RESEARCH PAPER

Historical and phylogenetic constraints on the incidence of entire leaf margins: insights from a new South American model

Luis Felipe Hinojosa[1,2]*, Fernanda Pérez[2,3], Aurora Gaxiola[2,3] and Isabel Sandoval[1,2]

[1]*Laboratorio de Paleoecología, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425 Ñuñoa, Santiago, Chile,* [2]*Instituto Milenio de Ecología y Biodiversidad, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425 Ñuñoa, Santiago, Chile,* [3]*Center for Advanced Studies in Ecology and Biodiversity (CASEB), Departamento de Ecología, Pontificia Universidad Católica de Chile, CP 6513677, Santiago, Chile*

ABSTRACT

Aim The relationship between the proportion of species with an entire leaf margin (pE) and mean annual temperature (MAT) is one of the most powerful tools for estimating palaeotemperatures. However, phylogenetic and phytogeographic constraints on this relationship have remained unexplored. Here we investigate the pE–MAT relationship for modern floristic assemblages from southern South American forests, assess its conformity to other models and test for the existence of historical constraints on pE–MAT models.

Location South America.

Methods We used samples from 30 sites located in Chile between 32° and 44° S to test for a pE–MAT relationship and compared it with four regional models. We assessed the reliability of these models for predicting MAT from instrumental records in eight modern temperate-forest localities in Chile. Additionally, palaeotemperatures for Cenozoic fossil floras were estimated. To assess historical constraints in pE, we measured the phylogenetic signal in leaf margin type and the association between leaf margin and phytogeographic affiliation, defined by the distribution of genera.

Results We found a significant pE–MAT relationship for Chilean forest species that differed from Australia and Northern Hemisphere models, but not from tropical South America (TSA). Temperatures for southern South American localities predicted from the new regional model – combining Chilean and TSA datasets – were more accurate than those from previous models. We also showed that leaf margin type has a strong phylogenetic signal, which was further confirmed by the highly significant effect of phytogeographic element on leaf margin type.

Main conclusions Differences between the Chilean and other regional models are explained by historical legacy, as Chilean leaf margin types are strongly affected by phylogenetic closeness and phytogeographic elements. We highlight that leaf margin analyses should be conducted within the context of a flora with a shared history. Thus, we propose a new model for South America to estimate palaeotemperatures for regional fossil floras.

Keywords
Cenozoic, Chile, CLAMP, foliar physiognomy, Gondwana, palaeoclimate, palaeotemperature, phylogenetic signal, phytogeographic affiliation.

*Correspondence: Luis Felipe Hinojosa, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Casilla 653, Ñuñoa, Santiago, Chile. E-mail: lfhinojosa@abello.dic.uchile.cl

INTRODUCTION

Since the pioneering work of Bailey & Sinnott (1915, 1916), who first explored the correlations between leaf margin type of woody dicots and climatic variables, the relationship between the proportion of leaves with an entire margin (pE) and mean annual temperature (MAT) has become a powerful tool for reconstructing continental palaeoclimates (Wolfe, 1971, 1979, 1995; Wing & Greenwood, 1993; Wing, 1996; Gregory-Wodzicki et al., 1998; Wilf et al., 2003a,b, 2005; Greenwood et al., 2004; Hinojosa, 2005). This relationship, known as leaf margin analysis (LMA), is considered the most reliable proxy for estimating Cenozoic palaeotemperatures (Wolfe, 1979; Wilf, 1997). Although positive linear pE–MAT relationships were established from data for contemporary plant communities with distinctive biogeographical contexts, such as Asia (Wolfe, 1979), Australia (Greenwood et al., 2004), North and Central America (Wolfe, 1993; Wiemann et al., 1998), Europe (Traiser et al., 2005), Africa (Jacobs & Deino, 1996) and tropical South America (Gregory-Wodzicki, 2000a; Kowalski, 2002), significant differences among them have been found. A common explanation for these differences is the alleged influence of biogeographic history and phylogenetic inertia on leaf morphology (Jordan, 1997; Kowalski, 2002; Greenwood et al., 2004; Aizen & Ezcurra, 2008). Nevertheless, the influence of phylogenetic closeness of a regional flora on LMA has not been assessed in previous studies.

Cenozoic palaeotemperatures for southern South America have been estimated by combining data from floras of the Northern Hemisphere, Australia and tropical South America, without consideration of biogeographical and historical differences among regions (Hinojosa, 2005; Hinojosa & Villagrán, 2005). Southern South American forests are a strongly endemic plant assemblage with a complex biogeographic history (Armesto & Rozzi, 1989; Arroyo et al., 1995; Villagrán & Hinojosa, 1997; Aizen & Ezcurra, 1998). Therefore errors in the estimated palaeotemperatures could be expected when using models derived from other world floras. Accordingly, Aizen & Ezcurra (2008) showed that equations developed from data for Northern Hemisphere floras tend to overestimate palaeotemperatures of southern temperate rainforests by 6–10 °C. These authors developed a new pE–MAT model for southern South American forests by dividing the whole temperate forests range (between 40 and 55° S) in 16 slices of 1° of latitude, and calculating pE for each interval using species latitudinal ranges. This slice approach, however, could bias the model given the increasingly smaller area involved in each interval from north to south. Hence, a more extended regional dataset, using classical LMA based on leaves and temperatures of local floras, is necessary to obtain reliable estimates of palaeotemperatures for the region.

Southern South American forests represent a biogeographic 'island' surrounded by arid and semi-arid ecosystems, the Pacific Ocean, the Andean mountain range and the Patagonian steppe on the western side of the Andes, and are therefore characterized by a uniquely high proportion (32%) of endemic genera (Arroyo et al., 1995; Villagrán & Hinojosa, 1997; Hinojosa et al., 2006a). Furthermore, one third of the temperate forest woody genera are related to tropical and subtropical floras from Australasia and the Neotropics. The blend of Austral-Antarctic, Australasian, Neotropical and endemic phytogeographic elements that make up the flora of southern temperate forests can be traced back to the ancient palaeofloras that occupied southern South America mainly in pre-Pleistocene times (Villagrán & Hinojosa, 1997; Wardle et al., 2001; Hinojosa et al., 2006a). Some notable features of these temperate rain forests, such as the unexpectedly high incidence of plant–animal mutualisms (Armesto & Rozzi, 1989; Aizen & Ezcurra, 1998) or the persistence of many original foliar physiognomies (Hinojosa et al., 2006a), have been attributed to their persistence under past warmer tropical-like conditions. Aizen & Ezcurra (2008) suggested that the high incidence of woody species with entire leaf margins is also a legacy of this warmer past. The rich combination of taxa from different biogeographic origins makes southern South American temperate forests a great natural laboratory for gaining new insights into the historical effects that influence leaf margin types.

