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Isozyme Studies of New Zealand Nothofagus Species (Southern Beech) Using Leaf Extracts

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Summary

Leaf extracts of the New Zealand species of *Nothofagus* (southern beech) were subjected to horizontal starch gel electrophoresis and stained gel slices were scored for 12 enzyme systems segregating into 22 putative loci. Results of isozyme analysis are supportive of the present taxonomic concept and reveal a major genetic divergence between *N. menziesii* and the remaining four species which belong to the *'fusca'* group and form natural hybrids among each other. Genetic variation in *N. menziesii* is distinctly higher than in any of the *'fusca'* group species where *N. fusca* shows the lowest levels of variation. Hierarchical analysis of gene diversity for each species shows that 84% to 100% of the total gene diversity resides within populations while only 0% to 16% are due to variation between populations. In the *'fusca'* group as a whole, the between-population component of gene diversity amounts to 52.0% and is much higher than in any single species including *N. menziesii* (12.3%). Comparison of observed and expected frequencies of heterozygotes gave no significant deviation from HARDY-WEINBERG equilibrium indicating predominant cross-pollination in both *N. menziesii* and the *'fusca'* group species.

Key words: *Nothofagus*, southern beech, genetic variation, isozyme analysis, electrophoresis, leaf tissue, New Zealand, Fagaceae.

Introduction

The native species of *Nothofagus* (southern beech) are New Zealand's most common and widespread forest trees and a large amount of information on their ecology, distribution, and physiology has already been published (WARDLE, 1984). There has been one study on provenance variation of *Nothofagus* seedling growth and morphology (WILCOX and LEDGARD, 1983), but at the biochemical level, genetic analyses of provenance variation, breeding system, and taxonomical relationships are lacking so far. The present taxonomic concept (WARDLE, 1984; POOLE, 1987) distinguishes four native species, the crenate to dentate-leaved *N. menziesii* (HOOK. f.) OERST. (silver beech), *N. fusca* (HOOK. f.) OERST. (red beech), and *N. truncata* (COL.) CKN. (hard beech), and the entire-leaved *N. solandri* with the recognized varieties *solandri* (HOOK. f.) OERST. (black beech), a lowland form with a small-leaved, divaricating juvenile growth habit, and the montane to subalpine *cliffortioides* (HOOK. f.) POOLE (mountain beech) with generally smaller leaves and lacking the juvenile characteristics of the former. All species except *N. menziesii* form natural hybrids among each other and are collectively classified as the *'fusca'* group. Species and varieties are both referred to as 'species' in the present study.

A comparatively quick and inexpensive method for preliminary investigations of the breeding system, genetic diversity, and provenance variation is isozyme analysis via starch gel or polyacrylamide gel electrophoresis. Isozyme analysis has been widely employed to investigate genetic variation of native forest trees in North America (e. g. WHEELER and GURIES, 1982; CHELIAK and PITEL, 1984) and Australia (e. g., MORAN and HOPPER, 1983; COATES and SOKOLOWSKI, 1989; PETERS et al., 1990), and there have been similar studies on European beech (*Fagus sylvatica* L.) (THIEBAUT et al., 1982; MÜLLER-STARCK, 1985), but in New Zealand research on genetic variation of native trees has only recently been initiated (HAWKINS and SWEET, 1989; HAWKINS et al., 1991; BILLINGTON, 1991).

Imbibed or germinating seeds are the preferred source material for isozyme studies because they are easily stored and extracted and usually contain high concentrations of enzymes. Seed collection of New Zealand *Nothofagus* presents a major problem in this respect because all species flower and reproduce only intermittently and not always throughout their range rendering provenance studies difficult and time-consuming. Since all native species are evergreen, foliage provides an alternative source material which is available throughout the year and easily collected. As in many other species, however, phenolic compounds in the foliage are rapidly oxidized to form quinones, condensed tannins, and brown pigments which inactive most enzymes when the tissue is homogenized during conventional extraction procedures (browning effect; ANDERSON, 1968). This problem has been overcome in recent years by adding a range of chemical agents to the extraction buffer which inhibit phenol oxidase activity and reduce or bind the products of the reaction (quinones).

