NATURAL HISTORY AND GENETIC STRUCTURE OF RAULÍ (*NOTHOFAGUS NERVOSA* (PHIL.) DIM. ET MIL.)

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ABSTRACT

We estimated allele frequencies for 10 polymorphic allozyme loci in samples from 18 natural populations of *Nothofagus nervosa* (Fagaceae), analyzed the genetic structure and inferred the recent evolutionary history of this southern Beech. Genetic variation in *N. nervosa* is organized as in most widely distributed, allogamous, anemochoric, anemophilic trees: high levels of within population polymorphism (A = 3.04, H_e = 0.28), considerable deficiency of heterozygotes (mean F_{IS} = 0.186) and moderate differentiation among populations (F_{ST} = 0.051). The UPGMA dendrogram revealed five groups of populations, two of which may have been isolated since before the last glacial period. Correlations among alleles at multiallelic loci do not follow the pattern expected for random differentiation of populations from a single source. The presence of a number of alleles with low frequencies, moderate genetic diferentiation among populations and high levels of genetic variation within populations, all argue that *N. nervosa* populations survived the last glaciation in large refuges, and probably colonized in waves. Our results suggest at least 4 refuges from which the current populations of raulí have derived; the current genetic structure of the species is best explained by post-glacial expansion.

Key words: allozymes, glacial refuges, recent evolutionary history, population differentiation.

INTRODUCTION

Genetic variation patterns in plants have been associated with several life history and ecological attributes HAMRICK *et al.* 1979; 1992). These authors pointed out that woody plants with large and relatively continuous populations, allogamy and whose pollen and seeds are dispersed by wind, maintain higher levels of allozyme variation within populations and low genetic differentiation among populations, compared to other plants which do not have this combination of characteristics. However these traits explain only 50 % of the genetic variation in those woody plants, the remainder would be explained by their evolutionary history (HAMRICK *et al.* 1979, 1992; HAMRICK & GODT 1989); the latter may be particularly important for species recently affected by climatic changes (BARRETT & HUSBAND 1989; TOMARU *et al.* 1997).

Nothofagus nervosa (Fagaceae) has the reproductive characteristics mentioned above, allogamy, anemophily and anemochory. Locally called raulí (and *N. alpina*), the species is presently restricted to part of the two mountain ranges of central Chile. In the Andes range its distribution is more or less continuous, from 35° 11' S to 40° 22' S, while in the Coast Range only about 6 fragmented populations remain, between 36° 41' S and 41° S (ORMAZABAL & BENOIT 1987; Fig. 1). In the southern part of its range, *N. nervosa* forms mixed forests with *N. obliqua* at lower altitudes, and with *N. dombeyi* at higher altitudes ORMAZABAL & BENOIT 1987); it always occurs in mixed stands.

The generalizations of HAMRICK *et al.* (1979, 1992) predict high intrapopulation genetic variability and little differentiation among populations. However, if we consider the recent evolutionary history of *N. nervosa*, the expected pattern of allozyme variability may have suffered modifications. The paleobotanic history indicates that before the last glaciation populations were distributed along both Andean and Coast Ranges. At the maximum glacial period ⅔ of Chile was under glacial ice, including most of the present range of the species, it was obliged to take refuge in the unglaciated Coast Range, and perhaps the Central Valley and more northerly parts of the Andes (VILLAGRÁN 1991, 2001, Fig. 1). Palynological studies have identified one probable refuge for *N. nervosa*, the Nahuelbuta (Coast) Range, located between 37–38 °S. When the climate warmed, this refuge would have been the principal resource for the re-expansion of *N. nervosa* towards the South along the Coast and Andean Ranges, although the species may also have survived in other Coast Range sites and North of 37 °S (VILLAGRÁN 1991). The

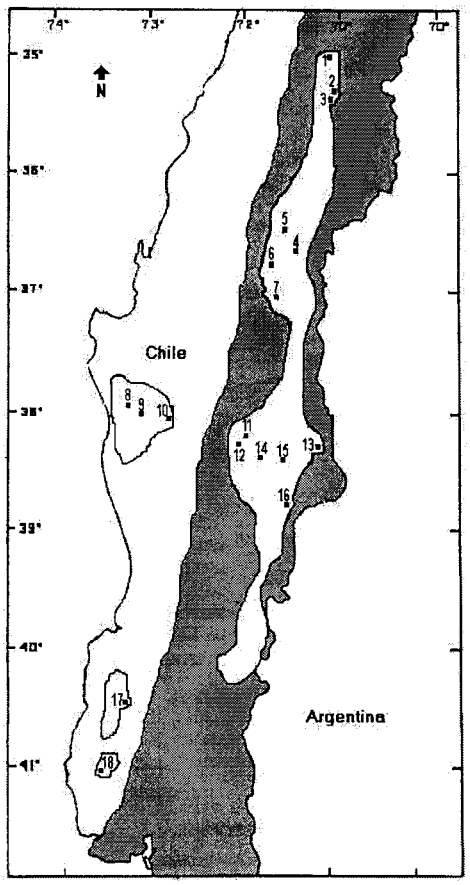

Figure 1. Map of central Chile showing the present range of of raulí (*Nothofagus nervosa* ((Phil.) Dim. et Mil.) (black lines around collection sites), collection sites (black dots with numbers), and the maximum extension of glaciers during the last glacial period (shaded area).

uncertainty is due to the fact that these studies do not distinguish between the pollen of *N. nervosa* and *N. obliqua*.

