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Genetic structure of *Podocarpus nubigena* (Podocarpaceae) provides evidence of Quaternary and ancient historical events

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ABSTRACT

Evolution of ancient lineages inhabiting austral latitudes of the southern Andes has taken place in response to historical processes occurring at distinct spatial and temporal scales. We tested the hypothesis that distribution patterns of genetic polymorphisms within *Podocarpus nubigena* Lindl. (Podocarpaceae) were shaped by contemporary events acting at local scales such as Quaternary glaciations as well as pre-Quaternary palaeogeographical features of the landscape. We predict that such cold hardy species was able to survive locally in refugia during ice ages. As a result of local long-lasting persistence, its gene pool may also reflect the pre-Quaternary palaeogeography of southern South America. We collected fresh leaves from 30 randomly selected individuals at 14 populations. Leaf tissue was analyzed by isozyme electrophoresis using 12 putative loci most of which (92%) were polymorphic in at least one population. Elevated total genetic diversity was recorded $H_T = 0.275$ which was similarly high throughout populations with an overall significant mean among-population isolation ($F_{ST} = 18\%$). Multivariate cluster and Bayesian analyses yielded significant latitudinal divergence at 43°S associated to a palaeobasin that produced a barrier for gene flow. Populations south of 43°S and those growing in lowland areas on Quaternary substrates of the North Patagonian Massif (Q NPM) present the highest genetic diversity suggesting long-lasting persistence. In contrast, northern mountain populations on pre-Quaternary substrates (pre-Q NPM) hold reduced genetic variation due to altitudinal movements in response to climate shifts. We suggest that palaeogeography of southern South America in addition to Neogene glaciations have shaped the genetic makeup of such ancient lineage as *Podocarpus*.

1. Introduction

Palaeogeographical features of southern South America have affected regional patterns in plant species diversity (Hinojosa and Villagrán, 2005) and within-species genetic characteristics of widespread ancient lineages (Acosta and Premoli, 2009). The geologic history of Patagonia is complex. During the early Tertiary, emergent land was probably scant due to higher sea level (Miller et al., 2005) and the presence of numerous palaeobasins (Romero, 1986; Lisker et al., 2006). Elevated terrain driving early evolution of extant lineages in Patagonia consisted of rock formations (Pankhurst et al., 2006) that existed prior to the Miocene uplift of the main Andes Cordillera. These were the plutonic basement of the North Patagonian Massif (NPM) and exposed areas of the South Patagonian Massif (SPM). Therefore southern South America provides an opportunity to test the hypothesis that large discontinuities in the within-species genetic structure arise from long-term extrinsic barriers for gene flow (Avise et al., 1987). Spatially concordant phylogeographic patterns among extant species within subgenus *Nothofagus* call for a shared ancient past controlled by palaeogeography (Acosta and Premoli, 2009). However, the question still remains if gene pools and thus genetic architectures of early evolving taxa as *Nothofagus* and *Podocarpus* have been conserved throughout contemporary processes affecting plant distribution patterns such as the last glaciations in Patagonia.

During the Quaternary, tree species from temperate regions have been subjected to changes in their distribution ranges. In particular, populations have suffered from fragmentation due to increased ice extent during glacial periods (Hewitt, 1996, 2000). However, the impact of climate on vegetation will be dependent on species' life history traits (McLachlan et al., 2005). As such, different species would respond according to their ecological tolerances that will also be reflected in their genetic makeup (Premoli et al., 2007). Therefore, unrelated taxa with similar ecological tolerances and responding in a concordant fashion to processes such as Quaternary glaciations should show similar well differentiated clades if deep genetic structures were established sometime in the past.

Palynogical records from mid latitudes of Patagonia documented the persistence during glacial periods of cold hardy conifers in ice-free lowland refugia (Markgraf et al., 1995; Villagrán, 2001; Moreno and León, 2003; Moreno, 2004) whereas during interglacials tree populations migrated to high elevations in mountain areas (Villagrán, 2001). In addition, genetic studies of conifers in the Cupressaceae

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family have suggested that forest survival occurred in multiple ice-free glacial refugia (Premoli et al., 2000) some of which were located in high latitudes (Premoli et al., 2002; Allnutt et al., 2003). Similar scenarios were also described for northern refugia in Europe and North America (Stewart and Lister, 2001; McLachlan et al., 2005; Bhagwat and Willis, 2008). However, most genetic evidence for different species from the Northern Hemisphere reported high latitude populations to be the result of long-distance migration from refugia located in warmer areas south of the ice sheets (Hewitt, 2000; Petit et al., 2002). Under this scenario, populations became locally extinct within glaciated areas and wide ranging post-glacial dispersal is advocated to explain current distribution patterns. As a result, more recently colonized populations are expected to hold reduced genetic polymorphism due to bottlenecks and founder events (Hewitt, 2004). In contrast, no such post-glacial long-distance dispersal has been recorded for widespread South American cold-tolerant taxa (Premoli et al., 2002). Overall, glacial/interglacial cycles were of smaller amplitude in southern South America related to the oceanic setting and the physical heterogeneity of the landscape, including coastal mountain ranges that provided a great diversity of habitats for forest survival in small stands for prolonged periods (Markgraf et al., 1995).