This paper describes the extraction and assay of enzymes from *Nothofagus* foliage and discusses the results of isozyme analysis of 12 enzyme systems segregating into 22 putative loci.

Materials and Methods

Three populations each of all 5 native *Nothofagus* species were sampled from various provenances in the South

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Table 1. — Provenance and sample size of 15 analysed *Nothofagus* populations.

	Population	Species	N	Latitude S	Longitude E	Altitude (m)
1	Pigeon Saddle	*truncata*	57	40°49'	172°57'	200–300
2	Reefton Saddle	*truncata*	55	42°08'	171°49'	300–370
3	Lake Haupiri	*truncata*	62	42°34'	171°42'	210
4	Rough Stream	*fusca*	60	42°12'	171°56'	260
5	Lake Haupiri	*fusca*	56	42°33'	171°40'	210–270
6	Upper Taramakau	*fusca*	60	42°46'	171°40'	275
7	Pigeon Saddle	*solandri*	57	40°49'	172°57'	200–300
8	Hundalee Hills	*solandri*	60	42°13'	173°25'	150
9	Coopers Creek	*solandri*	60	43°17'	172°06'	320
10	Bell Hill	*cliffort.*	50	42°32'	171°38'	250
11	Craigieburn Range	*cliffort.*	55	43°08'	171°44'	880
12	McFarlane Mound	*cliffort.*	59	44°02'	168°44'	30–60
13	Rough Stream	*menziesii*	55	42°12'	171°56'	260
14	Blackwater Creek	*menziesii*	55	42°37'	171°15'	60
15	Haast	*menziesii*	60	43°58'	169°13'	60–90

Island (*Table 1*). For each sample population, branch tips bearing 5 to 20 leaves were harvested from 50 to 60 trees located along up to 50 m wide and approximately 1 km long transects. Samples were kept cool and moist in sealed plastic bags and were normally extracted within 1 to 5 days of collection. C. 50 mg (fresh weight) of leaf lamina were crushed with 0.5 ml Tris-sucrose-polyvinyl-pyrrolidone (PVP) extraction buffer pH 7.5 (Appendix I) and 20 mg to 30 mg acid washed sand in a cooled mortar until all tissue was homogenized. The slurry was transferred into 0.5 ml auto-analyser cups with a plastic scraper and centrifuged at 6000 rpm for 2 minutes. The supernatant was absorbed onto 15 mm x 2 mm filter paper wicks (Whatman No. 3). Extracts were stored in a refrigerator if analysed on the same day or in a conventional freezer for overnight or longer storage.

Diaphorase (DIA, EC 1.6.4.3), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), and superoxide dismutase (SOD, EC 1.15.1.1) were assayed on sodium-borate pH 8.6/7.8, esterase (EST, EC 3.1.1.1) and 6-phosphogluconate dehydrogenase (6-PG, EC 1.1.1.44) on lithium-borate pH 8.3/8.3, and aconitase (ACO, EC 4.2.1.3), aldolase (ALD, EC 4.1.2.13), glyceraldehyde-3-phosphate dehydrogenase (G3PDH, EC 1.2.1.12), hexokinase (HEX, EC 2.7.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), and shikimate dehydrogenase (SKDH, EC 1.1.1.25) on a Tris-histidine buffer system pH 7.0/7.0 (Appendix II).

The starch gels (230 mm x 70 mm x 7 mm, 11.5% w/v) were loaded with 60 to 65 sample wicks and subjected to horizontal electrophoresis by applying an initial current of 35 mA. After 30 minutes the wicks were removed and the current increased to 50 mA. The gels were disconnected after 2 to 4 h and sliced horizontally into 1 mm thick slices which were incubated at 37° C in specific stain solutions (Soltis et al., 1983). After staining, gel slices were scored visually by measuring the distance of each band.