The current pattern of genetic variation in many forest species has been related to the effects of the last glaciation and to the process of postglacial expansion, examples in the Fagaceae include *Fagus sylvatica* (COMPS *et al.* 1990; COMPS *et al.* 2001), *Quercus petraea* (ZANNETO & KRAMER 1995), and *Fagus crenata* (TOMARU *et al.* 1997). In these cases, the species survived the glacial period in one or more large population(s), and gene flow seems to have been more important than bottlenecks in determining present genetic variation. A contrasting case is provided by New Zealand species of *Nothofagus* (HASSE 1992), which apparently persisted in small, isolated refuges; in these species bottlenecks have increased population differentiation and purged low-frequency alleles, and gene flow has had little importance in determining present genetic variation.

In this investigation, we use allozyme information to study the genetic structure of *N. nervosa* and its relationship with life history and ecological traits of this species, in particular to infer the effects of the last glacial cycle on the species. Moreover, we use genetic diversity and paleobotanical data to infer its recent evolutionary history. Since the species has colonized most of its present range in the last 10,000 years or less, we expect this history to be reflected in the distribution of genetic variation.

MATERIALS AND METHODS

Studied Populations

We sampled 18 natural populations of raulí, whose geographic locations are indicated in Table 1 and Fig. 1. In each population 42 individuals were selected randomly, always allowing a minimum of 5 m between sampled trees to be sure that we did not sample two stems of the same individual; distances between sampled trees were usually at least 15 m., and the total area sampled was at least 4 ha. We collected a terminal lateral branch including at least 6 leaves from each individual. The bases of the branches were wrapped in wet paper towels and kept over ice in a cooler until return to the laboratory, after which the branches were kept at 4° C with the bases in water until processed.

Starch Gel Electrophoresis

We stripped 3–5 of the newest leaves of their main veins and ground them along with scrapings of the cortex, in about 2 ml of an extraction buffer pH 7.5 containing: Trisma base 6.5 g, citric acid, 1.5 g, cisteine 1.0 g, ascorbic acid 1.0 g, polyethylene glycol 5.0 g, 2-mercaptoethanol 0.1 ml, soluble PVP (mol. wt. 360,000) 13 g in 1 l of distilled water. The resultant slurry was centrifuged at 5000 rpm for 4 min, then the supernatant was poured into Eppendorf tubes in duplicate and stored at –80 °C until used for electrophoresis.

Electrophoretic and staining procedures followed CONKLE *et al.* (1982). Gels were 11% starch by weight. We used their buffer system D ("morpholine citrate") for malate dehydrogenase (MDH, EC 1.1.1.37), menadione reductase (MNR, EC 1.6.99.2) and shikimate dehydrogenase (SKDH, EC 1.1.1.25); their buffer system A (lithium borate) for alanine amino peptidase (AAP, EC 3.4.11.1), alanine amino transferase (AAT, EC 2.6.1.1), acid phosphatase (ACP, EC 3.1.3.2) and phosphogluco isomerase (PGI, EC 5.3.1.9); and histidine pH 8.0 for peroxidase (PER, EC 1.11.1.6). Chemical

Table 1. Geographic location and alloenzyme genetic variability for 18 natural populations of Raulí (*Nothofagus alpina (Poepp. et Endl (Oerst)*). N = Mean sample size locus; A = Mean number of alleles/locus; H_o = Observed heterozygosity; H_e = Expected heterozygosity (unbiased estimation of NEI 1978) (standard errores in parentheses).

Population	Range	LAT	LONG	ALT	N	A	H_o	H_e
1 Curicó	Andes	35°07'	71°58'	800	37.9	2.9	0.188 (0.044)	0.230 (0.060)
2 Agua Fría	Andes	35°21'	71°04'	650	38.1	3.2	0.244 (0.048)	0.335 (0.064)
3 Radal-7 Tasas	Andes	35°25'	71°03'	800	37.2	3.4	0.291 (0.058)	0.317 (0.063)
4 San Fabian Alto	Andes	36°39'	71°23'	750	32.4	2.8	0.199 (0.061)	0.275 (0.087)
5 San Fabian	Andes	36°30'	71°38'	450	37.0	2.9	0.244 (0.073)	0.269 (0.077)
6 Bajo	Andes	36°59'	71°41'	750	40.7	2.9	0.238 (0.059)	0.282 (0.075)
7 Recinto	Andes	37°01'	71°44'	850	37.4	2.6	0.193 (0.059)	0.256 (0.081)
8 Monte León	Coast	37°55'	73°13'	950	20.0	2.6	0.200 (0.073)	0.235 (0.060)
9 Nahuelbuta	Coast	38°01'	73°08'	725	37.4	3.1	0.254 (0.057)	0.292 (0.068)
10 Chacay-Nahuel	Coast	38°05'	72°54'	860	42.7	3.5	0.306 (0.045)	0.346 (0.048)
11 Vega Blanca	Andes	38°14'	71°57'	850	38.4	3.0	0.213 (0.061)	0.250 (0.071)
12 Malleco A	Andes	38°13'	71°50'	1000	45.4	3.2	0.237 (0.048)	0.282 (0.065)
13 Malleco B	Andes	38°19'	72°05'	550	31.5	3.1	0.235 (0.045)	0.284 (0.049)
14 Selva Oscura	Andes	38°27'	71°44'	750	39.5	3.4	0.203 (0.055)	0.287 (0.064)
15 Curacautín	Andes	38°28'	71°31'	1050	38.9	3.5	0.258 (0.041)	0.337 (0.056)
16 Malalcahuello	Andes	38°51'	71°28'	750	43.2	3.1	0.246 (0.056)	0.307 (0.064)
17 Melipeuco	Coast	40°27'	73°16'	150	38.2	3.1	0.234 (0.058)	0.275 (0.046)
18 Las Trancas El Colegual	Coast	41°02'	73°29'	223	39.7	2.5	0.144 (0.047)	0.222 (0.053)
Mean Values					35.5	3.04	0.228 (.055)	0.289 (0.064)

products were purchased from Sigma-Aldrich.