In this context, this work seeks to: (1) assess the pE–MAT relationship for woody species from the southern South American forest region using data from 30 Chilean localities; (2) compare the new Chilean pE–MAT relationship with previous models from other regions of the world; (3) test the accuracy of the new and previous models for predicting modern MAT for eight forest localities in Chile; (4) assess the similarities between previous palaeotemperatures for fossil floras of southern South America and those obtained using the new pE–MAT model; and (5) evaluate the influence of historical constraints on the pE–MAT relationships for southern South America, measuring the phylogenetic signal in leaf margin type, and the association with phytogeographic affiliation, defined by distribution of genera.

METHODS

Chilean dataset

We used the dataset from the Paleoecology Laboratory at the University of Chile, which includes 30 localities (Appendix S1 in Supporting Information). The dataset consists of field and herbarium collections (Rodriguez et al., 1983; Pliscoff, 2003), taken within 32° and 44° S, covering the temperature and rainfall gradient from sclerophyllous to mediterranean forests, to Valdivian and north Patagonian rainforests (Fig. 1), including sites in the Coastal Range and the Andes (Fig. 2). MAT values for each location (n = 30) were obtained from instrumental records for meteorological stations located near each site (Di Castri & Hajek, 1976; Luebert & Pliscoff, 2006). Four sites that comprised dry as well as wet forest ecosystems did not have a meteorological station in their vicinity, so MAT was obtained from an interpolated climate surface model, WORLDCLIM (Hijmans et al., 2005). Because pE can be affected by both temperature and precipitation (Royer et al., 2009), we regressed pE versus precipitation and temperature separately to assess which variable had stronger effects on pE. As we found no significant effects of
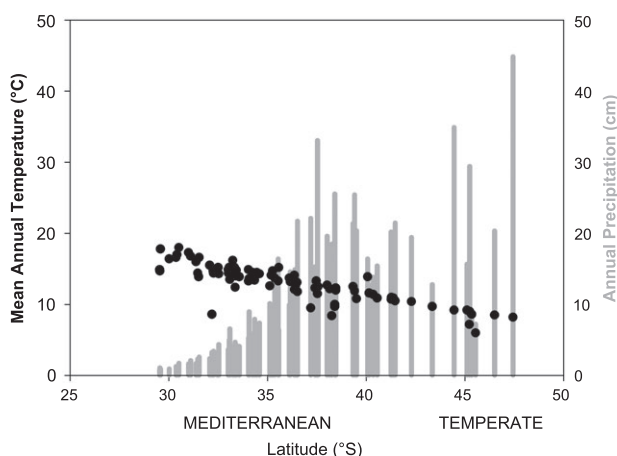
Figure 1 Latitudinal trend of mean annual temperature (solid dots) and annual precipitation within the range of Chilean forest (grey bars) from Mediterranean to cool-temperate climates.

precipitation on pE ($\beta = -0.006 \pm 0.17$; $t_{28} = 0.03$, $P = 0.97$), we felt confident about including sites with contrasting precipitation regimes. Leaf margin analyses were done for dicotyledonous woody taxa only (Appendices 1 & S1).

Regional datasets

For North and Central America and Japan data are from 144 localities included in the CLAMP dataset (Climate Leaf Analysis Multivariate Program; Wolfe, 1993). This dataset is a subset of the original of 173 localities, from which the coldest and driest sites were removed by Wing & Greenwood (1993) and Wilf (1997). For East Asia the original linear regression is from Wolfe (1979) later derived by Wing & Greenwood (1993). The dataset contains 34 sites. Data included for tropical South America are from 44 sites in Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru and Venezuela (Fig. 2), published by Gregory-Wodzicki (2000a), Kowalski (2002) and Wilf (1997). Australian data are from 74 sites and were published by Greenwood *et al.* (2004).

Modern and fossil assemblages used for MAT prediction

To test the accuracy of the new and previous models for predicting modern Chilean MATs, we used, as a control, leaf-litter that could represent a 'fossil' sample of modern vegetation; such a sample was recovered from an area of 6 m² in the bed of the Rio Caunahue. In addition, we included seven other sites as modern controls (Rodriguez *et al.*, 1983; Pliscoff, 2003), corresponding to an area of 0.5×0.5 degrees of latitude and longitude, between 35° and 38° S. We also considered published fossil data for Palaeogene sites, including Palacio de Los Loros, Palaeocene, Argentina (Iglesias *et al.*, 2007), Laguna del Hunco, Eocene, Argentina (Wilf *et al.*, 2005), Ligorio Marquez, Eocene, Chile (Hinojosa *et al.*, 2006b), and the Neogene sites of Matanzas and
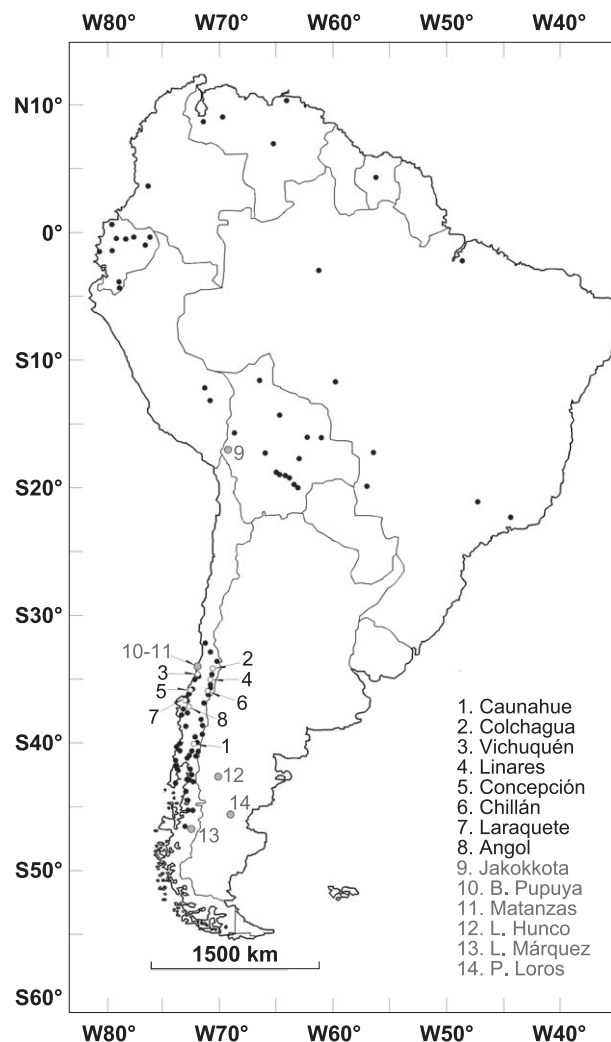

Figure 2 Locations used for physiognomic leaf margin analysis and mean annual temperature (MAT) predictions. Solid dots indicate Chilean localities sampled in this study and tropical South American localities taken from Wilf (1997), Gregory-Wodzicki (2000a) and Kowalski (2002). Numbered dots indicate modern localities (white dots, 1–8) and fossils floras (grey dots, 9–14) used for MAT predictions.

