Shrinking Forests under Warming: Evidence of *Podocarpus parlatorei* (pino del cerro) from the Subtropical Andes

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Abstract

Phylogeography in combination with ecological niche modeling (ENM) is a robust tool to analyze hypotheses on range shifts under changing climates particularly of taxa and areas with scant fossil records. We combined phylogeographic analysis and ENM techniques to study the effects of alternate cold and warm (i.e., glacial and interglacial) periods on the subtropical montane cold-tolerant conifer *Podocarpus parlatorei* from Yungas forests of the central Andes. Twenty-one populations, comprising 208 individuals, were analyzed by sequences of the trn*L*–trn*F* cpDNA region, and 78 sites were included in the ENM. Eight haplotypes were detected, most of which were widespread while 3 of them were exclusive of latitudinally marginal areas. Haplotype diversity was mostly even throughout the latitudinal range. Two distribution models based on 8 bioclimatic variables indicate a rather continuous distribution during cooling, while under warming remained within stable, yet increasingly fragmented, areas. Although no major range shifts are expected with warming, long-lasting persistence of cold-hardy taxa inhabiting subtropical mountains may include in situ and ex situ conservation actions particularly toward southern (colder) areas.

Key words: *cold-tolerant conifer, cpDNA phylogeography, long-term persistence, montane species,* Podocarpus, *South America, Yungas*

Mountain habitats of the subtropics can be considered relatively continuous areas that facilitated the migration of elements of austral origin that, in association with vicariant events, produced lineage diversification. In addition, the impact of varying environmental conditions with elevation resulted in complex ecological and evolutionary processes and distribution patterns (Graham 2009). Profound regional climatic changes may modify the range of montane taxa. For example, species living near the tropical tree line may face "mountaintop extinction" or may survive in disjunct populations at higher mountains or cooler latitudes (Colwell et al. 2008). Such scenarios will influence the gene pool and long-lasting persistence of any cold-tolerant species.

Range shifts in response to past climates can provide an excellent calibration for predictions on the consequences of present-day climate change. Range movements were identified by pollen studies that indicate population descent and northward migration of cold-adapted species such as *Podocarpus*, *Araucaria*, *Weinmania*, and *Drimys* (Bush et al. 1990, 1992; Colinvaux et al. 1996, 2000; Behling and Lichte 1997; Pennington et al. 2000, 2004) from tropical distribution. In

contrast, during warmer periods, these were confined to relatively isolated populations on mountain peaks and to the south (Colinvaux et al. 2000). Therefore, distribution ranges of cold-adapted species varied during cold and warm periods according to their ecological tolerances.

Phylogeographic hypothesis can be used to track range shifts in response to changes in climate, particularly in areas where continuous pollen records are scant. For example, cold-adapted species may consist of isolated warm-refugial populations during interglacials, which could be vulnerable to stochastic forces such as genetic drift and increased inbreeding that would result in the loss of genetic diversity, increased among-population divergence, and potential local extinction. Phylogeographic methods can thus be used to identify signatures of historical genetic discontinuities within contemporary populations.

The integration of a phylogeographic approach with independent proxy data such as ecological niche modeling (ENM) provides a rigorous assessment of alternative spatially explicit scenarios during different time periods and offers the ability to identify processes operating at the

level of individual populations. The use of ENM can be projected onto paleoclimate reconstructions to identify past potential distributions and suitable areas to be potentially colonized at present (Waltari et al. 2007). These extrapolate from associations between point occurrences and environmental data sets to identify areas of predicted presence on the map (Soberón and Peterson 2005). Such analyses are important especially when complementary information from the fossil record is not available as in the study area of this work. In addition, fragmentation analysis under past and present climatic scenarios may provide valuable input on range continuity. In particular, spatial patterns by distribution maps for past and present time provide a quantification of land cover change and forest loss which allow to configure grid covers for the application of landscape spatial indices.

Climate oscillations are expected to affect altitudinal and latitudinal movements. This will be particularly the case of cold-tolerant species inhabiting montane subtropical forests, which are known to have gone through range modifications in response to past climate changes (Cárdenas et al. 2011). Members of the genus *Podocarpus* inhabit tropical mountains of distinct regions of the world. Such woody elements may well be considered as paleotemperature indicators. Abundant *Podocarpus* fossils, including pollen, macrofossils, and wood, as well as tree rings have been extensively used to reconstruct vegetation history and climate at distinct time scales in other tropical and temperate forests. However, no such evidence from southern-most subtropical montane forests is yet available to be used for biogeographic reconstruction within our study area. For that reason, we hereby combined molecular data with ENM and fragmentation analyses, which can provide information on range shifts.