Percentage of polymorphic loci (P; frequency of the most common allele $\leq$ 0.99), mean number of alleles per locus (A; measured over all loci), Nei's (1978) unbiased estimate of expected heterozygosity (H_e), and observed heterozygosity (H_o) were calculated using the BIOSYS-1 computer package (Swofford and Selander, 1989). Total gene diversity (H_T) at each locus was partitioned into hierarchical components (Nei, 1973).

Results

Sample extraction, storage, and stainability

The Tris-sucrose-PVP buffer proved to be the only suitable extraction buffer for *Nothofagus* foliage. Tris-maleate and phosphate extraction buffers containing sodium tetraborate, sodium metabisulfite, sodium diethyldithiocarbamate as well as PVP, which were often successfully employed for foliage extraction of conifers, ferns, and some tropical hardwood trees (e. g., Pitel and Cheliak, 1984; Soltis et al., 1983; Liengsiri et al., 1990), produced only very unsatisfactory stain results.

Although usually extracted within a few days of sampling, enzyme activity of foliage samples stored in a cold room (2° C to 4° C) had not noticeably decreased when extracted after up to 6 weeks. If leaf extracts were stored in a conventional freezer (— 18°C), some enzymes, e. g., ACO, HEX, SKDH, lost activity after a few days, while EST, PGI, and SOD could still be assayed after several weeks.

Enzymes of the amino acid metabolism, commonly assayed in other studies, e. g., aspartate-aminotransferase (AAT), leucine aminopeptidase (LAP), and glutamate dehydrogenase (GDH), did not produce stains on any of the buffer systems employed. Only unscorable stains were obtained for peroxidase (PER) and isocitrate dehydrogenase (IDH).

Genetic variation and gene diversity

Ten enzyme systems produced two scorable loci (loci are distinguished in italics with hyphenated numerals; 1 designating the most anodal locus) which usually represent cytosolic and plastid isozymes (e. g., Gottlieb, 1982). One locus was scored for DIA and G3PDH. All recognized allozymes (electrophoretic variants) at each locus were consecutively numbered according to their migration distance, 1 designating the most anodal allozyme. The species of the *'fusca'* group and *Nothofagus menziesii* show a complete segregation of alleles at all but two of the 22 analysed loci. Only the *Sod-2* locus of the *'fusca'* group and allele 1 of the *Mdh-1* locus in *N. solandri* have the same electrophoretic mobility as the respective alleles in *N. menziesii*. In all species of the *'fusca'* group, 15 of the 22 loci are fixed (homozygous) for one allele in all analysed populations. *Aco-1* and *Aco-2* are only variable in *N. solandri* var. *solandri*, but *Dia* and *Pgm-1* are

Table 2. — Genetic variation (mean ± s. e.) in New Zealand *Nothofagus* species, based on 22 loci; (N) sample size, (P) mean percentage of polymorphic loci, (A) mean number of alleles per locus, (H_e) mean expected heterozygosity, (H_o) mean observed heterozygosity.

No.	Species	N	P	A	H_e	H_o
1	*truncata*	57	4.6	1.23±0.15	0.035±0.033	0.036±0.034
2	*truncata*	55	9.1	1.27±0.15	0.039±0.033	0.043±0.036
3	*truncata*	62	13.6	1.32±0.17	0.037±0.029	0.034±0.026
4	*fusca*	60	4.5	1.14±0.14	0.028±0.028	0.030±0.030
5	*fusca*	56	4.5	1.18±0.14	0.026±0.025	0.020±0.019
6	*fusca*	60	4.5	1.14±0.14	0.027±0.027	0.026±0.026
7	*solandri*	57	18.2	1.32±0.14	0.026±0.015	0.026±0.014
8	*solandri*	60	22.7	1.36±0.17	0.034±0.017	0.030±0.016
9	*solandri*	60	18.2	1.18±0.08	0.020±0.014	0.020±0.013
10	*cliffort.*	50	18.2	1.18±0.08	0.039±0.021	0.033±0.017
11	*cliffort.*	55	13.6	1.23±0.11	0.027±0.017	0.028±0.018
12	*cliffort.*	59	18.2	1.23±0.11	0.035±0.020	0.037±0.022
13	*menziesii*	55	22.7	1.32±0.14	0.095±0.039	0.097±0.041
14	*menziesii*	55	22.7	1.36±0.15	0.103±0.044	0.102±0.047
15	*menziesii*	60	18.2	1.45±0.18	0.099±0.045	0.117±0.055
Population mean						
	truncata	58	9.1	1.27	0.037	0.038
	fusca	59	4.5	1.15	0.027	0.025
	solandri	59	19.7	1.29	0.027	0.025
	cliffort.	55	16.7	1.21	0.034	0.033
	menziesii	57	21.2	1.38	0.099	0.105