Usually 36 individuals were analyzed in each run; we always included individuals from at least three different, geographically separated populations, to avoid misinterpretation of band positions. Banding patterns were interpreted as products of single genes, based upon previous experience and consistency with information in the literature on related species.

Statistical Analyses

Allele frequency differences among populations were evaluated using the exact tests incorporated in the program GENEPOP 3.1 (RAYMOND & ROUSSET 1997). The number of alleles per locus (A), percent polymorphic loci, observed and expected heterozygosities (H_o and H_e) were determined using the program BIOSYS 1.7 (SWOFFORD & SELANDER 1981). We estimated WRIGHT's (1965) F statistics, calculated according to WEIR & COCKERHAM (1984). The significance of the F statistics was estimated by the method of LI & HORVITZ (1953) and WORKMAN & NISWANDER (1970). We used BIOSYS 1.7 to calculate NEI's (1978) genetic distances and to produce a UPGMA dendrogram. We made a simple jackknife test of the branching point confidence by eliminating one locus at a time and recalculating the dendrogram.

We compared the observed correlations of alleles (for loci with more than two) with the distribution expected under random differentiation of populations, using theory developed by Nei (NEI 1965; NEI 1987). Finally, we tested for bottlenecks using the program written by PIRY *et al.* (http://www.ensam.inra.fr/URLB, CORNUET & LUIKART 1996).

RESULTS

Heterozygosity and Allelic Variation

The 8 enzymatic systems studied revealed 10 variable presumptive loci with an average of 92.8 % polymorphism (Tables 1 and 2). Although we did not do the formal genetics, banding patterns corresponded with the number of subunits reported in the literature for other species, for example MNR is tetrameric, MDH is dimeric, SKDH is monomeric, and were completely consistent with a Mendelian interpretation. (a good revision of isozyme banding in plants can be found in WEEDEN & WEEDEN 1989). Uncommon alleles were almost always present as heterozygotes.

Populations of *N. nervosa* have a high number of alleles per locus (mean 3.04, range 2–6). However, a number of these alleles are present in low frequencies, as is the case for most forest species (CONKLE 1992). (Tables 1 and 2).

Table 2. Observed presumptive allele frequencies for 10 enzyme loci from 18 natural populations of *Nothofagus nervosa* ((Phil.) Dim. Et. Mil).

Locus	Allele	Population								
		1	2	3	4	5	6	7	8	9
Mnr	1	0.034	0.026	0.026	0.012	0.057	0.014	0.	0.184	0.047
	2	0.636	0.750	0.513	0.390	0.414	0.486	0.600	0.526	0.686
	3	0.114	0.079	0.184	0.146	0.129	0.122	0.050	0.039	0.580
	4	0.216	0.145	0.263	0.451	0.400	0.378	0.350	0.250	0.209
	5	0.	0.	0.	0.	0.	0.	0.	0.	0.
	6	0.	0.	0.	0.	0.	0.	0.	0.	0.
Mdh	1	0.045	0.118	0.081	0.078	0.029	0.073	0.050	0.132	0.081
	2	0.318	0.408	0.230	0.219	0.147	0.229	0.375	0.342	0.384
	3	0.625	0.382	0.581	0.594	0.529	0.583	0.575	0.421	0.500
	4	0.011	0.920	0.108	0.109	0.294	0.115	0.	0.105	0.035
	5	0.	0.	0.	0.	0.	0.	0.	0.	0.
Per-2	1	0.	0.021	0.	0.	0.	0.	0.075	0.066	0.140
	2	0.917	0.649	0.663	0.974	1.	1.	0.775	0.816	0.616
	3	0.083	0.330	0.315	0.026	0.	0.	0.150	0.118	0.221
	4	0.	0.	0.022	0.	0.	0.	0.	0.	0.023
Per-3	1	0.	0.	0.031	0.	0.	0.	0.025	0.	0.012
	2	1.	0.979	0.896	0.976	0.986	0.969	0.975	0.961	0.860
	3	0.	0.021	0.052	0.024	0.014	0.031	0.	0.	0.035
	4	0.	0.	0.021	0.	0.	0.	0.	0.039	0.093
Per-4	1	0.	0.875	0.	0.012	0.	0.979	0.	0.026	0.
	2	0.	0.104	0.896	0.927	0.972	0.021	1.	0.961	0.907
	3	1.	0.021	0.094	0.024	0.028	0.	0.	0.013	0.093
	4	0.	0.	0.010	0.	0.	0.	0.	0.	0.
Aap	1	0.068	0.141	0.059				0.050	0.013	0.036
	2	0.864	0.718	0.765				0.925	0.829	0.845
	3	0.068	0.026	0.029				0.025	0.158	0.119
	4	0.	0.115	0.147				0.	0.	0.
Aat(Got)	1	0.102	0.	0.	0.	0.019	0.	0.075	0.092	0.081
	2	0.080	0.125	0.	0.057	0.096	0.077	0.050	0.	0.058
	3	0.807	0.688	0.923	0.929	0.808	0.846	0.875	0.908	0.860
	4	0.011	0.188	0.077	0.014	0.077	0.077	0.	0.	0.
Skdh	1	0.091	0.036	0.026	0.	0.	0.018	0.	0.078	0.049
	2	0.852	0.946	0.974	0.786	0.020	0.929	0.925	0.859	0.841
	3	0.023	0.018	0.	0.214	0.900	0.054	0.075	0.047	0.085
	4	0.034	0.	0.	0.	0.080	0.	0.	0.016	0.024
Acp	1	0.034	0.125	0.060	0.	0.	0.085	0.050	0.053	0.093
	2	0.943	0.830	0.881	1.	1.	0.080	0.950	0.947	0.884
	3	0.023	0.045	0.024	0.	0.	0.085	0.	0.	0.023
	4	0.	0.	0.036	0.	0.	0.	0.	0.	0.
Pgi	1	0.047	0.	0.	0.073	0.069	0.033	0.050	0.066	0.081
	2	0.023	0.058	0.128	0.012	0.014	0.022	0.025	0.053	0.070
	3	0.930	0.919	0.851	0.902	0.889	0.880	0.875	0.829	0.791
	4	0.	0.023	0.021	0.012	0.028	0.065	0.050	0.053	0.058

