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Adaptive and Neutral Variation of the Resprouter *Nothofagus antarctica* Growing in Distinct Habitats in North-Western Patagonia

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N. antarctica occurs in the widest range of habitat types among all South American *Nothofagus*. The aim of this study is to investigate adaptive responses by variation in morphological (tree form and leaf characters), and environmental traits (soils) of the polymorphic *N. antarctica*. Also we analyze the effect of genetic drift and limited gene flow in such predominantly apomict by means of neutral variation (isozymes). We studied four potentially different morphological variants each associated with a separate habitat 1) an arboreal variant growing in optimal environments; 2) a sparsely branched variant of temporarily flooded basins or flats; 3) a dwarf variant growing at high elevation, and 4) a shrub-like variant inhabiting matorral environments. The study was restricted latitudinally to Nahuel Huapi National Park, Argentina. For each habitat type we investigated two sites. *Nothofagus antarctica* shows locally occurring phenotypes. The forest and the high elevation variants were morphologically distinct from the matorral and the basin types. The latter were undistinguishable except for more profuse branching in the matorral type as a result of sprouting due to recent fires. Isozyme evidence indicates a great deal of within-population genetic diversity which is maintained by outcrossing and significant among-site divergence (F_{ST} = 18%) that reflects limited gene flow.The apparent high phenotypic and genetic variability in *N. antarctica* is due to both plasticity and genotypic effects as a result of stable population structure and long periods of isolation which may be reinforced by selection at diverse biotopes.

Keywords isozymes, morphological variation, leaf traits, South America, temperate forest
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1 Introduction

Resprouters are plants able to survive fire and other disturbances that destroy above-ground parts and recover by sprouting from unaffected buds (Lamont and Wiens 2003). The majority of species that resprout also produce seeds which can contribute to species recovery (Clarke and Knox 2002). But in contrast to nonsprouters, resprouters do not depend on seed production and seedling recruitment for persistence. Plants with sprouting abilities can be thus considered predominantly apomicts. As such, they are thought to be distributed over broad latitudinal ranges than their sexual relatives. The capacity of an apomict species to inhabit different environments will be then a function of the breaths and number of individual genotypes and the evolutionary divergence among them mainly shaped by natural selection and genetic drift.

The *main objective* of this study is to investigate intraspecific variation patterns of a polymorphic resprouter inhabiting distinct habitat types. The *hypothesis* tested is that within-species polymorphism is the result of the effects of natural selection by means of adaptive traits and limited gene flow and genetic drift that can be measured by genetically neutral markers. Adaptive responses will be analyzed by morphological characters (gross individual morphology and leaf traits) in relation to distinct environmental conditions (soils). Also we analyze the effect of genetic drift and limited gene flow in such predominantly apomict by means of neutral variation using isozymes. Morphological characters often display a different structure than isozymes (Wright 1931, Falconer 1989). In particular, a tendency exists for morphological traits as an indication of the effects of natural selection to be more differentiated than neutral characters which are often a reflection of random events such as drift (Merilä and Crnokrak 2001).

The study species is *Nothofagus antarctica* that occurs in the widest range of habitat types among all South American southern beeches. Distinct morphotypes have been identified for *N. antarctica* in Chile (Ramírez et al.1985) and different ecotypes have been suggested (Romero 1986). Within Nahuel Huapi National Park in northwestern Patagonia, Argentina *N. antarctica* grows in four habitat types and distinct architectural features have been described for individuals growing in such contrasting physical settings. These were meant to be the result of a species-specific endogenous, i.e. genetically-determined, growing pattern modelled by environmental conditions (Stecconi 2006). This intraspecific polymorphism displayed by *N. antarctica* provides the ideal natural setting to quantify gross individual and leaf morphological features along with potential genetic differences using neutral markers which to date have not yet been explored. This experimental design allows the analysis of trait variation in species with limited production of viable seed as *N. antarctica* (Premoli 1991) on which studies such as common gardens are often difficult. The questions to be addressed by this study are: "do previously described morphotypes present significant differences in gross individual and leaf morphology?", "do morphological differences correlate to environmental conditions, i.e. soils?", "are among-population genetic differences in neutral markers significant?", "to what extent phenotypic and genetic differences are associated in *N. antarctica*?".