Similarly to *Nothofagus*, *Podocarpus* is a genus of austral (i.e., cold) origin. While *Nothofagus* is considered a key genus that portrays patterns of vicariance and dispersal from southern land masses (Van Steenis 1971), *Podocarpus* can be used as a model to analyze wide-ranging biogeographical questions. This is because it is widespread in all austral territories once united in Gondwana, including Africa, and although nowadays mostly restricted to mountain areas, it also enters into tropical latitudes where it may occur as the unique conifer. Profound regional climatic changes may modify the range of montane taxa. For example, species living near the tropical treeline may face "mountaintop extinction" or may survive in disjunct populations at higher mountains or cooler latitudes (Colwell et al. 2008). Such scenarios will influence the gene pool of any widespread species. This is particularly the case of *Podocarpus parlatorei* Pilg. that inhabits montane Yungas forests of the central Andes of northern Argentina and southern Bolivia. Genetic evidence by means of isozyme polymorphisms on *P. parlatorei* provided evidence of altitudinal descent and northern expansion during cooling (Quiroga and Premoli 2007). However, the question still remains if current populations are the remnants of a previously extended natural range over glacial periods that became locally fragmented during warming, and thus if

historical patterns of gene flow and genetic drift were preserved through time. In particular, previous isozyme results reflect contemporaneous movements associated with warmer trends, such as the current interglacial. In this paper, we included the organellar data to test if *P. parlatorei* 1) had a formerly widespread and more continuous range in relation to cold weather with the intent to determine the distribution of the species during the Last Glacial Maximum (LGM), 2) suffered from distribution shifts through time by local migrations from relatively stable areas that have existed throughout its current range, and 3) underwent a natural fragmentation process under warming. The integration of molecular data with ENM is relevant because of the lack of fossil records within *P. parlatorei* current range.

In this study, we combine phylogeographical analysis by means of sequences of nonrecombinant regions of the chloroplast DNA with paternal inheritance, ENM, and fragmentation analysis of *P. parlatorei* to portray past, present, and future biogeographical settings.

Materials and Methods

Study Species

Podocarpus parlatorei is a cold-tolerant species and the unique conifer that occupies the eastern-most flanks of the Andes of northwestern Argentina and Bolivia. It occurs within Montane Yungas Forests, between 14° and 27°S and elevations that vary from 1200 to 3000 m above sea level (a.s.l.). Wide river valleys cross *P. parlatorei* distribution, latitudinally and longitudinally. As a result, populations are naturally fragmented and thus significantly controlled by Andean geomorphology. In Bolivia, it grows on the Peruano–Boliviano Yungas and continues to the south in Argentina, on the Boliviano–Tucumano formation (Navarro and Ferreira 2004). These areas are naturally fragmented by lowland Chiquitano subandino forest and interandino xeric valleys. In northern Argentina, the Boliviano–Tucumano formation is also fragmented in 3 ecological and latitudinal sectors, recognized as north, central, and south (Brown and Ramadori 1989).

Podocarpus parlatorei has a variable height, between 15 and 30 m, and a diameter of up to 1.5 m. It is considered a pioneer species, and abundant regeneration is associated with large-scale disturbances of anthropogenic and natural origin (Arturi et al. 1998, Blendinger P, unpublished data). For example, it is the most abundant species in postgrazing secondary forests (Carilla and Grau 2010). At its southern limit, *P. parlatorei* generally occurs in pure forests, whereas in the north generally grows underneath the canopy of *Alnus acuminata*, *Cedrela lilloi*, and *Juglans australis* (Morales et al. 1995). The species is wind pollinated and fruits are dispersed by birds such as guans (*Penelope*) (Blendinger P, personal communication) and band-tailed pigeon (*Patagioenas fasciata*), or mammals such as hog-nosed skunk (*Conepatus chinga*) (Salinas R, Environmental Secretary, Catamarca, Argentina, personal communication). *Podocarpus parlatorei* is an important commercial timber, banned from international commerce by

Table 1 Location of sampled populations of *Podocarpus parlatorei* from Bolivia (1–4) and Argentina (5–21)

Name	Population	Province	Latitude (S)	Longitude (W)	Elevation (m a.s.l.)	N cpDNA	N Isozymes	N Haplotypes/unique haplotype	h	π
1. BO	Mizque	Cochabamba	17° 48′	65° 28′	3100	10	30	2/0	0.20	0.12
2. VS	Villa Serrano	Chuquisaca	19° 03′	64° 15′	Missing data	10	—	2/0	0.36	0.11
3. MG	Monteagudo	Chuquisaca	20°	64°	1700	8	—	2/1 H8[c]	0.57	0.35
4. TJ	Tarija[a]	O'Connor/Arce	21° 25′	64° 16'	2200	10	—	1/0	0.00	0.00
5. EN	El Nogalar[b]	Salta	22° 10′	64° 44′	1650	10	30	5/0	0.87	0.44
6. LT	Los Toldos	Salta	22° 17′	64° 41′	1600	10	30	3/0	0.51	0.25
7. SA	San Andrés	Salta	23° 04′	64° 51′	1647	10	30	4/1 H7[c]	0.71	0.34
8. CA	Calilegua[b]	Jujuy	23° 40′	64° 54′	1700	10	22	4/0	0.60	0.20
9. TX	Tiraxi	Jujuy	23° 59′	65° 18′	1500	10	20	3/0	0.71	0.27
10. TA	Tiraxi arriba	Jujuy	24° 00′	65° 23′	1900	10	21	2/0	0.53	0.16
11. EF	El Fuerte	Jujuy	24° 15′	64° 24′	1445	10	30	3/0	0.51	0.17
12. SL	San Lorenzo	Salta	24° 42′	65° 42′	2047	10	26	3/0	0.62	0.27
13. ER	El Rey[b]	Salta	24° 45′	64° 42′	1648	10	29	3/0	0.60	0.20
14. VR	Valderrama	Salta	25° 25′	65° 07′	1580	10	30	3/0	0.64	0.22
15. LC	La Candelaria	Salta	25° 59′	65° 27′	1800	10	30	3/0	0.38	0.17
16. SM	Sierra Medina	Tucumán	26° 25′	65° 21′	1400	10	30	3/0	0.71	0.27
17. TF	Taficillo[b]	Tucumán	26° 43′	65° 21′	1700	10	30	4/0	0.71	0.26
18. LB	La Banderita	Tucumán	27° 19′	65° 56′	1712	10	10	3/1 H1[c]	0.38	0.17
19. PG	Pinar Grande	Catamarca	28° 07′	65° 54′	1616	10	26	3/1 H1[c]	0.73	0.28
20. TG	Tintigasta	Catamarca	28° 24′	65° 28′	1398	10	30	3/1 H1[c]	0.20	0.12
21. CC	Concepción	Catamarca	28° 37′	66° 02′	1040	10	12	3/1 H1[c]	0.38	0.17