Table 2. (*Continued*).

Locus	Allele	Population								
		10	11	12	13	14	15	16	17	18
Mnr	1	0.103	0.021	0.035	0.	0.011	0.012	0.011	0.	0.
	2	0.538	0.500	0.523	0.653	0.648	0.631	0.585	0.372	0.800
	3	0.064	0.021	0.047	0.014	0.023	0.024	0.032	0.013	0.150
	4	0.295	0.458	0.384	0.319	0.318	0.333	0.372	0.603	0.050
	5	0.	0.	0.012	0.014	0.	0.	0.	0.	0.
	6	0.	0.	0.	0.	0.	0.	0.	0.013	0.
Mdh	1	0.095	0.064	0.087	0.036	0.100	0.013	0.122	0.	0.
	2	0.243	0.287	0.200	0.089	0.275	0.154	0.144	0.103	0.098
	3	0.608	0.606	0.688	0.768	0.512	0.654	0.644	0.872	0.902
	4	0.054	0.043	0.025	0.107	0.100	0.179	0.089	0.026	0.
	5	0.	0.	0.	0.	0.013	0.	0.	0.	0.
Per-2	1	0.	0.	0.	0.134	0.	0.098	0.031	0.	0.110
	2	0.8	0.917	0.965	0.646	0.965	0.965	0.854	0.962	0.854
	3	0.2	0.083	0.035	0.085	0.035	0.024	0.042	0.038	0.037
	4	0.	0.	0.	0.134	0.	0.183	0.073	0.	0.
Per-3	1	0.	0.010	0.	0.	0.	0.	0.021	0.026	0.049
	2	1.	0.979	0.988	0.923	0.977	0.952	0.948	0.923	0.829
	3	0.	0.010	0.012	0.051	0.011	0.012	0.031	0.051	0.122
	4	0.	0.	0.	0.026	0.011	0.	0.	0.	0.
Per-4	1	0.	0.010	0.	0.	0.	0.	0.021	0.026	0.049
	2	1.	0.979	0.988	0.923	0.977	0.952	0.948	0.923	0.829
	3	0.	0.010	0.012	0.051	0.011	0.012	0.031	0.051	0.
	4	0.	0.	0.	0.026	0.011	0.	0.	0.	0.
Aap	1	0.183	0.133	0.280	0.029	0.068	0.250	0.057	0.051	0.109
	2	0.533	0.522	0.549	0.897	0.648	0.643	0.739	0.756	0.781
	3	0.283	0.344	0.171	0.074	0.284	0.107	0.205	0.192	0.109
	4	0.	0.	0.	0.	0.	0.	0.	0.	0.
Aat(Got)	1	0.	0.011	0.037	0.200	0.012	0.037	0.066	0.	0.232
	2	0.018	0.089	0.074	0.	0.134	0.074	0.132	0.141	0.110
	3	0.893	0.844	0.0870	0.800	0.841	0.852	0.803	0.828	0.659
	4	0.089	0.056	0.019	0.	0.012	0.037	0.	0.031	0.
Skdh	1	0.025	0.017	0.	0.071	0.	0.033	0.	0.	0.092
	2	0.962	0.914	0.917	0.929	0.857	0.767	0.667	0.756	0.895
	3	0.013	0.069	0.083	0.	0.143	0.133	0.310	0.192	0.
	4	0.	0.	0.	0.	0.	0.067	0.024	0.051	0.013
Acp	1	0.	0.	0.	0.037	0.045	0.024	0.	0.013	0.
	2	1.000	0.917	1.	0.963	0.932	0.976	1.	0.910	1.
	3	0.	0.063	0.	0.	0.023	0.	0.	0.038	0.
	4	0.	0.021	0.	0.	0.	0.	0.	0.038	0.
Pgi	1	0.050	0.031	0.058	0.092	0.102	0.071	0.122	0.039	0.
	2	0.	0.	0.023	0.053	0.023	0.048	0.100	0.	0.012
	3	0.925	0.938	0.895	0.829	0.830	0.881	0.767	0.934	0.963
	4	0.025	0.031	0.023	0.026	0.045	0.	0.011	0.026	0.024

Table 3. Common alleles (frequency greater than 10%), widely distributed and local, and rare alleles (less than 10%) widely distributed and local for 10 enzymatic loci in 18 populations of Raulí (*Nothofagus alpina* (Poepp. et Endl (Oerst)).