2 Materials and Methods

Nothofagus antarctica (G. Forster) Oerst. (Nothofagaceae) has a wide latitudinal range from 36º30´ to 56º S latitude in mountain regions of Chile and Argentina (Donoso 1974, Moore 1983). It is found from sea level in the south to 2000 m a.s.l. in the north. It occurs in a variety of habitat types (Donoso 1987). These include poorly drained areas at low to high elevation, exposed sites of unstable substrate at the alpine timberline, areas of topographic depressions to cold air drainage, steep slopes with shallow soils, and dry open woodlands near the Patagonian steppe ecotone (Veblen et al. 1996).

In this study we analyze and compare quantitatively four morphological variants bound to different habitat types 1) a forest variant growing in optimal low-elevation and relatively humid environments (Ramirez et al. 1985, Veblen et al. 1996); 2) a high-elevation shrubby variant grow-

Table 1. Location and physical characteristics of sampled sites of *Nothofagus antarctica*. Capital letters denote different vegetation variants: M=Matorral, H=High elevation, F=Forest, B=Temporarily flooded basin or flat.

Site	Latitude (S)	Longitude (W)	Elevation (m)	Slope	Precip. (mm)	Temp. (ºC)	Exposure
1M	41º 12’ 56’’	71º 18’ 19’’	1025	6–10º	1400	7.2	N
2H	41º 15’ 57’’	71º 18’ 26’’	1500	20º	1400	4.4	NW
3M	41º 10’ 31’’	71º 26’ 17’’	1050	4º	2000	7.1	NE
4H	41º 14’ 36’’	71º 24’ 39’’	1700	21º	1700	3.2	NE
5F	41º 20’ 51’’	71º 45’ 6’’	800	0º	2200	8.6	–
6B	41º 13’ 53’’	71º 46’ 55’’	890	0º	1700	8.0	–
7F	41º 25’ 35’’	71º 32’ 8’’	990	0º	1400	7.4	–
8B	41º 27’ 21’’	71º 28’ 38’’	930	0º	1500	7.8	–

Annual precipitation from Barros et al. (1983)
Temperatures calculated from the mean temperature in Bariloche (Cabrera 1994)

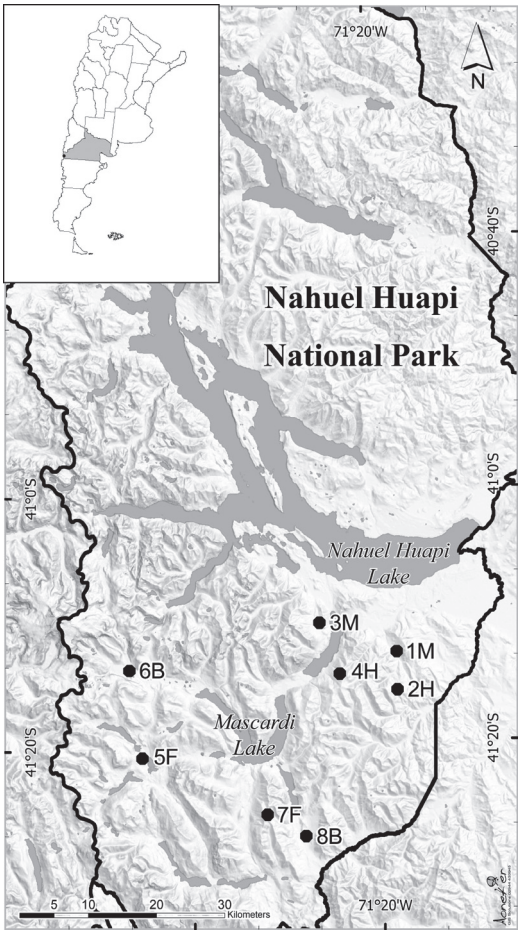

Fig. 1. Location of eight sampled populations of *Nothofagus antarctica* within Nahuel Huapi National Park, Rio Negro province, Argentina.

ing near timberline (Ramirez et al. 1985, Veblen et al. 1996); 3) a sparsely branched variant growing at temporally flooded basins or flats (Ramirez et al. 1985, Stecconi 2006), and 4) a matorral variant growing in transitional habitats between the forest and the steppe under Mediterranean climates of the eastern Andes called matorral environments (Veblen et al. 1996). In the south (around 50 to 52º S) the tree form is most common and in the north (40 to 43º S) on the eastern side of the Andes the matorral form is more usual (Ramirez et al. 1985, Veblen et al. 1996).

Nothofagus antarctica most often resprouts vigorously from base trunks after disturbance, producing c. 1 m tall shoots 60 d after fire (pers. obs., A.P.). Also individuals affected by grazing have profuse branching and compact growth (Stecconi 2006). *N. antarctica* is wind-pollinated and seed are mainly dispersed by gravity. Seed production and germination is reduced (Premoli 1991) and seedlings are occasionally seen in the wild (Marino 1996).