Boca Pupuya, Chile (Troncoso, 1991; Hinojosa & Villagrán, 2005), and Jakakkota, Bolivia (Gregory-Wodzicki *et al.*, 1998). The geographic locations of modern and fossil collection sites are shown in Fig. 2.

Statistical analysis

Simple linear regression analyses were used to explore the pE–MAT relationship for Chilean sites. For pairwise comparisons between Chile and other regional models, a general linear model $Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 XZ + \varepsilon$ was fitted to the data, where temperature is the response variable (Y), pE is the explanatory variable (X) and Z is a dummy variable that takes value of 1 for the Chilean dataset and 0 for a regional one.

Coincidence between regressions, parallelism and equality of intercepts tested the significance of Z and the interaction term XZ. Bonferroni corrections for multiple comparisons were calculated as $\alpha = 0.05/4$. Analyses were done in R (R Development Core Team, 2005).

Historical constraints on LMA

A phylogenetic tree of all the plant species in the Chilean dataset was assembled using the phylogeny of angiosperms at subfamily level from the Angiosperm Phylogeny Group (Stevens, 2001 onwards) as a backbone. The lineages for lower taxonomic levels were obtained from Landrum (1981), Olmstead & Palmer (1992), Hoot & Douglas (1998), Manos (1997), Chanderbali *et al.* (2001), Panero & Funk (2002), Goertzen *et al.* (2003), Sulaiman *et al.* (2003), Kim *et al.* (2004), Wilson *et al.* (2005) and Bottini *et al.* (2007). When relationships between genera or congeneric species were poorly resolved or unknown, clades were entered as polytomies and all genera and tribes were assumed to be monophyletic. The ancestor-states method of Maddison & Slatkin (1991) implemented in the program Mesquite (Maddison & Maddison, 2009), was used to measure phylogenetic signal in leaf margin type. Leaf margin type (entire versus toothed) was mapped on the assembled phylogenetic tree, the ancestral states were reconstructed using maximum parsimony, and the number of evolutionary transitions was then estimated. The observed number of transitions in leaf margin was compared with a null distribution using 1000 permutations. A *P*-value was calculated as the percentage of values of the null distribution that were less than or equal to the observed. If a trait evolves fewer times than expected by random, then it can be concluded that the trait has evolved sufficiently slowly to retain phylogenetic information and thus has been subject to historical inertia.

A second approach to evaluate the existence of historical constraints on the incidence of entire leaf margins in the Chilean flora was to test the effects of phytogeographic affiliation on leaf margin type. Because there may be climatic differences along the current distribution of phytogeographic groups, which could also affect the incidence of entire leaf margins, we decided to test for confounding effects of species thermal environment on the leaf margin–phytogeographic affiliation relationship. For this we first defined six phytogeographic elements in the Chilean forest assemblage based on their modern distribution, and classified species accordingly (from Willis, 1985; Mabberley, 1987; Villagrán & Hinojosa, 1997; Gayó *et al.*, 2005; Hinojosa *et al.*, 2006a) (for more details see Appendix 2). Secondly, as a proxy of species thermal environment we used the median of the MATs (mT) of the sites in which each species was present (Appendices 1 & S1). We used a generalized linear model with binomial error and a logit link; with leaf margin type as a response variable (entire = 1, toothed = 0) and phytogeographic affiliation and mT as predictor variables. Analyses were done in R (R Development Core Team, 2005).

RESULTS

Chilean model and inter-regional model comparisons

The pE for the Chilean forest flora, from mediterranean to sub-antarctic latitudes, ranged from 0.24 to 0.55, placing this regional flora towards the lower end of most regional databases (Fig. 3). Even for this short range of pE we found a significant pE–MAT relationship (Table 1), confirming the world-wide positive relationship between pE and MAT (Fig. 3). Notably, the Chilean model did not differ significantly from the tropical South American one, but it differed from other regional models (Table 2). We found significant differences between the slopes of the Chilean and East Asian models only. In contrast, intercepts were consistently higher for the Chilean model than for the other regional models (Table 2, Fig. 3). Given that Chilean and tropical South American models were fairly coincident, we combined these datasets into a single new model: the South American (SA) model (Table 1) which in turn comprised a range of pEs from 0.24 to 1.00. The new SA model also differed significantly from CLAMP, East Asian and Australian datasets (Table 2).

Differences in MAT estimates from pE of modern and fossil floras

Comparisons of MAT estimates for Rio Caunahue and seven other modern locations, showed that the CLAMP and East Asian models overestimate current MAT on average by 2.4 °C (range 0.9–3.6 °C) and 2.7 °C (1–4.4 °C), respectively (Table 3). In contrast, estimates from the new SA model differed from current temperatures by 1.1 °C (0.8–2.1 °C) on average. Palaeotemperature estimates for six fossil floras using the new SA model were on average 1.5 °C and 2.7 °C lower than those estimated using the CLAMP and East Asian models, respectively (Table 3).

Historical constraints

We found a highly significant phylogenetic signal in the leaf margin type of Chilean forest flora; the number of character transitions was lower than that expected by chance (observed 24 versus expected 55, $P < 0.001$; Fig. 4). This difference was maintained even when only one species per genus was included in the analyses (observed 20 versus expected 30, $P < 0.001$), indicating that the phylogenetic signal of leaf margin is not limited to the genus level, but it actually extends to more ancient and higher taxonomic levels. Finally, we found a strong effect of phytogeographic element ($P < 0.01$), and no effect of mT ($P = 0.59$) on leaf margin type in the Chilean forest flora, thus confirming that historical constraints are important.