Population diversity parameters by means of number of haplotypes, haplotypic diversity (*h*), and nucleotide diversity expressed in percent (π).

[a] Samples from BOLV and LPB Herbaria.

[b] Populations within protected areas National Park, National Reserve, Province Park.

[c] Haplotype following Table 2.

CITES (Appendix I) due to massive logging (CITES 2007) and listed as data deficient by the IUCN (Conifer Specialist Group 1998).

Population Sampling and DNA Extraction Protocol

We sampled 21 natural populations across the Yungas forest in northwestern Argentina and southern Bolivia, covering most of *P. parlatorei* natural range (Table 1). Samples at each population consisted of leaf tissue collected from at least 10 individuals. Five individuals from each population were kept dry in silica gel while the other five were stored as protein homogenates at −80 °C that were used in a population genetic study by means of isozymes (Quiroga and Premoli 2007). In addition, dry plant material was obtained from 3 other locations in Bolivia. These consisted of herbarium specimens from Herbaria at La Paz and Cochabamba, Bolivia. In total, we analyzed 208 individuals. DNA extraction was performed by using the DNeasy Plant-Mini-Kit (QIAGEN) following the manufacturer's instructions from dry tissue and isozyme homogenates following Arbetman and Premoli (2011).

DNA Isolation and cpDNA Polymorphisms

We amplified distinct DNA regions by polymerase chain reaction (PCR). These included 9 universal noncoding chloroplast, 6 mitochondrial and the ITS regions (Supplementary Material). Polymorphism was only obtained for the chloroplast intergenic spacer *trnL–trnF* with known paternal

inheritance within Podocarpaceae (Wilson and Owens 2003). Sequences were analyzed on an ABI 3100 Avant at Laboratorio Ecotono, Universidad Nacional del Comahue. Reduced sequence variation characterizes other Podocarpaceae. This was also the case with the temperate *Podocarpus nubigena* for which no polymorphism was obtained at any screened region of either the mitochondria or the chloroplast (Quiroga 2009). Some of these taxa have an evolutionary history of in situ long-lasting persistence, and therefore they consist of highly conserved genetic variants that have been preserved through time which possess a limitation for phylogeographic analyses by using cytoplasmically inherited markers.

Genetic Data Analysis

Sequences were aligned with Mega 4 (Tamura et al. 2007). For each population, we calculated different diversity parameters: alignment size, number of haplotypes (*H*), number of variable sites, number of transitions, transversions, and indels, haplotype or genetic diversity (*h*), and nucleotide diversity (π) using Arlequin v 3.0 (Excoffier et al. 2005). The relationships among different haplotypes were preformed by minimum spanning network of haplotypes with the program Network 4.5 (fluxus-engineering.com) under the assumption of Median Joining (Bandelt et al. 1999). To analyze the relationship between within-population nucleotide and haplotype diversity parameters with environmental factors, we used linear and quadratic regressions by

means of forward stepwise models using latitude as the independent variable. We compared the latitudinal distribution of genetic diversity (*h*) with that of expected heterozygosity (H_E) calculated with isozyme data (following Quiroga and Premoli 2007) from the same populations. We estimated the haplotype divergence among populations (φ_{PT}) under the model of molecular evolution (Kimura 1980) using Arlequin v.3.10 (Excoffier et al. 2005) that run 1000 permutations for statistical significance. We performed Mantel tests between population-pairs genetic distances by φ_{PT} (following Rousset 1997 as $\varphi_{PT}/[1 - \varphi_{PT}]$) with logarithmic geographical distances using GenAlEx v. 6 (Peakall and Smouse 2006).

Ecological Niche Modeling

Multiple information sources for presence localities of *Podocarpus parlatorei* were used in the distribution models. These were obtained from the sampled populations for genetic studies, the Subtropical Network of 1-ha Permanent Plots (RedSPP) belonging to Fundación ProYungas (Blundo and Malizia 2009), 0.1-ha circular plots from rapid presence surveys (Rivera L, Serrano M, unpublished data), public databases (Global Biodiversity Information facility, http://www.gbif.org/; Tropics, http://www.tropicos.org/Home.aspx), and the National Herbarium of Bolivia. All presence localities were thoroughly revised and doubtful herbarium specimens and/or those containing geographic coordinate problems were discarded. This resulted in a total of 78 presence localities throughout the latitudinal gradient of the species' distribution in Argentina and Bolivia.