The study was conducted within Nahuel Huapi National Park in north-western Patagonia, Argentina. A total of eight sites were investigated, two replicates of each contrasting habitat type: forest, matorral, temporally flooded basins or flats, and high-elevation environments (Fig. 1). These can all be found within the Park and possible latitudinal variations can therefore be controlled. Sampling on natural stands consisted of population pairs of different habitat types located in close proximity. These were 1M–2H, 3M–4H, 5F–6B, and 7F–8B (Table 1, Fig. 1). The *Matorral* sites (1M and 3M) were at an elevation of about 1000

m a.s.l. and had a slope of 5º exposed at north or northeast. The accompanying vegetation was mostly other shrub species, for example *Schinus patagonicus*, *Diostea juncea*, and *Berberis* spp. Both sites had rather dense vegetation. The *high elevation* sites (2H and 4H) were at an elevation of about 1600 m and had a slope of 20º exposed at northwest and northeast, respectively. *N. antarctica* was associated to *N. pumilio* and a few shrub species including *Chiliotrichum rosmarinifolium* and *Berberis* spp. These populations were very dense and form continuous mats. The *Forest* sites (5F and 7F) were at an elevation of 800–1000 m on flat terrain. The accompanying vegetation was other trees as *Maytenus boaria* and co-dominant shrub species such as *Schinus patagonicus*, *Diostea juncea* and understory vegetation mainly consisted of *Chusquea couleou*. These populations were relatively sparse although the understory vegetation was dense. The *temporarily flooded basin or flat* sites (6B and 8B) were at an elevation of about 900 m, accompanied by common heath-vegetation consisting of a few shrubs and abundant reeds such as *Juncus arcticus* and grasses. These sites were less dense than the other studied locations. Burned stumps were found in all studied sites and known fire history indicate that matorral sites1M and 3M burned as recent as during the summer 1996 while the forest in Guillelmo (7F) last burned in 1854 and the Fonk (5F) forest site in 1943 (pers. comm. N. Tercero-Bucardo and T. Kitzberger, Universidad Nacional del Comahue).

Physical characterization of field sites and collection of fresh leaf material for genetic and morphological analyses were conducted during March 2004 from the eight sites. At each site and within a 1 ha plot, ten 100 m^2 patches were randomly chosen. Within each patch five individuals were regularly sampled, which gives a total number of 50 sampled individuals at each of the eight sites. Although vegetative spread has been suggested to occur to some extent in *N. antarctica*, individuals were clearly identifiable in the field. Also in a companion paper Premoli and Steinke (submitted) using spatially explicit genetic analysis show that each 100 m^2 patch holds on average 80% different genets, therefore discarding the possibility of large-scale vegetative spread at most studied locations. Gross individual morphology was characterized on each individual by height and DBH (diameter at breast height) in forest types or DGH (diameter at ground height) in matorral, high-elevation, and basin sites where individuals have profuse branching from base trunks, hereafter considered as diameter (D). The ratio between height and diameter was calculated to estimate overall tree shape. Rotten centres characterize *N. antarctica* trunks and therefore individual tree age by cores taken using increment borers was not determined. Leaves on 15–20 cm long terminal branches were collected for leaf morphological and isozyme analyses. The leaves were sun exposed, north facing to control for shadow effects, and located at the same height on each individual. Depending on the vegetation type these were 0.8 m for high elevation and flooded basin, 1.5 m for matorral, and 5 m for forest variant. Leaf morphology analysis was performed on 10 leaves from each of five randomly chosen individuals at each site. Leaves were mounted on paper and scanned using a Cannon Scanner (Canoscan N656U). Digital images were used to measure size and shape components of leaf traits using the program SigmaScan Pro 5.0. Leaf size was quantified by length, width, area, and perimeter. Leaf shape was described by width to length ratio, length from the insertion point of the petiole to the widest point of the leaf (hereafter distance), and the dissection index dividing the perimeter by the square root of the area (hereafter toothiness).

Nitrogen economy between sites was investigated by comparing nitrogen content of fresh and senescent leaves collected from the same five individuals at each site. Fresh leaves were collected during summer in March and senescent leaves that were ready to fall to the ground when the individual was shaken, were collected during the autumn in May. Leaves were oven-dried for three days at 60ºC and then pulverized using a grinder. Nitrogen concentration in leaves were measured at the Soil Lab, Chemistry Department, Universidad Nacional del Comahue, Bariloche, Argentina using the regular semi-micro Kjeldahl method with a block digester (Bremner 1996).