Locus	Common alleles		Rare alleles	
	Widely distributed	Local	Widely distributed	Local
Mnr-1	2	0	2	2
Mdh-2	2	0	2	1
Per-2	1	0	3	0
Per-3	1	0	3	0
Per-4	1	0	3	0
Aap	2	0	2	0
Aat-2	1	0	3	0
Skdh	1	0	3	0
Acp-2	1	0	2	1
Pgi-2	1	0	3	0
Total	13	0	26	4

Table 4. Genotypic diversity for 10 enzymatic loci in 18 natural populations of Raulí (*Nothofagus alpina* (Poepp. et Endl (Oerst)) and levels of gene flow (*Nm*).

Locus	F_{is}	F_{it}	$1-F_{st}$ (%)	F_{st} (%)	*Nm*
Mnr-1	0.1766**	0.2159**	95.2	4.77**	4.99
Mdh-2	0.2740**	0.3075**	95.4	4.60**	5.18
Per-2	0.3161**	0.3905**	89.11	10.89**	2.05
Per-3	0.1089**	0.1332**	97.3	2.73**	8.90
Per-4	0.0244ns	0.0692ns	93.1	4.60**	5.18
Aap	0.1399**	0.1986**	93.2	6.83**	3.40
Aat-2	0.1975**	0.2165**	97.6	2.37**	10.30
Skdh	0.1341**	0.1741**	95.4	4.61**	5.17
Acp-2	0.2006**	0.2304**	96.3	3.73**	5.45
Pgi-2	0.0996**	0.1127**	98.6	1.45ns	17.24
Mean	0.1856**	0.2272**	94.9	5,11	4.64

ns = not significant ($P > 0.05$), ** = highly significant ($P < 0.01$); $Nm = (1/F_{st}-1)/4)$; Wright's F-statistics according to WEIR & COCKERHAM (1984)

BROWN (1981) as: common alleles (frequencies >10 %) which are (a) widely-distributed or (b) local, and rare alleles (frequencies <10 %) which are (c) widely-distributed or (d) local.

Applying the above definitions, we found one or two common widely distributed alleles at each locus (30.2 % of all alleles). Seven loci presented one common allele, while *Mnr-1, Aap* and *Mdh-2* each had two widely-distributed common alleles; we did not find common local alleles. The majority of the alleles were rare (69.8 %), with 60.4 % being widely distributed alleles and the remaining 9.4 % local rare alleles. (Table 3).

Although we found no locally common alleles, we observed four rare local alleles in six populations: allele *Mnr-1–5* was present in Radal-7 Tasas (0.013), Malleco A (0.012) and Selva Oscura (0.014), alleles *Mdh-1–5* and *Acp-2–4* were present in Curacautín (0.013) and Radal-7 Tasas (0.034) respectively and allele *Mnr-1–6* was observed only in Las Trancas (0.013).

We found high levels of heterozygosity (H_o) in all populations (Table 2), an average of 22.8 % with a range of 14.4–30.6 %. Only the two populations from the extremes of the species' range, El Colegual and Curicó, showed somewhat lower H_o.

N. nervosa has important levels of consanguinity in its populations. The average value of F_{IS} (= 0.186) was positive and highly significant ($P < 0.001$) (Table 4) and the observed heterozygosity was less than the expected in all populations (Table 1).

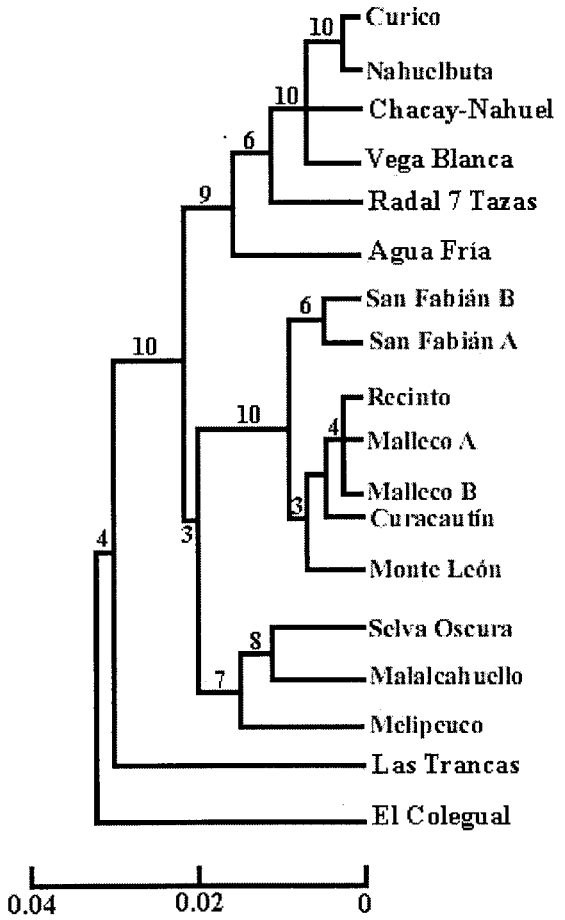

Figure 2. Dendrogram based on NEI (1978) unbiased genetic distances for 10 loci in 18 natural populations of raulí (*Nothofagus nervosa* ((Phil.) Dim. et Mil.). Numbers at the intersections represent the number of jackknife tests in which this group was maintained.