DISCUSSION

Phylogenetic closeness and phytogeographic affiliations had strong effects on leaf margin types, and thus historical constraints would explain the significant differences between the
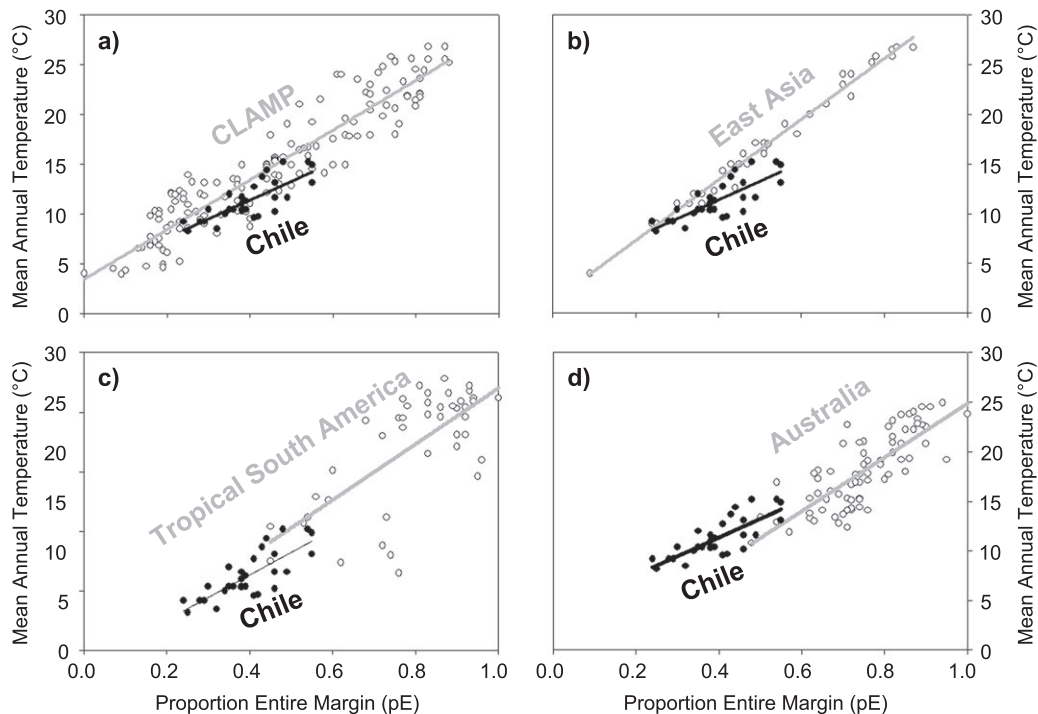
Figure 3 Panels showing comparisons among the proportion of leaves with an entire margin–mean annual temperature (pE–MAT) relationships for Chilean forests (solid dots) and Northern (a, b) and Southern Hemisphere models (c, d) (circles) Black and grey lines represent significant linear relationships. For statistics see Table 2. CLAMP, dataset for North and Central America and Japan.

Table 1 The relationship between the proportion of leaves with an entire margin (pE) and mean annual temperature (MAT) for Chilean forests and other regional datasets.

Dataset	Equation	r^2	SE	P
Chile	MAT = 18.85pE + 3.83	0.61	1.2	< 0.0001
CLAMP*	MAT = 25.00pE + 3.42	0.87	2.2	< 0.0001
East Asia	MAT = 30.55pE + 1.18	0.98	0.78	< 0.0001
TSA	MAT = 23.42pE + 3.60	0.48	3.5	< 0.0001
Australia	MAT = 27.07pE − 2.21	0.61	2.3	< 0.0001
South America	MAT = 26.03pE + 1.31	0.82	2.8	< 0.0001

CLAMP, dataset from North and Central America and Japan; TSA, dataset from tropical South America. Coefficients of determination (r^2), standard errors of the model (SE), and *P*-values are shown. South America is the new model for pE–MAT combining TSA and Chilean forest.

*Dataset comprising 144 sites taken from http://www.open.ac.uk/earth-research/spicer/CLAMP/Clampset1.html

Table 2 Comparisons of Chilean and South American regression models *vs.* regional models reported in Table 1.

Regional datasets	Chile	South America (Chile + TSA)
East Asia		
C	$F_{(2,60)} = 41.3; P < 0.001$	$F_{(2,104)} = 12.7; P < 0.001$
P	$F_{(1,60)} = 22.5; P < 0.001$	$F_{(1,104)} = 3.4; P = 0.07$
I	$F_{(1,60)} = 60.2; P < 0.001$	$F_{(1,104)} = 21.8; P < 0.01$
CLAMP		
C	$F_{(2,170)} = 12.8; P < 0.001$	$F_{(2,214)} = 8.6; P < 0.001$
P	$F_{(1,170)} = 1.7; P = 0.19$	$F_{(1,214)} = 0.5; P = 0.49$
I	$F_{(1,170)} = 23.7; P < 0.001$	$F_{(1,214)} = 16.8; P < 0.001$
Australia		
C	$F_{(2,100)} = 4.8; P < 0.01$	$F_{(2,170)} = 12.8; P < 0.001$
P	$F_{(1,100)} = 2.6; P = 0.11$	$F_{(1,170)} = 0.1; P = 0.74$
I	$F_{(1,100)} = 7.1; P < 0.01$	$F_{(1,170)} = 38.5; P < 0.001$
TSA		
C	$F_{(2,70)} = 1.3; P = 0.268$	
P	–	
I	–	

F-values testing coincidence between regressions (C), parallelism (P) and equality of intercepts (I) are shown. Significant levels were set as $\alpha = 0.05/4$ for Chilean, and $\alpha = 0.05/3$ for South American comparisons. CLAMP, dataset from North, Central America and Japan. TSA, dataset from tropical South America.

Chilean pE–MAT relationship and Australia, and Northern Hemisphere models (Table 2). In contrast, the coincidence between the Chilean and tropical South American models would reflect an ancient floristic connection that remained in place until the emergence of the modern arid and semi-arid climates during the Pliocene (Hinojosa & Villagrán, 1997, 2005; Gregory-Wodzicki, 2000b; Hinojosa *et al.*, 2006a; Garzione *et al.*, 2008). Nowadays, the evidence of a former floristic connection between the Chilean and tropical South American flora

is seen in shared plant genera such as *Myrceugenia* and *Drymis* (Landrum, 1981; Villagrán & Hinojosa, 1997; Hinojosa *et al.*, 2006a). We argue that the mosaic of phytogeographic elements that comprise South American and Chilean forests is represented in the new SA model. This new SA model, that includes 74 sites, from Colombia to Chile, comprises a wider range of South American ecosystems, with contrasting rainfall and temperature regimes, than previous models. Moreover, it provides more accurate predictions of current temperatures for southern South America than models based on the Northern Hemisphere floras.