Soil properties were investigated from samples taken from the eight study sites. These consisted of soil collected from a depth of 10 cm on three randomly chosen 10 cm^2 pits from which the

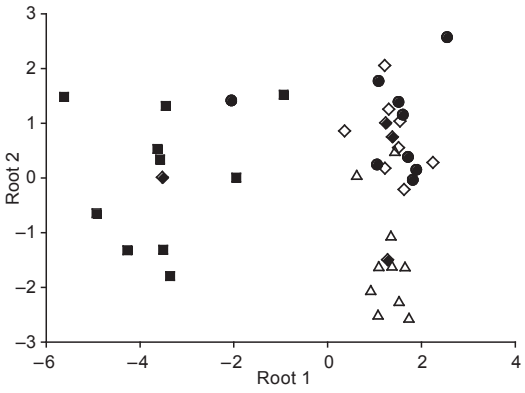

Fig. 2. Multivariate discriminant analysis of tree form and leaf characters of *Nothofagus antarctica* using a-priori morphotypes as classifying factor: forest by black squares ,matorral by black circles, high elevation by open triangles, and temporarily flooded basin or flat by open diamonds. Black diamonds represent population means.

organic matter had been removed. Soil samples were analysed at the Soil Lab, Chemistry Department, Universidad Nacional del Comahue, Bariloche, Argentina. They measured pH in H_2O (soil : water ratio = 1 : 2.5) (Thomas 1996), organic carbon (wet digestion by the Walkley-Black method) (Nelson and Sommers 1996), and total nitrogen (regular semi-micro Kjeldahl method using a block digester) (Bremner 1996).

Morphological, leaf nutrient, and soil data of all sites were analysed using one-way ANOVA. We assessed the degree to which a given population or groups of populations differed among each other by analyzing each of the variables by the Tukey post-hoc test for multiple comparison of means (Zar 1998), adjusting probability levels by the Bonferroni test (Rice 1989). An estimate of the population differentiation in quantitative traits (Q_{ST}), which is the analogue of F_{ST} based on molecular markers (see below), was obtained for single traits of leaf morphology as the ratio of within- and between-population variances following Merilä and Crnokrak (2001). To group individuals into different *a-priori* alternative habitat types based on tree form and leaf morphology and nutrients, we used multivariate discriminant analysis. This multivariate technique classifies populations on the basis of linear combinations of variables that represent the maximum possible separation among the groups while minimizing the variance within each group (Afifi and Clark 1990). Wilk's Λ (lambda) test was used to illustrate if the groups present significant differences in the position of their centroids; $\Lambda = 0$ indicates maximum dispersion of the centroids or perfect discrimination, whereas $\Lambda = 1$ indicates no dispersion between groups or no discrimination. Squared Mahalanobis distances between the group centroids (population averages) were calculated to study the discriminatory power between any two groups (Legendre and Legendre 1983).

Fresh leaf tissue collected for isozyme analysis was placed in plastic bags and kept on ice and stored at 5ºC in portable coolers until protein extraction in the laboratory. Leaves were ground in a mortar and proteins were extracted using a phosphate polyvinylpyrrolidone buffer (Mitton et al. 1979). Homogenates were stored at –80ºC until they were absorbed onto Whatman Nº 3 paper wicks that were loaded into 12% starch gels (Starch Art Corp., Smithville, TX, USA). Horizontal gel electrophoresis was used to separate isozymes according to protocols previously developed for *N. antarctica* (Vidal Russell 2000).

Eight enzyme systems were investigated in 400 individuals sampled; alcohol dehydrogenase (Adh, E.C.1.1.1.1), isocitrate dehydrogenase (Idh, E.C.1.1.1.42), malate dehydrogenase (Mdh, E.C.1.1.1.37), malic enzyme (Me, E.C.1.1.1.40), peroxidase (Per, E.C.1.11.1.7), phosphoglucoseisomerase (Pgi, E.C.5.3.1.9), 6-phosphogluconate dehydrogenase (6Pgd, E.C.1.1.1.44), and shikimate dehydrogenase (Skdh, E.C.1.1.1.25). They were all resolved using the morpholine-citrate gel and electrobuffer system (pH 7.5) (Ranker et al. 1989), running for 6 h at 30 mA. Five of the eight enzyme systems could be coded for nine loci which were consistently scored: Adh, Idh-1, Idh-2, Mdh-1, Mdh-2, Mdh-3, Pgi-1, Pgi-2 and Skdh.