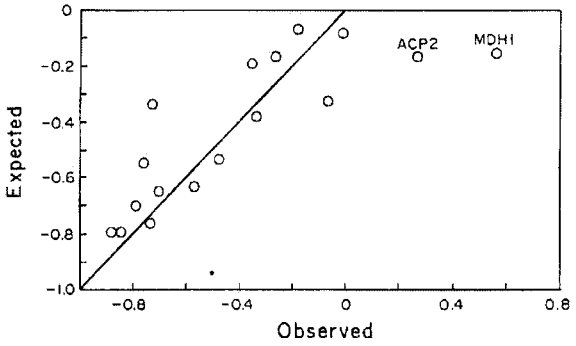

Figure 3. Correlations among alleles for loci with more than two alleles, compared to the expected under random differentiation for 18 natural populations of raulí (*Nothofagus nervosa* ((Phil.) Dim. et Mil.).

Distribution of genetic variability

Although only a little more than 5 % of the variability was distributed between populations (F_{ST} –Table 4), G test values from GENEPOP indicated an important heterogeneity in allele frequencies ($P < 0.01$). Part of the differentiation is due to the two samples from the southern Coast Range, Las Trancas (17) and El Colegual (18), however, the F_{ST} of the other 16 populations is still significant (0.041, $P < 0.05$). Using F_{ST} to estimate *Nm*, we obtained a value of 4.6 migrants per generation (Table 4).

Other genetic measures

The range of Nei's unbiased genetic distances was 0.001–0.076; the mean was 0.0215. Using these distances and UPGMA, populations cluster in five groups (Fig. 2), which roughly coincide with their geographic locations. However, the three samples from the northern Coast Range (8, 9 and 10) cluster with the populations from the northern extreme of its range in the Andes (1, 2 and 3), rather than with populations from the same latitude (11–16, see Fig. 1). The two populations from the southern Coast Range (17 and 18) are not similar to each other, nor to any of the other populations (checked with the matrix of genetic distances, data available upon request).

Allele correlations did not match those expected under random differentiation (Fig. 3). Although most values were close to the expected, two loci had alleles with positive correlations, alleles 1 and 2 of MDH1, and alleles 1 and 3 of *Acp-2*. Finally, no evidence of bottlenecks was found using Piry's program.

DISCUSSION

Recent History of Rauli Populations

In the interglacial period around 41,000 to 50,000 years before present (BP) there was abundant *Nothofagus* flora from at least 40° S as far North as the central zone of Chile (34° S). When the last glaciation advanced the changes in the climatic conditions permitted the hygrophilic flora including *N. nervosa* to migrate as far North as 33° S (VILLAGRÁN 1991). During the maximum glacial period about 28,000 to 14,000 years BP a large part of Chile, including most of the present range of *N. nervosa*, was covered by glacial ice (Fig. 1). Forest species were obliged to take refuge in the Coast Range, Intermediate Depression and the unglaciated part of the Andes foothills (VILLAGRÁN 1991).

During the glacial period, *N. nervosa* survived at

least in the Nahuelbuta (Coast) Range (37–38 °S). VILLAGRÁN (1991) points out that in the same period *N. nervosa* was present to 34° 30' S and represented by traces in a site at 39° 33' S. Nearly 15.000 years BP (Late Glacial Period) the temperature and precipitation began to increase, allowing the populations of Raulí to reach their present geographic distribution in Chile, and to cross the Andes into Argentina in the southern part of the range. Thus, except for populations which have remained in refuges, we should not expect present-day populations of *N. nervosa* to be older than about 10,000 years, and many may be much younger.

Population variation

The genetic structure of *N. nervosa* is similar to that of most wind-pollinated, wind dispersed, outcrossed and widely distributed tree species (HAMRICK *et al.* 1992); considerable within-population variability, measured by polymorphism, heterozygosity and number of alleles per locus. The number alleles per locus (3.04) was superior to published values for other species of the family (COMPS *et al.* 1990, VILLANI *et al.* 1991; HASSE 1992). Moreover, heterozygosity values were quite superior to the average for plants (H_e = 14.9 %, HAMRICK & GODT 1989), and are among the highest values published for Fagaceae: *Quercus* (H_e = 5.5–39.8 %), *Castanea* (18.3–30.5 %), *Fagus* (16.8–39.5 %), *Nothofagus* (2.7–9.9 %) (HASSE 1992; COMPS *et al.* 1990; TAKAHASHI *et al.* 1994; HUANG *et al.* 1994; VILLANI *et al.* 1991).