Similarities in pE–MAT relationships between Chilean and tropical South American datasets have previously been reported by Aizen & Ezcurra (2008) who developed a pE–MAT model for southern South American forests. The model of Aizen & Ezcurra (2008), however, underestimated the current MAT of eight modern floras by 4.8 °C on average (range 2.7–6.9 °C, data not shown). We believe that the use of the 'latitudinal slice approach' produces a bias in the model and thus in the temperature estimates. This is mainly because within 1° slices altitudinal gradients may remain undetected, and this is particularly relevant in southern South America where there is a strong vegetation gradient associated with altitude. For example, at 42° S, deciduous lowland forest, broad-leaved evergreen forest, north Patagonian and subantarctic forest occur within an altitudinal gradient of 1500 m. Consequently, differences in temperature estimates between the new SA model and this previous model reflect methodological differences.

Leaf margin seems to have changed sufficiently slowly in forest lineages as to retain phylogenetic information, and it is therefore subjected to strong phylogenetic inertia (Fig. 4).

Accordingly, MAT for Chilean sites are overestimated by other regional models (CLAMP and East Asia), revealing the historical legacy of past warm conditions on the foliar physiognomy of Chilean forests, as suggested by Hinojosa *et al.* (2006a) and Aizen & Ezcurra (2008). Northern Hemisphere models also overestimated MATs for Australian and New Zealand sites, probably a result of the historical differences between hemispheres (Jordan, 1997; Greenwood *et al.*, 2004). It is noteworthy that differences within the Southern Hemisphere are observed in modern Australian and Chilean models (Table 2), highlighting the effects of contrasting historical paths on the local flora of these former Gondwanan lands (Greenwood *et al.*, 2004). The influence of phylogeny on pE in Chilean flora is confirmed by the significant effects of phytogeographical elements on leaf margin type. For example, the proportion of leaves with entire margins in Pantropical and Neotropical elements exceeded 45% (Fig. 5): these elements originated under warm climates 45–60 Ma and are the oldest components of southern South American forests (see Fig. 1 in Hinojosa *et al.*, 2006a). In contrast, Austral–Antarctic plant genera exhibited the lowest incidence of entire leaves (15%; Fig. 5); these floristic elements became dominant 35 Ma, under cool conditions (Hinojosa *et al.*, 2006a). Undoubtedly, historical constraints at different temporal scales are a distinctive character of the southern South American biota highlighted in the current distribution of taxa with different phylogenetic histories and physiological requirements, such as plants, mammals and butterflies (Villagrán & Hinojosa, 1997; Samaniego & Marquet, 2009).

Our study supports the suggestion that leaf margin type is constrained by phylogeny, and hence leaf physiognomy of a given flora is not independent of floristic composition (Jordan,

Table 3 Predicted values of mean annual temperatures (pMAT) for eight modern localities and six southern South American fossil floras, estimated with the following proportion of leaves with an entire margin–mean annual temperature (pE–MAT) models: North, Central America and Japan (CLAMP), South America, and East Asia (Table 1).

Site	Age	Species	pE	cMAT	CLAMP			East Asia			South America		
					pMAT	SE	bE	pMAT	SE	bE	pMAT	SE	bE
Caunahue	Modern	36	0.44	12.3	14.4	2.2	2.1	14.6	0.8	2.5	12.8	2.8	2.2
Colchagua	Modern	32	0.48	12.1	15.4	2.2	2.2	15.8	0.8	2.7	13.8	2.8	2.3
Vichuquen	Modern	27	0.45	13.6	14.7	2.2	2.4	14.9	0.8	2.9	13.0	2.8	2.5
Linares	Modern	25	0.46	13.3	14.9	2.2	2.5	15.2	0.8	3.1	13.3	2.8	2.6
Concepción	Modern	47	0.49	12.7	15.7	2.2	1.8	16.1	0.8	2.2	14.1	2.8	1.9
Chillan	Modern	29	0.43	13.3	14.2	2.2	2.3	14.3	0.8	2.8	12.5	2.8	2.4
Laraquete	Modern	45	0.5	12.7	15.9	2.2	1.9	16.4	0.8	2.3	14.3	2.8	1.9
Angol	Modern	25	0.54	13.3	16.9	2.2	2.5	17.7	0.8	3.1	15.4	2.8	2.6
Jakokkota	Upper Miocene	31	0.68	8.3	20.4	2.2	2.1	21.9	0.8	2.6	19.0	2.8	2.2
B. Pupuya	Middle Miocene	27	0.88	14.6	25.4	2.2	1.6	28.1	0.8	1.9	24.2	2.8	1.6
Matanzas	Lower Miocene	28	0.54	14.6	16.9	2.2	2.4	17.7	0.8	2.9	15.4	2.8	2.5
L. Hunco	Middle Eocene	122	0.52	8.2	16.4	2.2	1.1	17.1	0.8	1.4	14.8	2.8	1.2
L. Márquez	Lower Eocene	55	0.62	6	18.9	2.2	1.6	20.1	0.8	2.0	17.4	2.8	1.7
P. Loros	Palaeocene	33	0.42	10.8	13.9	2.2	2.1	14.0	0.8	2.6	12.2	2.8	2.2

Geological age (Age), number of dicotyledonous species or palaeospecies (Species), proportion of leaves with entire leaf margins (pE) and current mean annual temperature (cMAT) are given for all localities. pMATs, one standard error (SE) and bivariate error (bE), of each prediction reported by Wilf (1997), are also given.

Figure 4 Maximum parsimony reconstruction of ancestral states for leaf margins. Solid lines represent species with entire leaf margin and light lines species with toothed leaf margins. Phylogenetic trees for the species in the Chilean dataset were assembled from published data (see Methods).
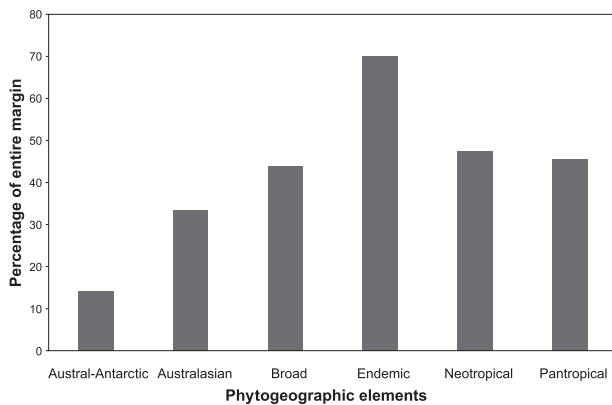
Figure 5 Incidence (%) of species with entire leaf margins in each phytogeographic element define for the Chilean forests dataset (*n* = 158 woody species) (for details see Methods and Appendix S1).