Based on electrophoretic phenotypes standard measures of within-population genetic variability were calculated for *N. antarctica*. For all sites these were percentage of polymorphic loci (P) (sensu stricto criterion), mean number of alleles per locus (A) (considering both monomorphic and polymorphic loci), effective number of alleles (A_E), number of alleles per polymorphic locus (A_P),

Table 2. Tree form, morphology and nutrient content of leaves of *Nothofagus antarctica* and soil characteristics at the eight sites.

Traits	Site								F ANOVA
	1M	2H	3M	4H	5F	6B	7F	8B	
Tree gross morphology									$F_{(7, 392)}$
Height (m)	1.47 ab (0.05)	1.51 ab (0.05)	1.88 a (0.08)	0.80 b (0.04)	8.88 c (0.43)	1.88 a (0.08)	6.48 d (0.32)	2.01 a (0.10)	57.8*
Diameter (cm)	3.25 a (0.11)	4.21 ac (0.20)	6.36 ac (0.34)	4.24 ac (0.22)	22.15 b (1.53)	6.58 ac (0.71)	21.73 b (1.45)	6.92 c (0.55)	89.4*
Heigh / Diameter	0.46a (0.01)	0.38b (0.02)	0.32b (0.01)	0.21c (0.01)	0.48ad (0.03)	0.40af (0.03)	0.38bf (0.03)	0.37bf (0.02)	12.6*
Number of branches	7.58 a (0.58)	5.92 b (0.54)	3.66 c (0.31)	1.52 d (0.09)	1.12 d (0.05)	1.64 d (0.13)	1.62 d (0.15)	1.96 d (0.12)	57.8*
Leaf morphology									$F_{(7, 32)}$
Length (cm)	1.60 a (0.13)	1.70 a (0.16)	1.46 a (0.13)	1.61 a (0.06)	2.40 b (0.09)	1.58 a (0.09)	2.28 b (0.13)	1.50 a (0.05)	10.7*
Width (cm)	1.04 a (0.04)	1.11 a (0.07)	1.04 a (0.14)	1.02 a (0.03)	1.65 b (0.02)	1.12 a (0.05)	1.72 b (0.08)	1.06 a (0.03)	17.7*
Area (cm^2)	1.21 a (0.14)	1.39 a (0.19)	1.16 a (0.26)	1.08 a (0.06)	2.66 b (0.10)	1.24 a (0.10)	2.68 b (0.24)	1.14 a (0.05)	17.7*
Perimeter (cm)	4.68 a (0.32)	5.02 a (0.44)	4.37 a (0.51)	4.58 a (0.16)	7.31 b (0.21)	4.75 a (0.26)	7.29 b (0.42)	4.51 a (0.09)	14.0*
Distance (cm)	0.55 a (0.04)	0.59 a (0.08)	0.50 ab (0.06)	0.61 a (0.03)	0.87 c (0.04)	0.56 a (0.04)	0.73 b (0.06)	0.56 a (0.02)	5.7*
Width / Length	0.88 a (0.07)	0.85 abc (0.09)	0.95 a (0.06)	0.94 a (0.02)	0.62 bc (0.02)	0.91 a (0.03)	0.65 c (0.04)	0.93 a (0.03)	6.3*
Toothiness	4.26 ab (0.05)	4.29 ab (0.07)	4.13a (0.05)	4.41 ab (0.05)	4.49b (0.05)	4.27 ab (0.09)	4.47b (0.06)	4.23 ab (0.06)	4.0*
Leaf nutrients									$F_{(7, 32)}$
Green leaves %N	1.87 a (0.07)	1.78 a (0.05)	1.84 a (0.09)	2.60 b (0.04)	2.01 a (0.08)	1.96 a (0.07)	1.73 a (0.05)	1.83 a (0.09)	15.2*
Senescent leaves %N	0.69 a (0.02)	1.03 c (0.09)	0.66 b (0.05)	0.48 ab (0.01)	0.73 a (0.02)	0.69 a (0.01)	0.67 ab (0.04)	0.72 a (0.02)	12.5*
Soils									Mean
pH (H_2O)	6.1	6.9	6.3	6.4	5.7	5.5	6.3	6.4	6.2
Corg (g/kg)	48.7	65.8	35.5	19.5	88.4	16.2	75.6	111.2	57.6
N (g/kg)	0.5	3.6	1.9	1.1	6.4	1.1	4.7	10.2	3.7