MARSHALL & BROWN (1981) suggested that populations with high frequencies of local alleles would reflect the action of evolutionary processes such as selection and genetic drift, while NEI *et al.* (1975) pointed out that the reduction of population size lowers the level of allozyme variability by eliminating rare alleles. The high levels of allozyme variability and presence of common and widely-distributed rare alleles in the studied populations is evidence that the species did not suffer important reduction in population size during the last glacial period, but rather persisted in large refuges. These results, plus the apparent absence of recent bottlenecks, also suggest that founder effects were not important in producing the present genetic structure, and that the species has colonized more as an advancing wave than by long-distance dispersal (FISHER 1930)

This variation pattern is similar to that which has been found for Northern Hemisphere species of Fagaceae (COMPS *et al.* 1990; VILLANI *et al.* 1991; ELENA-ROSELLO & CABRERA 1996; TOMARU *et al.* 1997), and contrasts markedly with that of the New Zealand species of *Nothofagus*, which suffered bottlenecks, isolation and population fragmentation during the last glacial period (HASSE 1992).

Levels of Consanguinity

High levels of consanguinity were present in almost all populations of *N. nervosa* studied. BROWN (1979) noted that allogamous species generally present heterozygote deficiency compared to the expectations of panmixia. This appears to be true in the Fagaceae, our average F_{IS} of 0.186 is not much higher than other reported values: *Fagus sylvatica* F_{IS} = 0.115 (CUGUEN *et al.* 1988); *Quercus suber* F_{IS} = 0.173 (1990; MICHAUD *et al.* 1995; ELENA-ROSELLO 1996); *Q. rubra* F_{IS} = 0.10 (SORK *et al.*1993).

As expected in the model of isolation by distance (WRIGHT 1943), this consanguinity appears to be largely due to neighbors being relatives; in a number of species of Fagaceae, groups of related individuals have been demonstrated at small spatial scales, 5 to 30 meters (COMPS *et al.* 1990; VILLANI *et al.* 1991; ELENA-ROSELLO & CABRERA 1996). Some authors suggest that the existence of this phenomenon would favor the formation of semi-isolated mating groups, which would be an effective mechanism for the maintenance of genetic polymorphism and adaptability (WRIGHT 1943, 1965).

Differentiation and gene flow

Population differentiation was also moderate if we compare with other Fagaceae, whose F_{ST} values range from 0.9 % to 16.9 % (COMPS *et al.* 1990; MICHAUD *et al.* 1995; ELENA-ROSELLO & CABRERA 1996). The genetic differentiation among populations is produced by heterogeneity of allele frequencies along the natural distribution of raulí (comparing Table 2 with Fig. 1). We did not find common local alleles that could have inflated the estimation of F_{ST}.

Our results support the idea that the expansion after glaciation was a cause of the of present genetic structure of raulí. Part of the genetic differentiation is due to the two isolated populations from the Coast Range in the southern extreme of the distribution, which are notably different from each other and from the rest of the sampled populations. These two populations have probably been isolated since before the last glacial period and probably were maintained as isolated populations during this period.

Although the other 16 populations only had an F_{ST} of 0.041, this differentiation is significant. We would not expect to find significant differentiation if they had all originated in the same refuge. Populations from the northern extremes of the range in the Andes (1–3) and

the Coast Range (8–10) appear to have spread out from one refuge, and the remaining 10 samples from the Andes from a second refuge. The pattern of correlations among allele frequencies (Fig. 3) also indicates that random differentiation from one ancestral population is not an adequate hypothesis to explain the current pattern of differentiation. These results strongly suggest that historical and/or selective factors have influenced current allele distribution.

Estimated gene flow (Nm = 4.6) was lower than other species with allogamy and anemochory (Nm = 5.3–7.8; HAMRICK & GODT 1990) and similar to other Fagaceae (Nm = 3.6–5.7; COMPS *et al.* 1990; CUGEN *et al.*, 1988; HOUSTON & HOUSTON 1994; HOKANSON *et al.* 1993). However, this is most probably an overestimate, estimates based on F_{ST} are inflated because the assumptions of the model (equilibrium between gene flow and genetic drift) are not met, and we cannot assure that the loci studied are all neutral. We suggest that the moderate level of alloenzymatic differentiation in the majority of the range is due the relatively short time span involved, and that current populations which persist should be more different in the future, especially because fragmentation of populations due to human intervention has certainly lowered the possibility of gene flow.

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REFERENCES

BARRETT, S. C. & HUSBAND, B. C. 1989: The genetics of plant migration and colonization. *In*: Plant population Genetics, Breeding and Genetic Resources. (eds. Brown, A., Clegg, M., Kahler, A. & Weir, B.) pp. 254–277. Sinauer Associates,. Massachusetts.

BROWN, A. 1979: Enzyme polymorphism in plant populations. *Theoretical Population Biology* **15**: 1–42.

COMPS, B., THIÉBAUT, B., PAULE, L., MERZEAU, D. & LETOUZEY, J. 1990: Allozymic variability in beechwoods (*Fagus sylvatica L.*) over central Europe: spatial differentiation among and within populations. *Heredity* **65**: 407–417.

CONKLE, M. 1992: Genetic diversity - seeing the forest through the trees. *New Forests* **6**: 5–22.

CONKLE, M., HODGKISS, P., NUNNALLY, L. & HUNTER, S. 1982: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA, Forest Service. 18 pp.

CUGUEN, J., MERZEAU, D. & THIÉBAUT, B. 1988: Genetic structure of European beech stands (*Fagus sylvatica* L.): F-statistics and importance of mating systems characteristics in their evolution. *Heredity* **60**: 91–100.

