 384 seedlings (right) of *Polylepis tarapacana*. The samples are color coded according to their geographic origins. Abbreviations: A, Argentina; BN, Bolivia North; BS, Bolivia South; C, Chile.

more likely to be caused by Pleistocene climatic oscillations rather than recent human disturbance.

Effects of human impact—Levels of genetic diversity for adults of *P. tarapacana* in the current study did not differ much from those found for *P. australis* adults in Argentina (Hensen et al., 2011), and they were higher than those found for *P. incana* in Ecuador (Hensen et al., 2012). In contrast to the results for *P. incana* in the latter study (Hensen et al., 2012), but in accordance with the results for *P. pauta* in Ecuador (Aragundi et al., 2011), we found similar levels of genetic diversity for *P. tarapacana* adults and seedlings. The result suggests that the genetic diversity of *P. tarapacana* has not been significantly affected by forest fragmentation during the last few hundred years. Nevertheless, as these genetic patterns are certainly also influenced by life history and ecological traits including population sizes, fecundity and longevity, we believe that they mainly reflect historical and geographical signatures in the intensity of human impact on these populations. The distribution of *P. tarapacana* covers the driest and least densely populated region of the (sub)tropical Andes. This region has been inhabited by humans for millennia (Kessler, 2002), but their effect on the distribution of *Polylepis* forests does not seem to have influenced their genetic diversity. Perhaps most importantly, vegetation cover here is so sparse that fires, the main reason for the large-scale destruction of these forests (Kessler, 1995, 2002), cannot spread. Also, grazing is largely done by native camelids and is concentrated in valley bottoms, such that human influence on *P. tarapacana* takes place mainly via timber extraction. In contrast, in the more humid Argentinean range of *P. australis*, human impact has greatly increased within the last few hundred years (Renison et al., 2006). Here, both the frequent use of fires and grazing with nonnative animals (cattle, sheep) within forest patches have had negative impacts on the *Polylepis* forests (Renison et al., 2015). In the Ecuadorian range of *P. incana*, human impact also has a long history, and much of the fragmentation probably predates Spanish colonization (Bush et al., 2015); current impacts are also rather pronounced (Cierjacks et al., 2008). Accordingly, the populations of *P. incana* studied in the country showed strong age-related signatures of human impact (Hensen et al., 2012).

We therefore propose that the history of human land use, modulated by different environmental conditions, has left a geographic signature in the age-related genetic diversity of *Polylepis* forests. While more studies are needed to verify our assumptions, they potentially provide a guideline to predict where human impact may be most detrimental and

TABLE 2. AMOVA of adults and seedlings for *Polylepis tarapacana* calculated with Arlequin ver. 3.5, respectively, partitioned by regions.

SV	Adults			Seedlings		
	df	PV	F statistic	df	PV	F statistic
All						
Among 4 clusters	3	7.11	$F_{CT} = 0.071^{***}$	3	6.6	$F_{CT} = 0.066^{***}$
Among populations within cluster	28	3.14	$F_{SC} = 0.034^{***}$	28	3.44	$F_{SC} = 0.037^{***}$
Within populations	324	89.75	$F_{ST} = 0.102^{***}$	328	89.96	$F_{ST} = 0.100^{***}$
Total	355			359		
Bolivia North						
Among populations	11	1.54		11	1.12	
Within populations	126	98.46	$F_{ST} = 0.015^{***}$	129	98.88	$F_{ST} = 0.011^{***}$
Total	137			140		
Bolivia South						
Among populations	6	4.88		6	5.23	
Within populations	60	95.1	$F_{ST} = 0.048^{***}$	62	94.77	$F_{ST} = 0.052^{***}$
Total	66			68		
Argentina						
Among populations	8	4.57		8	6.19	
Within populations	95	95.43	$F_{ST} = 0.046^{***}$	97	93.81	$F_{ST} = 0.062^{***}$
Total	103			105		
Chile						
Among populations	3	3.66		3	2.5	
Within populations	43	96.34	$F_{ST} = 0.037^{***}$	40	97.5	$F_{ST} = 0.025^{***}$
Total	46			43		

Notes: SV, source of variation; df, degrees of freedom; PV, percentage of variation; *** $P < 0.001$.

where corresponding management activities are thus most urgently needed.

Phylogeographic history—Greater genetic variability of genetic diversity was found within, rather than among populations, as found for other *Polylepis* species (Hensen et al., 2011, 2012; Gareca et al., 2013). Our overall fixation indices (F_{ST} values) provide evidence of weak spatial isolation, particularly when regarding the large scale of our study, and are much lower than those determined for *P. australis* ($F_{ST} = 0.165$ for adults, Hensen et al., 2011) and *P. incana* ($F_{ST} = 0.307$ for adults, $F_{ST} = 0.298$ for seedlings, Hensen et al., 2012). In our study, the low genetic differentiation may be due to the maintenance of large population sizes (field observation). Gene flow through seeds or pollen between populations might replenish alleles that have been lost through drift, which in turn reduces genetic differentiation between populations (Hensen et al., 2011). In addition, the effect of drift on population structure in tetraploids is probably reduced compared with diploids due to an effective population size twice as high as for *P. tarapacana* (Meirmans and Van Tienderen, 2013). It should be noted that the results in our study might be biased due to the unavoidable genotyping error because we used the dominant data. However, we attempted to limit the biases by (1) reducing the genotyping error as much possible following the suggestions of Pompanon et al. (2005) and by (2) comparing genetic

TABLE 3. Pairwise F_{ST} between the four regions for *Polylepis tarapacana*. The lower left part shows results for adults; the upper right shows seedlings. Bolded text indicates that the pairwise F_{ST} value is significant.