Past and present distributions were derived from models based on bioclimatic variables that best explained current occurrences of *P. parlatorei*. Current and LGM (approximately 21 000 years ago) climatic variables available on WorldClim (http://www.worldclim.org/download) with an approximate resolution of 0.86 km^2 within the study area represented annual and seasonal trends. These included temperature and precipitation extreme values for the period between 1950 and 2000 (Hijmans et al. 2005). Past LGM projections of these variables have an approximate spatial resolution of 4 km^2 in the study area. Therefore, the projections were brought to a spatial resolution of 0.86 km^2 using the Spline function at ArcGIS. For modeling climate conditions, we used Community Climate System Model (CCSM) and Model for Interdisciplinary Research on Climate (MIROC) scenarios, available on WorldClim.

From the 19 bioclimatic variables available on WorldClim, we selected those with lower self correlation (Pearson correlations <0.7, only considering presence cells) and that contributed significantly to the model. The distribution models were based on mean temperature of coldest quarter, mean precipitation of coldest quarter, precipitation of driest month, precipitation seasonality, precipitation of warmest quarter, isothermality, temperature seasonality, and mean temperature of warmest quarter. Contribution of each variable was evaluated with the Jacknife test, implemented by the program Maxent v3.0 (Phillips et al. 2006, Phillips

and Dudik 2008). These were used to model the present potential distribution (MIROC and CCSM scenarios) and projected into past climatic conditions to model the past potential distribution (MIROC and CCSM scenarios). We developed a total of 4 models: present–past distribution models based on the climatic CCSM scenario (2 models) and present–past distribution models based only on the climatic MIROC scenario (2 models).

Distribution models were developed using Maxent. This program works well with few presence data, does not require absence data, combines continuous and categorical variables, provides the contribution of each variable to the model, controls the excessive adjustment of distributions, and the output is a continuous occurrence probability. To obtain a better fit and a measure of the model dispersion, we performed 100 simulations using Maxent standard parameters. Thirty percent of presence data was used for internal validation and the remaining data was used for model construction. The overall efficiency evaluation of the model was made by the receiver operating curve, from which the indicator of the area under the curve (AUC) is derived (Fielding and Bell 1997). A value above 0.75 is considered adequate in studies oriented to management and conservation (Pearcy and Ferrier 2000). Finally, to classify the models in binary form of presence and absence areas, we determined a threshold. No exclusive procedure exists to determine this threshold (Liu et al. 2005). We used the pixel probability factor of 0.55 as cutting threshold for the current model plus its past projections. Pixels with a higher value correspond to areas with a higher probability of presence, which contain bioclimatic conditions typical for the species. Based on field information and expert knowledge, this model provides an adequate distribution of *P. parlatorei*.

Maxent generates distribution models based on present occurrences and extracts actual climatic variables and then projects the relationship of such variables using present and past climatic scenarios to develop potential present and past ranges, respectively. Therefore, each model used in this study has current and past potential ranges of *Podocarpus*. Quantification of current and past distribution areas and of stable, expansion, and retraction areas was performed with ArcGis 9.3 and the Spatial Analyst extension (ESRI, Redlands, CA). Stable areas correspond to those that were occupied in the past by *P. parlatorei* and where the species occurs today. Expansion areas are those that were not occupied by the species in the past but that currently harbor the species, and retraction areas are those that were occupied by the species in the past but where the species is absent today.

Fragmentation Analysis

Distribution maps of *P. parlatorei* were grouped into 2 types to create a binary forest/nonforest map. These maps were analyzed using ArcGIS software and its extension Spatial Analyst to quantify land cover change and forest loss and to configure grid covers for the application of landscape spatial indices. These indices were computed by FRAGSTATS vs3 (McGarigal et al. 2002) to compare the spatial pattern of

Table 2 Variable sites in eight cpDNA haplotypes at trn*L*—trn*F* of *Podocarpus parlatorei*. Indels are indicated by nucleotide initial or otherwise shown by I consisting of 13 bp

Haplotype	59	106	137	296	N	Accession number	Pattern in Figure 1
1	—	A	I × 2	A	122	JQ860351	Gray
2	—	A	I × 3	A	52	JQ860352	Vertical
3	T	A	I × 3	A	4	JQ860353	Forward diagonal
4	—	A	I × 5	A	1	JQ860354	Black
5	—	A	I × 4	A	8	JQ860355	Horizontal
6	—	A	I × 3	G	11	JQ860356	Backward diagonal
7	T	A	I × 2	A	3	JQ860357	White
8	—	C	I × 3	A	6	JQ860358	Cross diagonal

I = AGAAAGTAGAAGG.

distribution maps for past and present time. Although an extensive set of indices has been developed for quantifying patch composition and configuration at the landscape level, a number of researchers have demonstrated that most of them are redundant and only a few are required to characterize fragmentation (Imbernon and Branthomme 2001). Each of the following indices was calculated: 1) Patch area is considered as perhaps the single most important and useful piece of information contained in the landscape and has a great utility in ecology (McGarigal et al. 2002). 2) Largest patch index (LPI) approaches 0 when the largest forest patch is increasingly small and is equal to 100 when the entire landscape consists of a single forest patch. This index has recently been used to evaluate dominance of forest cover in landscapes (Armenteras et al. 2003). 3) Total edge length is important to many ecological phenomena because much of the presumed importance of spatial pattern is related to edge effects (Millington et al. 2003). 4) Aggregation index (AI) equals 0 when the focal patch type is maximally disaggregated (i.e., in the absence of like adjacencies), which rises as the focal patch type is increasingly aggregated and equals 100 when the patch type is maximum resulting in a single compact patch. 5) Patch number.