CORNUET, J. & LUIKART, G. 1996: Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.

ELENA-ROSSELLO, J. & CABRERA, E. 1996: Isozyme variation in natural populations of cork-oak (*Quercus suber* L.): Population structure, diversity, differentiation and gene flow. *Silvae Genetica* **45**: 229–235.

FISHER, R.A. 1930: The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

HAMRICK, J., LINHART, Y. & MITTON, J. 1979: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Ann. Rev. Ecol. Syst.* **10**: 173–200.

HAMRICK, J. & GODT, M. 1989: Allozyme diversity in plant species. *In*: Plant Population Genetics, Breeding and Genetics Resources. (eds. Brown, A, Clegg, M., Kahler, A. & Weir, B.). pp. 43–63. Sinauer Associates, Massachusetts.

HAMRICK, J., GODT, M., MURAWSKI, D. & LOVELESS, M. 1992: Correlations between species traits and allozyme diversity: Implications for conservation biology. *In:*. Genetics and conservation of rare plants. (eds. Falk, A. & Holsinger, K). pp. 75–86 Oxford University Press, New York.

HASSE, P. 1992: Isozyme variability and biogeography of *Nothofagus truncata* (Fagaceae). *New Zealand Journal of Botany* **30**: 315–328.

HUANG, H., DANE, F. & NORTON, J. 1994: Allozyme diversity in Chinese, seguin and American chestnut (*Castanea* spp.). *Theor. Appl. Genetics* **88**: 981–985.

HOKANSON, S., ISEBRANDS, J., JENSEN, R. & HANCOCK, F. 1993: Isozyme variation in oaks of the Apostle Island in Wisconsin: genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoidalis*. *Am. J. Bot.* **80**(11): 1349–1357.

HOUSTON, D. & HOUSTON, D. 1994: Variation in American beech (*Fagus grandifolia* Ehrh.): Isozyme analysis of genetic structure in selected stands. *Silvae Genetica* **43**: 277–284.

LI, C. & HORVITZ, D. 1953: Some methods of estimating the inbreeding coefficient. *Am. J. Human Genet.* 5:107–117.

MARSHALL, D. & BROWN, H. 1981: Wheat genetic resources. *In*: Wheat Science-Today and Tomorrow. (eds. Evans, L. Peacock, W.). pp. 21–40. Cambridge University Press, Cambridge, England.

MICHAUD, H., TOUMI, L., LUMARET, R., LI, T., ROMANE, F. & DI GIUSTO, F. 1995: Effect of geographical discontinuity on genetic variation in *Quercus ilex* L. (holm-oak): Evidence from isozyme polymorphism. *Heredity* **74**: 590–606.

NEI, M. 1965: Variation and covariation of gene frequencies in subdivided populations. *Evolution* **19**: 256–258.

NEI, M. 1987: Molecular Genetics. Columbia University Press, New York.

NEI, M., MURAYAMA, T & CHAKRABORTY, R. 1975: The

bottleneck effect and genetic variability in populations. *Evolution* **29**: 1-10.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-590.

ORMAZABAL, C. & BENOIT, I. 1987: El estado de conservación del género *Nothofagus* en Chile. *Bosque* **8**(2):109-120

RAYMOND, M & ROUSSET , F. 1997: GENEPOP 3.1: Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**:248-249.

SORK, V., HUANG, S. & WIENER, E. 1993: Macrogeographic and fine scale genetic structure in North American oak species, *Quercus rubra* L. *Annals Sciences Fores*tières 50 (supplement):261s-270s.

SWOFFORD, D. & SELANDER, R. 1981: BIOSYS-1: a FORTRAN program for the comprehensive analysis for electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**: 281-283.

TAKAHASHI, M., TSUMURA, Y., NAKAMURA, T., UCHIDA, K. & OHBA, K. 1994: Allozyme variation of *Fagus crenata* in northeastern Japan. *Can. J. Forest Research* **24**: 1071-1074.

TOMARU, N., MITSUTSUJI, T., TAKAHASHI, M., TSUMURA, Y., UCHIDA, K. & OHBA, K. 1997: Genetic diversity in *Fagus crenata* (Japanese beech): Influence of the distributional shift during the late Quaternary. *Heredity* **78**: 241-251.

VILLAGRÁN, C. 1991: Historia de los bosques templados del sur de Chile durante el tardiglacial y el postglacial. *Revista Chilena de Historia Natural* **64**: 447-460.

VILLAGRÁN, C. 2001: A model for the history of vegetation of the Costal Range of central-southern Chile: Darwin's glacial hypothesis. *Revista Chilena de Historia Natural* **74**: 793 -803.

VILLANI F., PAGLUCCI, M., BENEDETTELLI, S. & CHERUBINI, M. 1991: Genetic differentiation among Turkish chestnut (*Castanea sativa* Mill.) populations. *Heredity* **66**:131-136.

WEIR, B. & COCKERHAM, C. 1984: Estimating *F* statistics for the analysis of population structure. *Evolution* **36**:1358-1370.

WORKMAN, P. & NISWANDER, J. 1970: Population studies on Southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genetics* 22: 24-49.

WRIGHT, S. 1943: Isolation by distance. *Genetics* **28**: 114-138.

WRIGHT, S. 1965: The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* **19**: 395-420.

ZANETTO, A. & KREMER, A. 1995: Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. I. Monolocus patterns of variation. *Heredity* **75**: 506-517.