Note: Values are means (1 SE). Measures of leaf morphology and nutrients were performed on five randomly chosen individuals per site (N=40). Gross morphology of individuals were measured on all 50 sampled individuals at each site. Results of one-way ANOVA ($P < 0.05$) for each variable are shown in the last column (after Bonferroni correction *$P < 0.05$). Within each row different letters indicate significant differences (Tukey post-hoc test for multiple comparisons of means).

and observed (H_O) and expected (H_E) heterozygosity. Departure of genotypic frequencies from Hardy-Weinberg equilibrium and the significance of by-locus fixation indices (F) were analyzed by chi-square tests (Li and Horvitz 1953). Positive values are associated with deficiencies of heterozygotes and suggest inbreeding. In contrast, negative values indicate heterozygote excess as commonly found in apomict plant populations. Also, restricted seedling establishment results in

Table 3. Genetic variability parameters for nine loci at eight sites of *N. antarctica.* Population pairs located in close proximity were 1M–2H, 3M–4H, 5F–6B, and 7F–8B. Values are means (1SE).

Site	P	A	AE	AP	HO	HE
1M	56	2.2 (0.4)	1.3	3.2	0.127 (0.059)	0.186 (0.68)
2H	44	2.0 (0.4)	1.4	3.2	0.164 (0.080)	0.199 (0.086)
3M	56	2.2 (0.4)	1.6	3.2	0.236 (0.079)	0.266 (0.092)
4H	67	2.0 (0.3)	1.6	2.5	0.228 (0.073)	0.275 (0.087)
5F	56	2.2 (0.4)	1.5	2.8	0.146 (0.055)	0.225 (0.092)
6B	78	2.8 (0.4)	1.5	3.3	0.176 (0.074)	0.250 (0.070)
7F	67	2.3 (0.4)	1.4	3	0.149 (0.050)	0.230 (0.077)
8B	67	2.3 (0.4)	1.5	3	0.123 (0.047)	0.247 (0.089)

Note: P is the percentage of polymorphic loci using the sensu stricto criterion, A is the mean number of alleles per examined locus, A_E is the number of effective alleles, A_P is the number of alleles per polymorphic locus. H_O and H_E are observed and expected heterozygozity respectively.

limited gene flow that would tend to favor among-population genetic differentiation by genetic drift. This was tested by analyzing the heterogeneity of allele frequencies throughout populations using chi-square tests. Also mean and 95% confidence intervals (95%CI) of the among-site divergence F_{ST} and within-population inbreeding F_{IS} were calculated following Weir and Cockerham (1984) using polymorphic loci by FSTAT version 2.9.1 (Goudet 2000).

3 Results

Gross tree morphology and leaf traits of *Nothofagus antarctica* differed significantly among all study locations (Table 2). 1M and 3M correspond to matorral, 2H and 4H high-elevation, 5F and 7F forest type, and 6B and 8B are the temporarily flooded basin or flat. In addition, nearby populations are 1M–2H, 3M–4H, 5F–6B, and 7F–8B. Forest types (5F and 7F) were distinct from the other variants for both tree morphology and most leaf traits. The forest type is much taller, has wider DBH, and bigger leaves than the other morphological variants. Also forest types 5F and 7F differed significantly from their respective near-by flooded basin variants 6B and 8B in tree form and all leaf morphology measures except toothiness (Table 2). The ratio height/diameter was in all cases less than one attaining the smallest value, i.e. widest tree morphology, in high-elevation site 4H. Matorral and high-elevation types located in close proximity (1M–2H and 3M–4H) had similar leaf characteristics although they differed in tree morphology. As such, matorral 1M and 3M had more branches than high elevation 2H and 4H, respectively. In addition, individuals from matorral 3M were taller than those from the high elevation site 4H (Table 2).

Population differentiation in quantitative traits by means of leaf morphology was on average $Q_{ST} = 0.21$. For leaf size metrics (length, width, area, and perimeter) Q_{ST} values were on average 0.15 (range 0.11–0.19) while for leaf shape measures (W/L, distance, and toothiness) attained a relatively larger mean of 0.28 (range 0.27–0.29).