1997; Kowalski, 2002). Therefore, physiognomic methods used to reconstruct palaeotemperatures should be less reliable when large floristic differences between fossil and modern floras used to build the models are present. Hence, palaeoclimate estimates from LMA models should be corroborated with independent palaeontological or geological lines of evidence. This is particularly relevant for estimating pMATs when only few elements of current lineages are present in a fossil flora and/or in the absence of non-analogous modern environments. Error estimates from LMA, however, can be reduced when a flora derived from the regional fossil assemblage is used as the modern analogue, rather than an unrelated flora. In the case of southern South American forests, we argue that the new SA model presented here should provide more accurate estimates of palaeotemperatures for Cenozoic fossil floras than Northern Hemisphere models, as the latter floras share few floristic elements with South America. The SA model also provide better predictions than the tropical South American and Chilean models alone, because it yields a higher *r²* (0.83) than either of those two models alone and is based on a wider range of pE (Fig. 3). Palaeo-MAT estimates for pre-Pleistocene fossil floras from southern South America, using the SA model, are less divergent from present MATs than previously reported. For example, for the mid Eocene fossil flora from (current MAT of 8.2 °C), the estimated pMAT from the SA model was 14.8 °C; whereas Wilf *et al.* (2005), using the East Asian model, obtained a pMAT of 16.6 °C. For Jakokkota, a fossil flora from the Upper Miocene, the estimated pMAT from the new SA model was 9.6 °C higher than the current MAT (Table 3), whereas Gregory-Wodzicki *et al.* (1998), using the CLAMP model, estimated a pMAT 12.1 °C warmer than the present.

In summary, we show that leaf margin type in a given floristic assemblage is subjected to phylogenetic and historical constraints, and highlight the relevance of conducting LMA within the context of a shared phytogeographic history, particularly when predicting palaeotemperatures for recent periods. Therefore, we propose that the new SA model is more appropriate for estimating palaeotemperatures for Southern Hemisphere fossil floras, particularly for periods when common taxa were still present in former Gondwanan lands.

REFERENCES

Aizen, M.A. & Ezcurra, C. (1998) High incidence of plant–animal mutualisms in the woody flora of the temperate forest of southern South America: biogeographical origin and present ecological significance. *Ecología Austral*, **8**, 217–236.

Aizen, M.A. & Ezcurra, C. (2008) Do leaf margins of the temperate forest flora of southern South America reflect a warmer past? *Global Ecology and Biogeography*, **17**, 164–174.

Armesto, J.J. & Rozzi, R. (1989) Seed dispersal syndromes in the rain-forest of Chiloe: evidence for the importance of biotic dispersal in a temperate rain forest. *Journal of Biogeography*, **16**, 219–226.

Arroyo, M., Cavieres, L.A., Peñaloza, A., Riveros, M. & Faggi, A.M. (1995) Relaciones fitogeográficas y patrones regionales de riqueza de especies en la flora del bosque lluvioso templado de Sudamérica. *Ecología de los bosques nativos de Chile* (ed. by J.J. Armesto, C. Villagrán and M.T.K. Arroyo), pp. 71–99. Comité de Publicaciones Científicas, Vicerrectoría Académica, Universidad de Chile. Editorial Universitaria, Santiago.

Bailey, I.W. & Sinnott, E.W. (1915) A botanical index of Cretaceous and Tertiary climates. *Science*, **41**, 831–834.

Bailey, I.W. & Sinnott, E.W. (1916) The climatic distribution of certain types of angiosperm leaves. *American Journal of Botany*, **3**, 24–39.

Bottini, M., De Bustos, A., Sanso, A., Jouve, N. & Poggio, L. (2007) Relationships in Patagonian species of *Berberis* (Berbereidaceae) based on the characterization of rDNA internal transcribed spacer sequences. *Botanical Journal of the Linnean Society*, **153**, 321–328.

Chanderbali, A.S., Van der Werff, H. & Renner, S.S. (2001) Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden*, **88**, 104–134.

Di Castri, F. & Hajek, E. (1976) *Bioclimatología de Chile*. Editorial de la Universidad Católica de Chile, Santiago.

Garzione, C.N., Hoke, G.D., Libarkin, J.C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P. & Mulch, A. (2008) Rise of the Andes. *Science*, **320**, 1304–1307.

Gayó, E., Hinojosa, L.F. & Villagrán, C. (2005) On the persistence of tropical Paleofloras in central Chile during the early Eocene. *Review of Palaeobotany and Palynology*, **137**, 41–50.

Goertzen, L.R., Cannone, J.J., Gutell, R.R. & Jansen, R.K. (2003) ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny of the asteraceae. *Molecular Phylogenetics and Evolution*, **29**, 216–234.

Greenwood, D.R., Wilf, P., Wing, S.L. & Christophel, D.C. (2004) Paleotemperature estimation using leaf-margin analysis: is Australia different? *Palaios*, **19**, 129–142.

Gregory-Wodzicki, K.M. (2000a) Relationships between leaf morphology and climate, Bolivia: implications for estimating paleoclimate from fossil floras. *Paleobiology*, **26**, 668–688.

Gregory-Wodzicki, K.M. (2000b) Uplift history of the central and northern Andes: a review. *Geological Society of America Bulletin*, **112**, 1091–1105.

Gregory-Wodzicki, K.M., McIntosh, W.C. & Velasquez, K. (1998) Climatic and tectonic implications of the late Miocene Jakokkota flora, Bolivian Altiplan. *Journal of South American Earth Sciences*, **11**, 533–560.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hinojosa, L.F. (2005) Cambios climáticos y vegetacionales inferidos a partir de Paleofloras Cenozoicas del sur de Sudamérica. *Revista Geológica de Chile*, **32**, 95–115.

Hinojosa, L.F. & Villagrán, C. (1997) Historia de los bosques del sur de Sudamérica, I: antecedentes paleobotánicos, geológicos y climáticos del Terciario del cono sur de América. *Revista Chilena de Historia Natural*, **70**, 225–239.

Hinojosa, L.F. & Villagrán, C. (2005) Did South American mixed paleofloras evolve under thermal equability or in the absence of an effective Andean barrier during the Cenozoic? *Palaeogeography, Palaeoclimatology, Palaeoecology*, **217**, 1–23.

Hinojosa, L.F., Armesto, J.J. & Villagran, C. (2006a) Are Chilean coastal forests pre-Pleistocene relicts? Evidence from foliar physiognomy, palaeoclimate, and phytogeography. *Journal of Biogeography*, **33**, 331–341.

Hinojosa, L.F., Pesce, O., Yabe, A., Uemura, K. & Nishida, H. (2006b) Physiognomical analysis and paleoclimate of the Ligorio Márquez fossil flora, Ligorio Márquez Formation, 46°45′S, Chile. *Post Cretaceous floristic changes in southern Patagonia, Chile* (ed. by H. Nishida), pp. 45–55. Chuo University, Tokyo.