Results

DNA Sequence Analysis

Sequences of the fragment trn*L*–trn*F* on 208 individuals from 21 populations resulted in 330 bp with 4 polymorphic sites. Species' parameters of genetic diversity yielded values for haplotype and nucleotide diversity of $h = 0.5985$ and $\pi = 0.00024$, respectively (Table 1). Six sites were variable consisting of 1 transition, 1 transversion, and 4 indels. We detected a total of 8 haplotypes (Table 2). Two of those were common throughout *P. parlatorei* range. Haplotype H1 (gray) was present in all populations while haplotype H2 (vertical) tends to be less frequent at the extremes of the distribution. Two haplotypes were restricted to northern populations. These were haplotypes H3 (forward diagonal) and H4 (black), which were exclusive to MG and SA, respectively. Haplotypes H5, H6, and H7 (horizontal,

backward diagonal, and white) were mostly found in central populations. Haplotype H8 (cross diagonal) is restricted to populations of the south (Figure 1a). The minimum spanning network that was built with gaps recoded (Table 2) shows a peripheral distribution of low-frequency haplotypes (Figure 1b).

Geographic DNA Analysis

Linear regressions between haplotype and nucleotide diversities yielded nonsignificant results while quadratic regressions of haplotype diversity against latitude were marginally significant (Figure 2). No marked differences were found in the haplotype diversity with latitude, although it was relatively lower in latitudinally extreme populations. The degree of genetic differentiation among populations for haploid data was $\varphi_{PT} = 0.104$ ($P < 0.001$). The Mantel test between pairwise genetic distances by means of $\varphi_{PT}/(1 - \varphi_{PT})$ and logarithmic geographical distances yielded a significant relationship ($r = 0.166$ and $P = 0.03$).

Distribution Models

Present distribution models have an overall high efficiency (AUC values for MIROC and CCSM scenarios were 0.96 ± 0.023 and 0.96 ± 0.021, respectively). Current distribution areas predicted by both models accurately describe the actual distribution of *P. parlatorei* according to expert opinions and fieldwork evidence (Figure 3). However, the models tended to overrepresent the species distribution at some areas by approximately 25% of the current total distribution.

Past distribution models based on MIROC or CCSM scenarios show significant changes in *P. parlatorei* distribution compared with their present distributions, and these changes are similar for both models (Figure 3). Considering past LGM scenarios, the distribution was extended to the east and to lower elevations throughout Bolivia and Argentina. Current distribution changes imply a migration to higher altitudes, a retraction in the distribution toward the west, and a fragmentation at particular places along the latitudinal distribution (Figure 3). The present distributions correspond to 27% and 30% of the past distributions, for the MIROC and CCSM scenarios, respectively (Table 3).
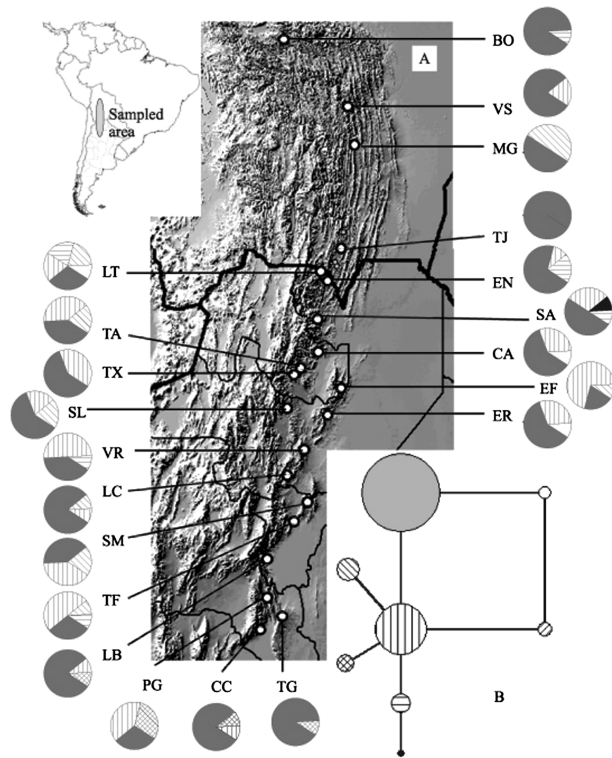
Figure 1. Haplotype distribution for cpDNA intergenic spacer trn *L*–trn *F* in 21 populations of *Podocarpus parlatorei*. (**A**) Circles represent the haplotype frequency in each population. (**B**) Median-Joining network showing the connections among haplotypes of cpDNA intergenic spacer trn *L*–trn *F* on 203 individuals from 21 populations of *P. parlatorei*. The diameter of the circles is proportional to their frequencies. Haplotype 1: gray, H2: vertical, H3: forward diagonal, H4: black, H5: horizontal, H6: backward diagonal, H7: white, and H8: cross diagonal.

Current climatic variables basically represent a marked increase in temperature with respect to the values of the LGM established for the MIROC and CCSM scenarios (Table 4). Both the average summer and winter temperatures increased 4 °C in comparison with those of LGM. As a consequence, present *P. parlatorei* distribution is 800 m a.s.l. higher than during the last glaciation.