Regions	BN	BS	A	C
BN	0	0.05895	0.0539	0.12649
BS	0.05475	0	0.05717	0.07943
A	0.0545	0.06093	0	0.08302
C	0.13166	0.09554	0.09988	0

Notes: BN, Bolivia North; BS, Bolivia South; A, Argentina.

diversity and differentiation with those of other *Polylepis* studies with the same AFLP markers.

Despite the overall low level of genetic differentiation, we found four distinct clusters for adults and seedlings (Figs. 3, 4). These four clusters were concordant with the four predefined and nonoverlapping geographic regions. We assume that particular Andean landscape structures hampering gene flow, such as high-mountain crests or lower elevation depressions, might explain the clusters. The role of such barriers for gene flow is impressively demonstrated by the highest pairwise F_{ST} values found between the Chilean forests and those of nearby northern Bolivia. While the four Chilean populations are located on the western side of the volcanic chain marking the borderline between Chile and Bolivia (Fig. 1), those of Bolivia and Argentina are located on the eastern side of the chain.

The low genetic differentiation between the four clusters may also be caused by the fact that the populations of *P. tarapacana* expanded to lower elevations and experienced gene flow in continuous populations during the cold glacial periods, with the Chilean populations shifting toward the Pacific and the Bolivian and

TABLE 4. Mantel tests for the individuals across all four regions for *Polylepis tarapacana*, each of the four regions, and different combinations of each region.

Region	r_m (Adults, Seedlings)	r_m (Adults)	r_m (Seedlings)
All	0.359**	0.342**	0.364**
BN	0.024	-0.138	0.113
BS	0.2	0.175	0.194
A	0.797*	0.807*	0.621+
C	-0.423	0.53	0.514
BN, BS, C	0.484*	0.835*	0.500*
BN, BS	0.843*	0.576*	0.760*
BS, C	0.544*	0.614*	0.479*
BN, C	0.602*	0.835*	0.585*

Notes: BN, Bolivia North; BS, Bolivia South; A, Argentina; C, Chile; * $0.05 \leq P \leq 0.1$; ** $0.01 < P \leq 0.05$; *** $P \leq 0.01$.

Argentinean populations migrating toward lower elevations within the Altiplano. During warm interglacial periods, the populations may have persisted only in isolated populations in the highlands. In accordance, the fossil record of *Polylepis*-type pollen covering multiple glacial/interglacial periods in the central Andes confirms several vertical migration cycles of *Polylepis* species in response to past climate change events, and a wider distribution during glacial periods (Gosling et al., 2009).

Focusing on within-cluster differentiation, we only detected significant isolation by distance for the Argentinean cluster. Thus, the Argentinean forest plots were somewhat more genetically differentiated than those of Bolivia and Chile, suggesting slightly stronger spatial isolation and enhanced genetic drift. In northern Argentina, topography is more divided, and higher mountain crests may represent barriers to pollen and seed flow (Hensen et al., 2011). The absence of isolation by distance across Bolivia and Chile could be accounted for by the relatively weaker landscape boundaries and the more continuous habitat of *P. tarapacana*, and thus pollen could disperse over long distances as found for *P. australis* (Seltmann et al., 2009).

We did not find a correlation between genetic diversity and elevation, which contrasts with previous results for *P. australis* and *P. incana* (Hensen et al., 2011, 2012). We assume that this contrasting pattern is the result of the more continuous elevational distribution of *P. tarapacana*, compared with *P. incana* and *P. australis* where fragments were sampled. Thus, although *P. tarapacana* most probably migrated up- and downslope during the Pleistocene interglacial–glacial periods, such patterns are not evident in its genetic signature, due to the extensive gene flow in continuous populations along elevations.

In accordance with our initial hypothesis, genetic diversity of *P. tarapacana* decreased toward the equator. In part, this decrease may be related to decreasing aridity because we also found a negative relationship between annual mean precipitation and genetic diversity in *P. tarapacana*, which explains variation in genetic diversity better than latitude. The pattern of decreasing genetic diversity toward the north is consistent with our previous study of *P. australis*, which has both higher genetic diversity (Hensen et al., 2011) and higher variability of ploidy levels (Kessler et al., 2014) in the southern extent of its range. The genetic pattern of *Podocarpus parlatorei* also revealed the long-term persistence of cold-tolerant elements in the northern Argentinean Andes adjacent to our Argentinean cluster (Quiroga and Premoli, 2007). These findings suggest that southern populations of these species persisted during glacial periods, while northern populations would have been relatively young. A possible interpretation is that *P. tarapacana* and other cold-adapted high Andean plant populations may have persisted in northern Argentina because here, the high western Andean range is closer to the eastern cloud forests and is separated by a lower eastern range in comparison to the Bolivian and Chilean populations. In addition, the northern Andes of Argentina are known to harbor higher levels of bird endemism than adjacent regions of Bolivia, which has been linked to historical ecoclimatic stability (Sandel et al., 2011). It thus seems likely that this geographical region would have served as a refugium for montane taxa during past climatic shifts.

In conclusion, the geographical patterns of genetic diversity, in particular the high levels of genetic connectivity found in our study, support the idea of a historically more widespread distribution of the treeline species *P. tarapacana*, possibly during cooler Pleistocene

periods. In addition, we suggest that the four geographical clusters have and could serve as putative refugium areas or reservoirs of genetic diversity, particularly for Argentinean populations of *P. tarapacana* under a global climate-change scenario.

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