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HISTORICAL EVENTS IN PATAGONIA REFLECTED IN PHYLOGEOGRAPHIC STRUCTURE OF THE WIDESPREAD \textit{EMBOITHRIUM COCCINEUM} (PROTEACEAE)

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The evolutionary history of populations and species play a major role in determining the levels and distribution patterns of genetic variants. Particularly range contractions during Pleistocene glaciations (c. 18,000 yr. bp) followed by recolonization after glacial retreat are thought to affect genetic patterns. Hotspots of genetic diversity and among-population divergence are predicted in refugial areas whereas low polymorphism is expected in newly colonized areas due to bottlenecks through the migratory process. We tested the hypothesis that cold-tolerant elements were able to withstand glacial conditions in multiple Pleistocene refugia using phylogeographic methods in the widespread \textit{Embothrium coccineum} Forst (Proteaceae). This species is distributed along 20° latitude in temperate forests of Argentina and Chile.

We sampled 34 populations along its entire range, resolving 16 isozyme loci assayed in 934 adult trees. We also explored the chloroplast genome with trnL-F spacer to track the maternal biogeographic history in 22 individuals representing 15 of the sampled populations. Cavalli-Sforza & Edwards (1967) distances between allele frequencies were used to perform parsimony analysis using PAUP* (Swofford, 2003). Weight changes among populations were implemented using step matrices (Wiens, 2000). Heuristic search was performed with a starting tree obtained via stepwise addition. DNA sequences were aligned by eye in BioEdit (Hall, 1999), consisting in 873 bp with no ambiguities. A parsimony network was performed using TCS (Clement et al., 2000).

The most parsimonious tree (L= 3,386) shows two clades (Fig. 1). One (denoted as A in Fig. 1) includes north-central populations; however, populations Bahía Inútil (S33) and Lapataia (S34) from the south (53° - 54° S) are also members of this group. The other major clade depicts two subclades, one contains populations located south of 43° S (denoted as B in Fig. 1) with the exception of populations Quillén (N6) and Huechulaufquen (N8) located north but at higher altitudes (above 1000 m a.s.l.). The other subclade has populations located north of 41° S (denoted as C in Fig. 1). Maternal inherited cpDNA haplotypes show some congruencies with isozyme generated tree. Seven haplotypes were found (Fig. 1), four characterize northern populations and the remaining are in southern ones. Four populations were unique in their haplotypes: Troomen (N7), Nahuelbuta (N1), Alerce Andino (C15) and Cucao (C23).

Phylogeographic analysis suggests that the
Fig. 1. Most parsimonious tree from allozyme frequencies for 34 populations of *E. coccineum*. Abbreviations: N, population located north of 41°S latitude; C, population located between 41° and 43°S latitude; S, population located south of 44°S latitude. Haplotype numbers are indicated where appropriate.
presented groups of populations are indicative of multiple forest refugia where *E. coccineum* survived during the Last Glacial Maximum. Current processes such as gene flow and selection seem to confound the historic signal via isozyme. Nevertheless, palynological records advocate for local topographical heterogeneity to provide a wide range of microhabitats favourable for woody taxa to persist in numerous glacial refugia (Markgraf et al., 1995). In addition to refugial areas suggested by the pollen record on western slopes (Villagrán et al., 1996), our data adds a potential glacial refugium for *E. coccineum* at mid latitudes on the highlands of the eastern slopes of the Andes. Populations of *E. coccineum* located south of 40° S on the eastern Andes hold the highest number of private alleles and have elevated allelic diversity by isozymes. The network analysis of cp DNA supports this hypothesis placing haplotype 2 as the basal one including populations Limonao (C19), Lago Verde (C18), and Manso inferior (C17). Also, in southern South America, mid latitudes (36° - 44° S) were characterized by the smallest amplitude of climatic change and westerly storm tracks allowed the persistence of forest vegetation throughout glacial times (Markgraf et al., 1995). Therefore, distinct centres of genetic diversity can be hypothesized for *E. coccineum*. One refugium probably was located around 38° S on the western Andes. At mid latitudes, two potential refugial areas can be found: one on the eastern highlands and another on the western lowlands, and at least one austral refugium could be located south of 44° S.

Our results are in contrast to many of the northern hemisphere post-glacial phylogeographic reconstructions (Comps et al., 2001; Dumolin-Lapegue et al., 1997; Heuertz et al., 2004) which show clear historical tracks of northern range expansion from southern refugia, suggesting contrasting historical patterns between hemispheres as previously suggested (Premoli et al., 2000).

Post-glacial migration from different refugia and selection at regional scale are proposed as the main processes that shape distribution patterns of genetic variation of *E. coccineum* in Patagonia.

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**BIBLIOGRAPHY**


Villagrán, C.; P. Moreno & R. Villa. 1996. Antecedentes palinológicos acerca de la historia Cuaternaria de los bosques Chilenos (Palynological evidences about the Quaternary history of Chilean forests). In *Ecología de los bosques nativos de Chile*, Ed. Universitaria, Santiago de Chile.