variable in all 4 species of the group. *Nothofagus truncata* is characterized by a fast *Dia* variant (allele 1) which is rare in the other 3 species. *Nothofagus truncata* and *N. fusca* are fixed for allele 2 at the *Mdh-1* locus. All 4 '*fusca*' species share the most common allele 2 of *Pgi-2* which is fixed in *N. fusca*, but *N. truncata* has 3 (3, 5, 6) and *N. solandri* var. *solandri* and var. *cliffortioides* posses 2 additional, less common alleles (1, 4). *Pgm-1* is highly polymorphic in *N. fusca* which has 4 alleles, sharing alleles 1, 3, and 4 with *N. truncata* and alleles 2, 3, and 4 with *N. solandri* var. *solandri* and var. *cliffortioides*. Six of the 22 loci are variable in *N. menziesii (Est-2, Mdh-1, Pgi-2, Pgm-1, Pgm-2, Skdh1)*, all except the latter are polymorphic.

Nothofagus menziesii exhibits the highest population means of all measures of genetic variation (*Table 2*). Most of the genetic variation in the '*fusca*' species arises from only one polymorphic locus with 2 to 4 common alleles. In *N. menziesii*, on the other hand, each population has 4 to 5 polymorphic loci with 2 to 4 common alleles each which accounts for the much higher measures of genetic variation. Within the '*fusca*' species percentage of polymorphic loci and average number of alleles per locus are highest in *N. solandri* var. *solandri*, but heterozygosity is highest in *N. truncata* which possesses the only highly polymorphic locus (*Pgi-2*) in the group. *Nothofagus fusca* has the lowest percentage of polymorphic loci and average number of alleles per locus and, together with *N. solandri* var. *solandri*, also the lowest levels of heterozygosity in the group.

Measures of gene diversity provide estimates of population differentiation and allow partitioning of genetic variation among subpopulations or different taxonomic levels. Total gene diversity (H_T) was calculated for each of the 22 loci and partitioned into hierarchical components at the group, species, and population level. Single locus diversity for each species is given in *table 3*. Between 84.0% and 100.0% of the total gene diversity is contained within populations. *Nothofagus menziesii* (12.3%) and *N. solandri* var. *cliffortioides* (16.0%) possess a much higher between-population variation than *N. truncata (1.0%)*, *N. fusca* (0.0%), and *N. solandri* var. *solandri* (0.7%). This may partly reflect the wider geographical sampling range of the two former species where one of the sample populations originated from the southern South Island (south Westland, latitude 44° S) which constitutes a disjunct distribution range for all *Nothofagus* species (e. g., WARDLE, 1984). In *N. solandri* var. *cliffortioides* another part of the interpopulational variation results from divergence in *Mdh-1* allele frequencies between individual populations which were also sampled across the largest altitudinal range (*Table 1*).

Hierarchical components of total gene diversity of all 15 *Nothofagus* sample populations are given in *table 4*. Only 12.8% of the total variation resides within populations; of the 87.2% interpopulational variation, 1.8% are due to variation within and 85.4% to variation between species. The between-species component can be further partitioned into 11.6% within-group and 73.8% between-group variation. The latter figure reflects the major genetic differences between *N. menziesii* and the '*fusca*' group. Results of a separate hierarchical analysis of the '*fusca*' group (*Table 5*) shows that 48.0% of the total gene diversity resides within populations, 47.9% between species, and 4.1% between populations within species. The interpopulational component of variation (52.0%) is much higher than the corresponding figure in *N. menziesii* (12.3%; *Table 3*) indicating that the '*fusca*' group as a whole contains more genetic variation than a single variable species. On the other hand, analysis of gene diversity between all 6 populations of the *N. solandri* 'complex' (includes both varieties) amounts to 18.1% which is only slightly higher than the figure for var. *cliffortioides* alone (16.0%) and stresses the close genetic relationship between the two varieties of *N. solandri*.