Fragmentation

A fragmentation process is observed in the present distribution of *P. parlatorei* with respect to the LGM considering the CCSM and MIROC scenarios. This is expressed as an increase in the extension of small patches (i.e., <500 km^2), from 68 to 150 small patches in the CCSM scenario and from 72 to 172 small patches in the MIROC scenario. The other fragmentation indexes also illustrated a fragmentation process (Table 5). The largest patch index currently decreases with respect to the past distribution, indicating that during the LGM, *P. parlatorei* consisted of
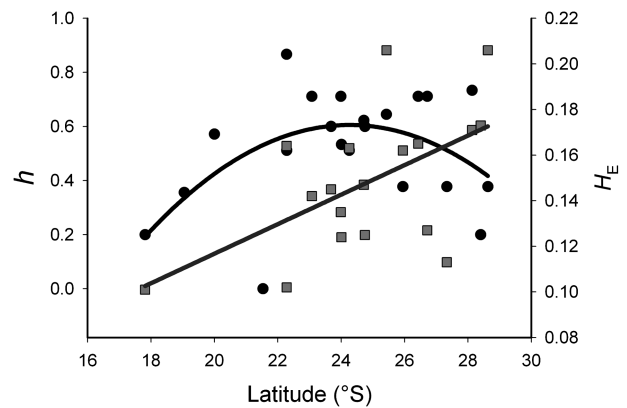

Figure 2. Distribution of genetic diversity by means of cpDNA (*h*, black circles) and expected heterozygosity (H_E, gray squares) by means of isozymes (data from Quiroga and Premoli 2007) for populations of *Podocarpus parlatorei*. Quadratic regression of cpDNA genetic diversity (*h*) in black ($R^2 = 0.25$, $P = 0.07$). Lineal regression of isozyme expected heterozygosity (H_E) in gray ($R^2 = 0.31$, $P = 0.01$).

a more continuous distribution with one or few dominant patches. The edge length increases as a consequence of the current larger amount of patches with respect to the LGM distribution. The AI decreases as a consequence of the current fragmentation and shows more dispersed patches in comparison with the past distribution.

Discussion

Presence of common haplotypes and similarly diverse populations throughout the entire range of *P. parlatorei* indicates a historically large widespread distribution. A history of continuous gene flow by means of common haplotypes possibly occurred during cold periods of the Pleistocene and early Holocene. While *P. parlatorei* is restricted at present to montane habitats, in the central Argentinean Boliviano–Tucumano sector it forms continuous and large forest stands. In contrast, it is found as isolated populations particularly at the southern and northern range margins. Although marginally significant, these populations harbored relatively lower haplotype diversity yet geographically restricted haplotypes. Probably, if population sizes continue to shrink due to warmer climates, they would suffer the effects of genetic drift.

The local presence of unique haplotypes reflects a contemporaneous signal of isolation similar to the significant geographical structure detected by nuclear markers (Quiroga and Premoli 2007). The glacial condition of a Milankovitch cycle lasts much longer than a brief interglacial, like the Holocene (Imbrie et al. 1984). Most cold-tolerant taxa of the modern forests are still well adapted even into an interglacial such as nowadays conditions, probably due to the fact that cooler times have comprised most of the last million years. The significant environmental stress to these forests comes
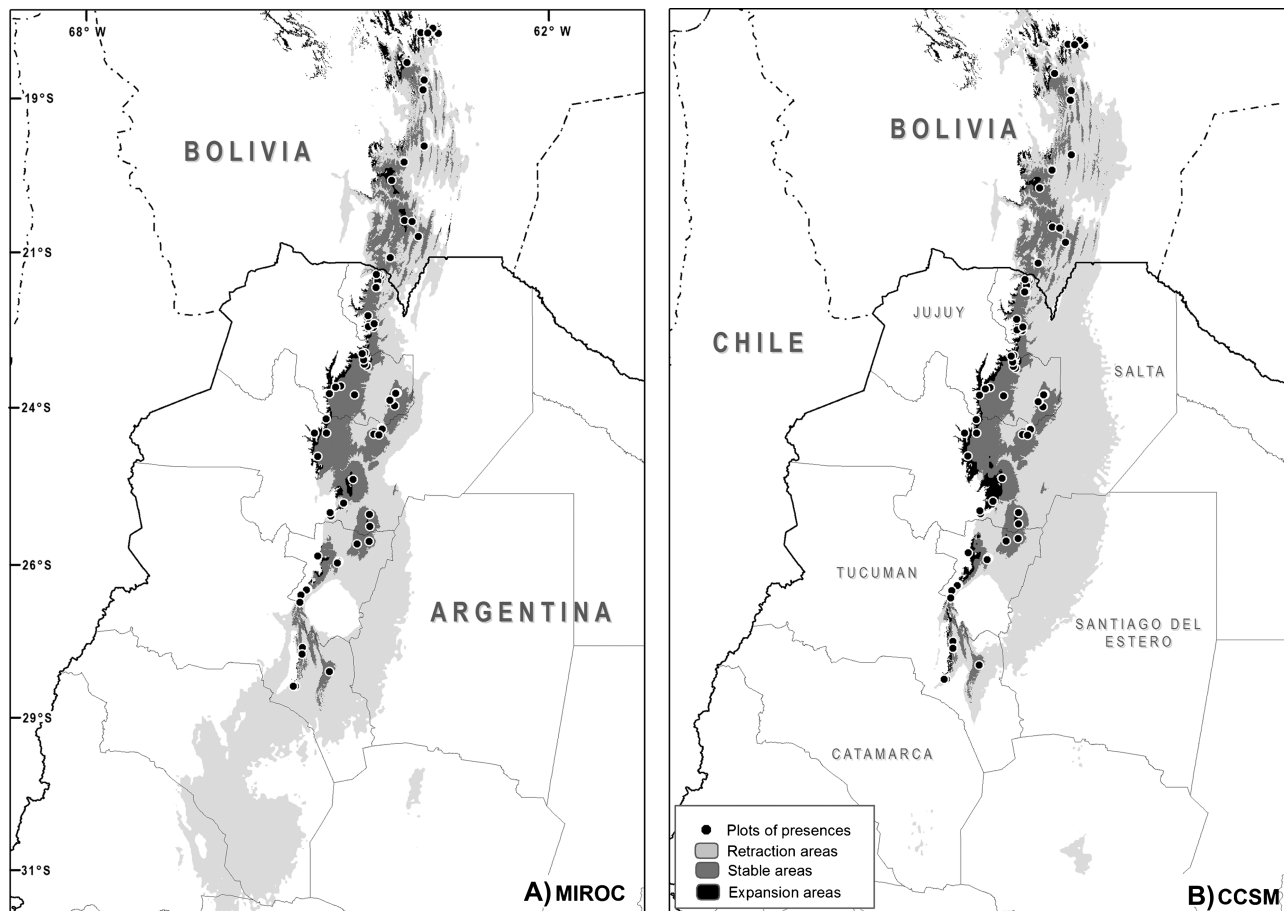
Figure 3. Present and past potential distributions of *Podocarpus parlatorei* using climatic scenarios MIROC (**A**) and CCSM (**B**). Retraction areas (light gray) are those that were occupied by the species in the past but where the species is absent today. Stable areas (dark gray) correspond to distribution areas of *P. parlatorei* that were occupied in the past and where the species occurs today. Expansion areas (black) are those that were not occupied by the species in the past but that currently harbor the species.