Hoot, S.B. & Douglas, A.W. (1998) Phylogeny of Proteaceae based on atpB and atpB-rbcL intergenic spacer region sequences. *Austral Systematic Botany*, **11**, 301–320.

Iglesias, A., Wilf, P., Johnson, K.R., Zamuner, A.B., Cuneo, N.R., Matheos, S.D. & Singer, B.S. (2007) A Paleocene lowland macroflora from Patagonia reveals significantly greater richness than North American analogs. *Geology*, **35**, 947–950.

Jacobs, B.F. & Deino, A.L. (1996) Test of climate-leaf physiognomy regression models, their application to two Miocene floras from Kenya and 40Ar/39Ar dating of the Late Miocene Kapturo site. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **123**, 259–271.

Jordan, G.J. (1997) Uncertainty in palaeoclimatic reconstructions based on leaf physiognomy. *Australian Journal of Botany*, **45**, 527–547.

Kim, Y.-D., Kim, S.-H. & Laundrum, L. (2004) Taxonomic and phytogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). *Journal of Plant Research*, **117**, 175–182.

Kowalski, E. (2002) Mean annual temperature estimation based on leaf morphology: a test from tropical South America. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **188**, 141–165.

Landrum, L. (1981) The phylogeny and geography of *Myrceugenia* (Myrtaceae). *Brittonia*, **33**, 105–129.

Luebert, F. & Pliscoff, P. (2006) *Sinopsis bioclimática y vegetacional de Chile*. Editorial Universitaria, Santiago.

Mabberley, D.J. (1987) *The plant book. A dictionary of higher plants*. Cambridge University Press, New York, Melbourne.

Maddison, W.P. & Maddison, D.R. (2009) *Mesquite: a modular system for evolutionary analysis.v.2.72*. Available at: http://mesquiteproject.org/mesquite/mesquite.html (accessed January 2010).

Maddison, W.P. & Slatkin, M. (1991) Null models for the number of evolutionary steps in a character on a phylogenetic tree. *Evolution*, **45**, 1184–1197.

Manos, P. (1997) Systematics of *Nothofagus* (Nothofagaceae) based on rDNA spacer sequence (ITS): taxonomic congruence with morphology and plastid sequences. *American Journal of Botany*, **84**, 1137–1155.

Olmstead, R.G. & Palmer, J.D. (1992) A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. *Annals of the Missouri Botanical Garden*, **79**, 346–360.

Panero, J. & Funk, V.A. (2002) Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). *Proceedings of the Biological Society of Washington*, **115**, 909–922.

Pliscoff, P.A. (2003) *Priorización de áreas para fortalecer la conservación de la flora arbórea nativa en la zona Mediterránea de Chile*. Magister, Universidad de Chile, Santiago.

R Development Core Team (2005) *R: a language and environment for statistical computing, reference index version 2.10.1*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org

Rodriguez, R., Matthei, O. & Quezada, M. (1983) *Flora arbórea de Chile*. Universidad de Concepción, Concepción.

Royer, D.L., Kooyman, R.M., Little, S.A., & Wilf, P. (2009) Ecology of leaf teeth: a multi-site analysis from an Australian subtropical rainforest. *American Journal of Botany*, **96**, 738–750.

Samaniego, H. & Marquet, P.A. (2009) Mammal and butterfly species richness in Chile: taxonomic covariation and history. *Revista Chilena de Historia Natural*, **82**, 135–151.

Stevens, P.F. (2001 onwards) *Angiosperm phylogeny website*. Available at: http://www.mobot.org/MOBOT/research/APweb/ (accessed Version 9, January 2010).

Sulaiman, S.F., Culham, A. & Harborne, J.B. (2003) Molecular phylogeny of Fabaceae based on rbcL sequence data: with special emphasis on the tribe Mimoseae (Mimosoideae). *Asia Pacific Journal of Molecular Biology and Biotechnology*, **11**, 9–35.

Traiser, C., Klotz, S., Uhl, D. & Mosbrugger, V. (2005) Environmental signals from leaves – a physiognomic analysis of European vegetation. *New Phytologist*, **166**, 465–484.

Troncoso, A. (1991) Paleomegaflora de la Formación Navidad, miembro Navidad (Mioceno), en el área de Matanzas, Chile central occidental. *Boletín Museo Nacional de Historia Natural, Chile*, **42**, 131–168.

Villagrán, C. & Hinojosa, L.F. (1997) Historia de los Bosques del sur de Sudamérica, II: análisis fitogeográfico. *Revista Chilena de Historia Natural*, **70**, 241–267.

Wardle, P., Ezcurra, C., Ramirez, C. & Wagstaff, S. (2001) Comparison of the flora and vegetation of the southern Andes and New Zealand. *New Zealand Journal of Botany*, **39**, 69–108.

Wiemann, M.C., Manchester, S.R., Dilcher, D.L., Hinojosa, L.F. & Wheeler, E.A. (1998) Estimation of temperature and precipitation from morphological characters of dicotyledonous leaves. *American Journal of Botany*, **85**, 1796–1802.

Wilf, P. (1997) When are leaves good thermometers? A new case for leaf margin analysis. *Paleobiology*, **23**, 373–390.

Wilf, P., Johnson, K.R. & Huber, B.T. (2003a) Correlated terrestrial and marine evidence for global climate changes before mass extinction at the Cretaceous–Paleogene boundary. *Proceedings of the National Academy of Sciences USA*, **100**, 599–604.

Wilf, P., Cuneo, N.R., Johnson, K.R., Hicks, J.F., Wing, S.L. & Obradovich, J.D. (2003b) High plant diversity in Eocene South America: evidence from Patagonia. *Science*, **300**, 122–125.

Wilf, P., Johnson, K., Cúneo, R., Smith, M., Singer, B.S. & Gandolfo, M. (2005) Eocene plant diversity at Laguna del Hunco and Río Pichileufú, Patagonia, Argentina. *The American Naturalist*, **165**, 634–650.

Willis, J.C. (1985) *A dictionary of flowering plants and ferns*, 8th edn. Cambridge University Press, Cambridge.

Wilson, P.G., O'Brien, M.M., Helsewood, M.M. & Quinn, C.J. (2005) Relationships within Myrtaceae *sensu lato* based on a matK phylogeny. *Plant Systematics and Evolution*, **251**, 3–19.

Wing, S.L. (1996) Global warming and plant species richness: a case study of the Paleocene/Eocene boundary. *Biodiversity II: understanding and protecting our biological resources* (ed. by M.L. Reaka-Kudla, D.E. Wilson and E.O. Wilson), pp. 163–185. Joseph Henry Press Book, Washington, DC.