The breeding system

Deviation of observed from expected heterozygote frequencies (HARDY-WEINBERG equilibrium) was analysed sep-

Table 3. — Distribution of gene diversity in New Zealand *Nothofagus* species, based on 2 to 7 variable loci; (H_T) total gene diversity, (H_S) mean gene diversity within populations, ($D_{ST} = H_T - H_S$) gene diversity between populations; ($G_{ST} = D_{ST}/H_T$) proportion of gene diversity between populations.

Species	Locus	H_T	H_S	D_{ST}	G_{ST}
truncata	*Dia*	0.02719	0.02704	0.00015	
	Est-2	0.01717	0.01717	0.00000	
	Pgi-2	0.70281	0.69566	0.00715	
	Pgm-1	0.07234	0.07171	0.00063	
	Mean	0.20488	0.20290	0.00198	0.010
fusca	*Dia*	0.00593	0.00593	0.00000	
	Pgm-1	0.58048	0.58048	0.00000	
	Mean	0.29320	0.29320	0.00000	0.000
solandri	*Aco-1*	0.03278	0.03193	0.00085	
	Aco-2	0.01163	0.01159	0.00004	
	Dia	0.01682	0.01682	0.00000	
	Est-2	0.06073	0.06064	0.00009	
	Mdh-1	0.30313	0.30313	0.00000	
	Pgi-2	0.08310	0.08211	0.00099	
	Pgm-1	0.07554	0.07343	0.00211	
	Mean	0.08339	0.08281	0.00058	0.007
cliffort.	*Dia*	0.09625	0.09463	0.00162	
	Est-2	0.08898	0.08640	0.00258	
	Mdh-1	0.49355	0.37041	0.12314	
	Pgi-2	0.10283	0.09459	0.00824	
	Pgm-1	0.09255	0.08864	0.00391	
	Mean	0.17483	0.14693	0.02790	0.160
menziesii	*Est-2*	0.47868	0.45415	0.02453	
	Mdh-1	0.35425	0.32058	0.03367	
	Pgi-2	0.45207	0.33902	0.11305	
	Pgm-1	0.57850	0.52196	0.05654	
	Pgm-2	0.61397	0.53521	0.07876	
	Skdh1	0.00554	0.00554	0.00000	
	Mean	0.41384	0.36274	0.05109	0.123

Table 4. — Distribution of gene diversity among different taxonomic levels in New Zealand *Nothofagus*; (H_T) total gene diversity, (H_S) mean gene diversity within populations, (D_{ST}) gene diversity between populations, (D_{CT}) gene diversity between species, (D_{RT}) gene diversity between groups.

Locus	H_T	H_S	D_{ST}	D_{CT}	D_{RT}
Aco-1	0.32531	0.00638	0.31893	0.31864	0.31862
Aco-2	0.32187	0.00232	0.31955	0.31953	0.31953
Dia	0.56755	0.02890	0.53865	0.53801	0.07131
Est-2	0.36628	0.12372	0.24256	0.23393	0.23626
Mdh-1	0.53246	0.19925	0.33321	0.28592	-0.00483
Pgi-2	0.53164	0.24229	0.28935	0.24960	0.15648
Pgm-1	0.49875	0.26780	0.23095	0.21168	0.16831
Pgm-2	0.34456	0.10704	0.23752	0.21342	0.22176
Skdh1	0.32022	0.00111	0.31911	0.31911	0.31911
Sod-2	0.00000	0.00000	0.00000	0.00000	0.00000
others	0.32000	0.00000	0.32000	0.32000	0.32000
Mean	0.34767	0.04449	0.30317	0.29681	0.25666
(%)	100.0	12.8	87.2	85.4	73.8

arately for the '*fusca*' group (41 variable loci from 12 populations) and *N. menziesii* (16 variable loci from three populations) and gave means of —0.92 ± 2.13% and 6.90 ± 7.33%, respectively. These only insignificant overall deviations from HARDY-WEINBERG equilibrium indicate that a random mating breeding system with predominant cross-pollination operates in both groups.