with interglacial warming which devastates populations that tend to migrate toward higher elevations (Colinvaux and De Oliveira 2001). As a consequence, cold-hardy biota is adapted to "normal" glacial conditions, and interglacial warm peaks can be viewed as "disturbances" (Colinvaux and De Oliveira 2001; Rull 2009). Therefore, sensitive taxa to warming had to endure upward migration to montane refugia (Bush 2002). For that reason, *P. parlatorei* shows a genetic structure due to the effects of climatic events on demography and niche conservatism.

The ENM suggests that *P. parlatorei* could have had a wide and continuous past distribution, occupying in part

what is now the Chaco forest. This is similar to the Atlantic forest that was able to maintain a reasonable degree of continuity during cooling due to the complex topography of the region and the potential for vertical migration (Carnaval and Moritz 2008). A possible advantage of cold-hardy taxa over broadleaf species during cooling is that the latter are not sufficiently tolerant to cold climates. As a result, they would be forced to descend to the lowlands, leaving a suitable niche for the development of cold-hardy species as *P. parlatorei*. However, range shifts of such taxa and its potential competitors can only be speculated, given that

Table 3 Present and past distribution of *Podocarpus parlatorei* using climatic scenarios MIROC and CCSM

	Area (km²) MIROC	Area (km²) CCSM
Past distribution	200 930	185 367
Present distribution	54 506 (27% past distribution)	56 158 (30% past distribution)
Retraction areas	157 818	142 640
Stable areas	43 113	42 727
Expansion areas	11 393	13 431

Table 4 Climatic variables along *Podocarpus parlatorei* distribution, for present and LGM past conditions (MIROC and CCSM scenarios)

	LGM	Present	Change
MIROC scenario			
Precipitation seasonality	88.62 (±7.51)	87.51 (±7.51)	1.11
Mean temperature of coldest quarter (°C)	8.77 (±2)	12.78 (±2.1)	−4.01
Mean precipitation of coldest quarter (mm)	17.23 (±10.01)	18.15 (±9.85)	−0.92
Mean precipitation of warmest quarter (mm)	325.14 (±126.4)	317.77 (±112.5)	7.37
Temperature seasonality (°C)	4455 (±1069)	4470 (±1066)	−15
Mean temperature of warmest quarter (°C)	19.76 (±2.41)	23.81 (±2.38)	−4.05
Isothermality	5.14 (±0.54)	5.13 (±0.59)	0.01
Precipitation of driest month (mm)	3.78 (±2.21)	4.09 (±2.26)	−0.31
CCSM Scenario			
Precipitation seasonality	88.78 (±6.57)	88.05 (±6.61)	0.73
Mean temperature of coldest quarter (°C)	10.10 (±2.13)	14.37 (±2.17)	−4.27
Mean precipitation of coldest quarter (mm)	20.21 (±11.1)	20.47 (±11.3)	−0.26
Mean precipitation of warmest quarter (mm)	409.89 (±84.83)	390.05 (±73.18)	19.84
Temperature seasonality (°C)	3888 (±791)	3903 (±791)	−15
Mean temperature of warmest quarter (°C)	19.63 (±2.18)	23.93 (±2.38)	−4.3
Isothermality	5.29 (±0.5)	5.37 (±0.5)	0.08
Precipitation of driest month (mm)	3.78 (±2.29)	3.89 (±2.46)	−0.11