Wing, S.L. & Greenwood, D.R. (1993) Fossils and fossil climate: the case for equable continental interiors in the Eocene. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **341**, 243–252.

Wolfe, J. (1971) Tertiary climatic fluctuations and methods of analysis of Tertiary floras. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **9**, 27–57.

Wolfe, J. (1979) Temperature parameters of humid to mesic forests of eastern Asia and relation to forests of other regions of the Northern Hemisphere and Australasia. *US Geological Survey Professional Paper*, **1106**, 1–37.

Wolfe, J. (1993) A method of obtaining climatic parameters from leaf assemblages. *US Geological Survey Bulletin*, **2040**, 1–71.

Wolfe, J. (1995) Paleoclimatic estimates from tertiary leaf assemblages. *Annual Review of Earth and Planetary Science*, **23**, 119–142.

SUPPORTING INFORMATION

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

Appendix S1 Chilean dataset for both leaf margin and biogeographic analysis in 30 localities of Appendix 1.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

BIOSKETCHES

Luis Felipe Hinojosa is an Associate Professor at the Ecology Department, Universidad de Chile and at the Millennium Institute of Ecology and Biodiversity. He is interested in the historical biogeography of southern South American forest, particularly in the palaeoecology of the Cenozoic period, based on plant systematics and leaf physiognomic analysis.

Editor: Matt McGlone

Appendix 1

Chilean dataset: Loc, localities; Nsp, number of species per site; LON, longitude; LAT, latitude; ALT, altitude; pE, proportion of species with entire margin; MAT, mean annual temperature; MAP, mean annual precipitation. W.S: Meteorological station close to the each site. For those sites where it was not possible to find a meteorological station, temperature was estimated from WorldClim (Hijmans *et al.*, 2005).

N	Loc	Nsp	LON	LAT	ALT	pE	MAT	MAP	W.S
1	Zapallar	31	−71.4	−32.55	200	0.55	14.9	374	Zapallar
2	LCampana	36	−71.13	−32.98	500	0.44	14.4	501	Quilpue
3	Vilches	40	−71.07	−35.61	1200	0.43	13.7	1380	Colorado
4	Lruiles	41	−72.50	−35.83	200	0.54	15.2	642	Cauquenes
5	L.Qeules	33	−72.69	−36.98	400	0.48	15.2	642	Cauquenes
6	Hualpen	33	−73.15	−36.79	10	0.55	13.1	1294	Concepcion
7	Llico	24	−73.59	−37.23	200	0.46	13.1	1294	Concepción
8	Nahuelbuta	29	−72.96	−37.76	600	0.38	10.3	1961	Angol
9	Contulmo	36	−73.18	−38.02	439	0.41	12.7	1961	Contulmo
10	Rucamanque	37	−72.61	−38.66	339	0.35	12	1191	Temuco
11	Villarrica	24	−71.56	−39.54	775	0.25	8.2	1298	worlclim
12	Mochochoshuenco	36	−72.13	−39.93	200	0.39	11.3	2096	worlclim
13	Cordillera Pelada Este	37	−73.35	−40.24	500	0.38	11.6	1267	La Union
14	Cordillera Pelada W	35	−73.35	−40.24	340	0.46	11.6	1267	La Union
15	Cordillera Pelada Wb	35	−73.34	−40.26	150	0.49	11.6	1267	La Union
16	Puyehue	32	−72.31	−40.74	600	0.41	9.6	2500	worlclim
17	Perez Rosales	32	−72.54	−41.02	134	0.34	10	2460	Ensenada
18	Petrohue	22	−72.41	−41.13	350	0.36	10.4	2363	worlclim
19	Valle de los Ulmos	31	−72.58	−41.29	400	0.35	10.4	2363	worlclim
20	Chiloe Senda Darwin	46	−73.67	−41.88	20	0.46	10.2	1982	Ancud
21	Horno Piren (Pichauco)	32	−72.46	−42.14	462	0.38	11	1982	Puerto Montt
22	Puente Tronador	20	−72.58	−42.66	20	0.3	10.4	1942	Castro
23	Puente Bonito	21	−72.69	−42.81	309	0.38	10.4	1942	Castro
24	Queilen	26	−73.57	−42.88	20	0.35	10.4	1942	Castro
25	Parque Tantauco	28	−73.80	−43.03	134	0.39	10.4	1942	Castro
26	P.R.Marín Balmaceda	33	−72.95	−43.77	15	0.42	9.7	1279	Isla Guafo
27	PN Quenlat	21	−72.41	−44.58	5	0.24	9.2	1565	Mañihuales
28	Mañihuales	24	−72.44	−44.64	350	0.29	9.2	1565	Mañihuales
29	Lago Riesco	25	−72.69	−45.44	500	0.28	9.2	3488	Puerto Cisnes
30	Bahia Exploradores	28	−73.16	−46.50	232	0.32	8.5	2941	Cabo Raper

Appendix 2

Phytogeographic affiliations. We defined six phytogeographic elements in the Chilean forest assemblage:

1. Austral-Antarctic – temperate elements (AA): genera distributed at mid and high latitudes of the Southern Hemisphere, including New Zealand, Tasmania, southern Australia and southern South America (e.g. *Eucryphia*, *Laurelia*).

2. Australasian tropical elements (AU): genera distributed in Southern Hemisphere territories, occasionally extending their range into tropical and subtropical regions of north-east Australia, New Guinea, Malaysia, Indomalaysia, Southeast Asia and the western Pacific islands (e.g. *Gaultheria*, *Weinmannia*). Some of these genera also occur in tropical regions of the Americas.

3. Neotropical elements (N): genera distributed in southern South American forests and in montane forests of the eastern Andes (north-west Argentina, Bolivia and Peru) and/or the Atlantic coast of southern Brazil, sometimes extending to Central America (e.g. *Azara*, *Myrceugenia*).

4. Pantropical elements (P): genera distributed in the circumtropical belt, incorporating South America, Indomalaysia, Africa and Madagascar (e.g. *Beilschmiedia*, *Cryptocarya*). Some of these genera are absent from tropical Australasia.

5. Widespread or cosmopolitan elements (W): genera with world-wide distributions, including tropical, subtropical and temperate regions (e.g. *Berberis*, *Senecio*).

6. Endemic elements (E): genera currently restricted to Chilean subtropical and temperate forests, south of 30° S. Some of these genera also occur along the western margin of the Andes in Argentina, south of 37° S (e.g. *Aextoxicon*, *Luma*; Appendix S1).