Multivariate discriminant analysis yielded a significant separation of morphotypes ($F_{(21,78)} = 5.37$, $P < 0.0001$). Except toothiness, seven variables were entered in the forward stepwise model. These were the number of branches, leaf size variables (area and perimeter), leaf shape traits

(distance and W/L), and leaf nutrients (%N in green and senescent leaves). A correct classification into *a priori* groups was obtained for 86% of the individuals. The first canonical root extracted, significantly discriminated between the forest type and the rest morphotypes (Wilk's $\Lambda = 0.06$, $P < 0.0001$) (Fig. 2). The second canonical root separated the high elevation type from the matorral and the basin types (Wilk's $\Lambda = 0.37$, $P = 0.002$). Significant squared Mahalanobis distances between group centroids (population averages) were obtained between the forest and all the other types ($P < 0.01$ in all cases) and between the high-elevation type with both the matorral ($P = 0.006$) and the basin type ($P = 0.017$). No significant separation was obtained between the matorral and the basin type ($P = 0.34$).

Leaf nutrients, soil characteristics, and variables of the physical environment were heterogeneous in terms of variant type and location (Table 2). Nearby populations 5F and 6B have similar soil pH (Table 2) and mean temperature inasmuch population pair 7F–8B (Table 1). In contrast, high elevation sites 2H and 4H have higher nitrogen content in senescent and fresh leaves than their respective matorral types 1M and 3M located in close proximity, respectively. On the other hand, organic carbon and nitrogen soil content were heterogeneous throughout sites. No correlation was found between the nitrogen content in soil and fresh leaves ($r = -0.34$, $P = 0.40$).

Among-population genetic differences in *N. antarctica* maybe the result of diversifying selection in contrasting habitats which is reflected in adaptive traits. Genetic differences among populations may also arise as a result of genetic drift and isolation which can be measured by neutral markers by means of isozyme electrophoresis. In addition, genetic diversity maybe a reflection of a predominantly outcrossing breeding system. Seven out of nine analysed loci were polymorphic under the sensu stricto criterion for at least one site. These were Adh, Idh-1, Idh-2, Mdh-2, Mdh-3, Pgi-2, and Skdh. Allele frequencies were significantly heterogeneous throughout populations for each of the seven polymorphic loci (chi-square, $P < 0.001$ for each locus). This is also reflected in a statistically greater than zero F_{ST} value of 0.18 (95%CI = 0.089 – 0.247). Although elevated genetic divergence existed among sites, similar genetic diversity was measured within studied locations. Populations of *N. antarctica* had relatively high levels of genetic variation (Table 3). The percentage of polymorphic loci at each population ranged from 40 to 80%. The mean number of alleles per locus was just above two in most sites which is about the same for most other woody species. The effective number of alleles was about 1.5 in sites of *N. antarctica* and the mean number of alleles per polymorphic loci was about 3 in most of the sites. For most sites the mean observed heterozygozity ranged from 0.120 to 0.231 and was slightly lower than the expected heterozygosity that ranged from 0.186 to 0.275. This resulted in an overall deficiency of heterozygous individuals as indicated by 25 significant departures from Hardy-Weinberg conditions that yielded positive F values out of a total of 44 possible tests (data not shown). Similarly, mean within-population inbreeding resulted in significantly positive F_{IS} of 0.283 (95%CI = 0.165–0.460).

4 Discussion

Nothofagus antarctica holds a great geographic variation both phenotypically and genetically. The multivariate analysis of morphological and environmental variables clearly depicted three groups: the forest variant, the high elevation, and the matorral that clustered together with the temporarily flooded basin or flat. However, matorral types 1M and 3M had more branches than basin types 6B and 8B which maybe an indication of a recent fire event in the former that results in more profuse branching due to sprouting. Near-by population pairs such as matorral-high elevation and forest-basin differed significantly either in tree morphology, leaf, and/or soil characteristics, suggesting that morphology is coupled with distinct environmental settings. In addition, similar soil and or environmental conditions such as temperature between population pairs located in close proximity (i.e. forest-basin pairs) suggest that these traits may be site-dependant.

Forest and high elevation types are clearly differentiated. The forest type is associated to temperature and precipitation conditions that are