We hereby predict that the gene pool of the widespread conifer *Podocarpus nubigena* Lindl. (following Marticorena and Rodríguez, 1995) will reflect both ancient regional events of dispersal and vicariance as well as local responses to glaciations in Patagonia. In this study, we examine amounts and distribution patterns of genetic variation within *P. nubigena* which is the southern-most and thus cold-tolerant species in the Podocarpaceae family inhabiting temperate rainforests of the southern Andes. Although latitudinally widespread, it occurs in relatively small and isolated populations with low density. Palynological records from southern Chile (between 37° and 40°S) indicate that podocarps and Magellanic Moorland elements occupied the lowlands of the Longitudinal Valley (Chile) during cold phases of the Last Glacial Maximum (LGM), ca. 22,000 ^{14}C years BP (Villagrán, 2001). During warming trends of the late glacial (14,600–10,000 ^{14}C years BP) and the Holocene (after 10,000 ^{14}C years BP) a rapid expansion of temperate taxa, including conifers, occurred to higher elevations and higher latitudes (Villagrán, 2001). We hypothesize that if cold-tolerant *P. nubigena* survived locally in multiple ice-free locations, then lowland refugial populations may hold increased genetic polymorphism. In contrast, high-elevation populations may be genetically impoverished due to founder events. Furthermore, we hypothesize that if *P. nubigena* survived the ice ages along its range, then the observed genetic structure will reflect ancient palaeogeographic features. These included major palaeobasins at mid latitudes of Patagonia such as the Chubut basin (Suárez and Márquez, 2007) which should have resulted in vicariance and thus producing allopatric divergence between mountain populations of the North and South Patagonic Massifs, respectively. In particular, while towards the south and along the northern Coastal Range and several areas of the Andes Cordillera consist of mountainous pre-Quaternary basement, northern lowland areas within the Central Valley of Chile and the eastern sector of Chiloé Island were shaped predominantly by the Quaternary (Cembrano and Lara, 2009). We analyzed within-population diversity and among-population divergence patterns of *P. nubigena* using isozyme polymorphisms. Distinct chloroplast and mitochondrial DNA regions were screened in *P. nubigena* populations none of which resulted polymorphic and thus prevented phylogeographic analyses (Quiroga, 2009).

2. Materials and methods

2.1. Study area and the species

Podocarpus nubigena (Podocarpaceae), common name "mañio macho," is endemic to southern Chile and adjacent parts of Argentina. It has a variable height between 10 and 25 m and stem diameters can reach up to 0.9 m. *P. nubigena* consists of disjunct populations that grow under high precipitation regimes typical of oceanic climatic conditions with moderated temperatures in the north and lower temperatures in the south. The geographical range extends from 39° 50′ to 50° 23′ S latitude (Marticorena and Rodríguez, 1995). In southern Chile it is found on the Andes, the Coastal Range, and the lowland Central Valley. It can be found from sea level to 1000 m. It often occurs on humid soils with good drainage (Donoso et al., 2006). On the drier eastern slopes of the Andes in Argentina, it is restricted to Valdivian rainforests included areas within Nahuel Huapi and Los Alerces National Parks.

Podocarpus nubigena is rarely dominant and typically occurs in mixed forests with a reduced density of approximately <10% of the total tree species present per stand and a basal area of 6–11%/ha (Donoso et al., 2006) It usually coexists with other conifers such as *Saxegothaea conspicua* (Podocarpaceae), *Pilgerodendron uviferum* and *Fitzroya cupressoides* (both in the Cupressaceae family), and the broadleaf *Embothrium coccineum*, *Laureliopsis philippiana*, *Drymis winteri*, and *Nothofagus dombeyi*. On western slopes of the southern Andes in Chile it is also found with *Nothofagus nitida* and *Nothofagus betuloides*. *P. nubigena* is wind pollinated and disperses primarily locally either by gravity and/or animals such as the *Turdus falcklandii* (austral thrush) (Willson et al., 1996).

2.2. Population sampling

During the austral summers (January to March) 2002–2004, 14 natural populations between 39° 45′ and 47° 39′ S latitude were sampled covering a wide variety of habitats and elevations (Fig. 1, Table 1). At most populations 30 individuals were randomly sampled within an area of c. 1 ha, separated by a minimum of 10 m to avoid sampling of closely related genotypes. From the small populations on Antillanca, Lago Menéndez, Río Frío, and Mañihuales sample size was small (Table 1). From a total of 355 individuals we collected one terminal twig of approximately 30 cm length bearing fresh leaf tissue that was kept cool until the arrival to the laboratory.

2.3. Isozyme electrophoresis

Protein extraction took place usually within a week after sample collection. Enzyme extracts were prepared by grinding approximately 1 g of leaf tissue using liquid nitrogen (−180 °C) and adding 1–1.5 ml of extraction buffer (Mitton et al., 1979). Homogenates were stored at −80 °C until they were absorbed onto Whatman No. 3 paper wicks that were loaded into 12% starch gels (Starch Art Corporation, Smithville, TX, USA).

A total of 12 isozyme loci were resolved using three buffer systems which were previously used for *Podocarpus parlatorei* (Quiroga and Premoli, 2007). These buffers included the type by Clayton and Treliak (1972), pH 6.1, which resolves Malate dehydrogenase (*Mdh*); the one by Poulik (1957), pH 8.2, to resolve Malic enzyme (*Me*), Menadione reductase (*Mnr*1, *Mnr*2), cathodic Peroxidase (Per_C1, Per_C2, Per_C3, Per_C4) and Phosphoglucoisomerase (*Pgm*); and the one by O'Malley et al. (1979), pH 8.5, to resolve anodic Peroxidase (Per_A1, Per_A2, Per_A3).

2.4. Data analysis

Levels of within-population isozyme variation were described by standard measures using POPGENE v. 1.32 (Yeh et al., 1999). These were the total number of alleles (A_T), mean number of alleles (A), effective number of alleles (A_E), number of low frequency alleles ($A<0.05$), the proportion of polymorphic loci using the *sensu stricto* criterion (P_{SS}), and the observed and expected under Hardy–Weinberg equilibrium conditions heterozygosities (H_O and H_E, respectively). To determine whether a population has experienced a demographic