The variables are organized according to their importance in the formulation of the model.

limited information on their biotic requirements is available. Pollen data from Amazonian and Tropical Andes postulated the altitudinal descent of cold-tolerant plants and movements to lower latitudes during cooling (Bush et al. 1990; Behling and Lichte 1997; Colinvaux et al. 1996, 2000; Hooghiemstra and van der Hammen 1998; Colinvaux and De Oliveira 2001; Urrego et al. 2005; Bush and De Oliveira 2006; Ledru et al. 2007). No such information exists for subtropical areas of Yungas. The most surprising result of ENM was to detect stable areas along the latitudinal range. Sediments in lowland western Amazonia yielded a continuous glacial record of a montane cloud forest, which reveals the constancy of these communities during periods of major global climate change (Urrego et al. 2005). As in the case of *P. parlatorei*, cold-tolerant taxa from subtropical mountains could have perhaps survived locally the glacial eras in the same, or nearby, areas that currently occupies without major latitudinal movements. This is consistent with widely distributed haplotypes that suggest local expansion–retraction from long-lasting core areas through time.

The fragmentation analysis indicates that populations at present tend to be fragmented by the availability of suitable niches for survival, which are naturally sheltered by the

mountainous terrain. Therefore, *P. parlatorei* consists of relatively isolated populations. These are distributed through a widespread latitudinal range and located toward the west of the ancient distribution. Current distribution is shaped by higher precipitations on eastern flanks of mountains and lower temperatures of high elevation areas. As a result, present *P. parlatorei* distribution is fragmented partially due to warming. Also, its range maybe limited by competition. Montane subtropical forests at mid- to low-elevation slopes are inhabited by dense humid multicanopy rainforest composed primarily of evergreen species. Ecological studies on forest dynamics of *P. parlatorei* showed that it rarely recruits within the close canopy of mature forests. On the contrary, abundant regeneration occurs in open areas after large-scale disturbances. While recruiting is intense at forest edges, regeneration in contiguous grassland is nil due to the absence of major dispersal agents. However, once few individuals are able to get established, new recruits are commonly seen following a characteristic radial expansion from seed trees (Blendinger P, unpublished data). As a pioneer yet long-lived tree, it facilitates the establishment of many other shade-tolerant species. Also its fruits and seeds are a food source for endangered species and/or with restricted distribution as red-faced guan (*Penelope dabbenei*), Tucuman amazon (*Amazona tucumana*), and many other species of birds and mammals. Thus, *P. parlatorei* provides key ecological functions for the maintenance of montane forests and a great potential for recovery plans of degraded land by overexploitation and erosion of mountain habitats.

Montane tropical forests dominated by cold-tolerant elements have probably faced local range contractions during past warm periods without major latitudinal shifts and from long-lasting stable areas. However, increasing fragmentation under current warming trends, which in

Table 5 Fragmentation indices (LPI: largest patch index, TEL: total edge length, AI: aggregation index, PN: patch number) for present and LGM past distributions (CCSM and MIROC scenarios)

	LPI (%)	TEL (km)	AI (%)	PN
CCSM LGM	2.9	11.5	98.7	68
CCSM present	0.6	25.4	89.1	150
MIROC LGM	3.1	14.7	98.4	72
MIROC present	0.5	26.0	88.5	172

combination with human-induced disturbances such as logging for the paper industry and clearance for agriculture and cattle grazing, may impose new conditions. In particular, if the pace of climate change exceeds species' abilities to migrate above current tree line, species from high Andean forests will experience large population losses and consequently may face high risk of extinction (Feeley and Silman 2010). While this may threaten some populations, uphill population spreading and openings in the forest may favor colonization by light-demanding species as *P. parlatorei*. Therefore, conservation actions should include awareness of local authorities and managers to value and preserve even small populations of species inhabiting montane areas. This is a key issue to preserve population connectivity given that the degree of historical among-population admixture (i.e., by cpDNA reported here, $\varphi_{PT} = 0.104$) is similar to that depicted by nuclear, i.e., contemporaneous exchange rates, markers (i.e., isozymes $G_{ST} = 0.104$, Quiroga and Premoli 2007; $F_{ST} = 0.109$ Quiroga 2009). Alike average degree of among-population divergence yielded by nuclear and relatively conserved DNA sequences of the chloroplast suggests that *P. parlatorei* populations have maintained high gene flow rates during the last 20 000 years throughout its range. In order to preserve historical and contemporaneous gene flow rates, population connectivity should be considered into the design of conservation actions.

The presence of unique cpDNA variants toward the south, in areas where no protected areas exist deserve special attention. Such variants may reflect isolation during interglacials and/or the remains of the ancient northern expansion of hardy trees as *Podocarpus* from austral latitudes during the Miocene. Especially, southern, that is, colder, mountain areas maybe key potential source populations for future forest expansion of cold-tolerant species toward the south. Therefore, experimental trials containing distinct provenances maybe established in the south, which could be the source for ex situ conservation of genetically diverse central and northern populations and for restoration efforts in degraded areas.

Supplementary Material

Supplementary material can be found at http://www.jhered. oxfordjournals.org/.

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