All '*fusca*' species are able to form natural hybrids among the group which are usually recognized by their intermediate leaf shape (e. g. WARDLE, 1984). Species specif-

Table 5. — Distribution of gene diversity in the *Nothofagus fusca* group; (H_T) total gene diversity, (H_S) mean gene diversity within populations, (D_{ST}) gene diversity between populations, (D_{CT}) gene diversity between species, (D_{SC}) gene diversity between populations within species.

Locus	H_T	H_S	D_{ST}	D_{CT}	D_{SC}
Aco-1	0.00830	0.00798	0.00032	-0.00003	0.00035
Aco-2	0.00292	0.00290	0.00002	0.00000	0.00003
Dia	0.38680	0.03612	0.35068	0.34988	0.00080
Est-2	0.04240	0.04112	0.00128	0.00020	0.00109
Mdh-1	0.42950	0.16891	0.26059	0.21445	0.04614
Pgi-2	0.30244	0.21811	0.08433	0.07741	0.00692
Pgm-1	0.24315	0.20427	0.03888	0.03657	0.00231
Mean	0.20221	0.09706	0.10516	0.09693	0.00823
(%)	100.0	48.0	52.0	47.9	4.1

ic alleles at the *Dia*, *Pgi-2* and *Mdh-1* locus were occasionally found in a heterozygotic state in specimens of another species, normally monomorphic at the relevant locus. This phenomenon is more prominent in mixed *Nothofagus* stands where 2 or 3 species of the *'fusca'* group occur together and care must be taken to distinguish 'normal' and intermediate leaf shapes during sampling. Presumed hybrids between *N. truncata* and one of the other species of the *'fusca'* group are easily noticed on gels stained for DIA which has a tetrameric sub-structure and the heteromeric enzymes produce prominent five-banded stains.

Effect of sample size on results

An inventory of common alleles with a frequency $\geq$ 0.05 in a sample population can usually be established by analysing comparatively few individuals. Comparisons of allele frequencies between populations can only be made at an acceptable level of significance with somewhat larger samples, however. In an extensive study of genetic variation in *Nothofagus truncata*, which possesses 4 common alleles of *Pgi-2*, only minor changes in allele frequencies were noted after the sample size had exceeded 50 to 60 individuals (Haase, unpublished data). The sample size chosen for the present study is therefore believed to yield representative allele frequencies for the sampled populations.

Discussion

The present taxonomic concept with emphasis on the distinction between *Nothofagus menziesii* and the *'fusca'* group species, so far only based on pollen character and morphological features, is supported by genetical analysis. Only 2 of a total of 69 different alleles distinguished at 22 loci are shared between the 2 'groups', which reflects the long time since the distinct *'menziesii'* and *'fusca'* pollen types have been produced by fossil *Nothofagus* species (*Upper Cretaceous*). On the other hand, the 4 native species of the *'fusca'* group share all common alleles at each of the analysed loci except *Dia* (*N. truncata*) and *Mdh-1* (*N. solandri*) and each species only possesses additional, less common alleles at up to 7 of the 22 loci. This close genetic relationship probably allows the observed natural hybridization between all members of the *'fusca'* group although this does not appear to have caused introgression into the specific genepools. The fitness and fertility of hybrids therefore needs to be investigated.

Wilcox and Ledgard (1983) studied variation in *Nothofagus* seedling growth rates and morphology in replicated growth trials. *Nothofagus menziesii* and the *N. solandri* 'complex' were found to be genetically variable with strong differentiation into regional ecotypes, whereas *N. fusca* was described as a comparatively uniform species. *Nothofagus truncata* was poorly represented in the study and no conclusive results were obtained. This relative ranking of morphological variation, high in *N. menziesii* and *N. solandri* and lowest in *N. fusca*, agrees with the levels of genetic variation established by isozyme analysis.

The present study was based on analysis of a limited number of sample populations and covered only part of the complete geographical range of the various *Nothofagus* species. The only species which has been extensively studied is *N. truncata* where 30 populations from provenances throughout the geographical range were analysed at 15 loci (Haase, 1992). Compared with the present results for *N. truncata*, the country-wide survey found only 4 more, mostly geographically restricted, rare alleles, but the 4 common alleles of *Pgi-2* revealed characteristic regional frequencies. Measures of genetic variation and total gene diversity were comparable to present results after correction from 15 loci to 22 loci, whereas the between-population component of variation was higher than in the present study because of the wider sampling range.

Poole (1949, 1987) assumed that the New Zealand *Nothofagus* species may be self-sterile, because of very poor viable seed production in isolated trees, but experimental work has been lacking so far. The preliminary results of the present study indicate predominant cross-pollination; selfing appears to be unimportant if occurring at all. Possible self-incompatibility should be further investigated by artificial pollination experiments.

At the present stage of methodology, leaf extracts of *Nothofagus* yield only a limited number of enzymes. Compared with seed analysis or growth trials, however, isozyme analysis of leaf extracts is a seasonally independent and comparatively quick and inexpensive method of genetic analysis since results can be obtained immediately after sample collection. Further experimentation with foliage extracts may increase the number of enzyme systems which can be assayed and improve staining results for others.

Electrophoretically detectable variation in enzymes constitutes only a small fraction of the genetic diversity of populations and may or may not be a good predictor of variation in other, physiologically or ecologically im-

portant genes. However, the method can detect and quantify changes in genetic diversity or frequencies of certain alleles which may be related to environmental gradients or vegetation history. Verification of genetic diversity and genetic distinctness of regional or local tree populations would give priority to conservation of such stands as potential genetic resources. *Nothofagus* species are widespread and common forest trees in the southern temperate zone and may become more important for forestry in the future.

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Appendix I

Tris-sucrose extraction buffer; amounts for 100 ml are given in brackets. Based on Feret (1971), modified by Pitel and Cheliak (1984).

0.1 M Tris (1.212 g)
0.5 M Sucrose (17.12 g)
6 mM Ascorbic acid (0.118 g)
6 mM Cysteine (0.106 g)
1 mM Dithiothreitol (0.015 g)
0.05 mM EDTA (0.002 g)
1% (v/v) Tween 80 (1.0 ml)
4% (w/v) Polyvinylpyrrolidone (4.0 g)
0.25% 2-mercaptoethanol (0.25 ml)

The extraction buffer can be stored frozen in convenient aliquots with mercaptoethanol and PVP added before use. The final composition can be stored in a refrigerator for several days.

Appendix II

Electrode and gel buffer systems; amounts for 1 litre are given in brackets.

Electrode	pH	Gel	pH
0.100M sodium hydroxide (4.0 g) 0.300M boric acid (18.55 g)	8.6	0.015M Tris (1.84 g) 0.004M citric acid (0.69 g)	7.8
(System 6 of Soltis et al., 1983)			
0.038 M lithium hydroxide (1.60 g) 0.188 boric acid (11.60 g) adjust to pH 8.3 with dry components	8.3	0.004M lithium hydroxide 0.019M boric acid 0.042M Tris (5.04 g) 0.007M citric acid (1.25 g) dilute electrode buffer 1 : 9, add other components	8.3
(System 7 of Soltis et al., 1983)			
0.125M Tris (15.135 g) adjust to pH 7.0 with 1M citric acid	7.0	0.014M L-Histidine (2.1 g) 0.002M EDTA (0.08 g) adjust to pH 7.0 with 1M Tris	7.0
(System H of Pitel and Cheliak, 1984, modified)			