optimal (Ramírez et al. 1985, Premoli 1991). In contrast, extreme environmental setting characterizes the Andes elevational gradients, including decreases in soil temperature and the period with frost-free soils, as well as increased percentage of snowfall at high elevation (Barrera et al. 2000). Also, lack of available organogenic elements in the soil is particularly critical at higher elevation resulting in higher values of foliar N (Larcher 1995) which would explain the elevated nitrogen content of leaves in 2H and 4H (Table 2). Directional selection is probably responsible for the observed morphological differences of these high elevation types compared to low elevation forest types. These morphological differences with elevation were demonstrated by common garden experiments in the closely related *Nothofagus pumilio* (Premoli et al. 2007) which were also linked to functional traits (Premoli and Brewer 2007). However, no differences were found between the matorral and basin types. The main difference between the two is that basin types are usually found in flat terrain that could potentially accumulate water during the rainy season. It has been suggested that phenotypic plasticity is of advantage in ecophysiological characteristics related to water use in the closely related deciduous species *N. pumilio* if they allow reducing water loss during critical times (Premoli and Brewer 2007). Climate of the eastern Andes is Mediterranean-type. This means that most annual precipitation falls as snow in winter and summers are dry due to the presence of a subtropical high-pressure cell in the south-eastern Pacific (Veblen et al. 1996). Given that the growing season coincides with drought, we can hypothesize that variable responses will be favored in the matorral and basin types due to unpredictable water availability conditions.

Isozyme evidence indicates a great deal of within-population genetic diversity. This was so even in the high elevation sites which may have reduce polymorphism as a result of more intense selection in marginal environments, genetic drift, and/or greater inbreeding as measured in *Nothofagus pumilio* along elevation gradients (Premoli 2003). Levels of genetic diversity of *N. antarctica* is typical for long-lived woody perennial species. The average percentage of polymorphic loci and the average observed and expected heterozygozity in sites of *N. antarctica* (P = 57%, H_O = 0.168, H_E = 0.234) are comparable to woody species (P ~ 65%, H_E ~ 0.177) (Hamrick et al.1992), but very high compared to other *Nothofagus* species within the same *Nothofagus* subgenus (P ~ 30%, H_O ~ 0.05, H_E ~ 0.06) (extracted from Premoli 1996, 1997, 2003). *N. antarctica*, similarly to other closely related *Nothofagus*, is most probably self-incompatible (Riveros et al. 1995) and therefore outcrossing is maximized. However, positive inbreeding yielded by 60% of by-locus tests most probably indicates Wahlund effect by population admixture due to local recruitment of genetically similar propagules. The rest 40% tests did not departure from Hardy-Weinberg conditions suggesting that vegetative propagation may not be significant in *N. antarctica*. Therefore the calculated inbreeding is probably a reflection of occasional events of sexual reproduction that results in the formation of family groups consisting of long lasting genets. As a consequence we predict the existence of a significant fine-scale genetic structure within populations (Premoli and Steinke submitted).

Marked among-morphotype divergence was measured for leaf traits by Q_{ST} as a result of heterogeneous environments inhabited by *N. antarctica*. This among-site difference was also mirrored by isozyme data due to drift. While local selection pressures result in a consistent relationship between a character and environment, a tendency for morphological characters exist to be more differentiated than neutral traits as allozyme loci which in turn will be more affected by genetic drift (Merilä and Crnokrak 2001). Leaf shape traits attained a relatively larger between-site divergence value (Q_{ST} = 28%) than leaf size traits (Q_{ST} = 15%) and isozymes (see below). In addition to plasticity and genetic drift that primarily affect leaf size and isozymes, respectively, divergence in leaf shape characters is probably reflecting the effect of natural selection. The use of shape traits maybe valuable in species such as *N. antarctica* with reduced germinability on which common garden experiments are often difficult.

Nothofagus antarctica has an unusually high proportion of genetic variation partitioned among sites. The F_{ST} of 18% for *N. antarctica* is an order of magnitude larger than other *Nothofa-*

gus species including the deciduous *N. pumilio* studied across a similar area (F_{ST} = 2%, Premoli 2003), the evergreen *N. dombeyi*, *N. betuliodes* and *N. nitida* (range F_{ST} = 5–12%, extracted from Premoli 1996) as well as other long-lived woody perennial species in general (G_{ST} ~ F_{ST} = 8%) (Hamrick et al. 1992). This is clearly indicating limited gene flow which in addition to differential selective pressures in the variable environments in which *N. antarctica* occurs results in marked inter-site divergence. The morphological variants can become ecologically adapted due to genetic variation through habitat selection.The apparent high phenotypic and genetic variability in *N. antarctica* is due to both plasticity and genotypic effects, promoted by isolation mechanisms. *N. antarctica* has the ability to vigorously resprout after natural disturbances such as fire. Therefore, the high genetic differences among sites of *N. antarctica* can be a result of stable population structure and long periods of isolation which may be reinforced by selection at diverse biotopes.

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Appendix. Allele frequencies at seven polymorphic isozyme loci in eight sites of *Nothofagus antarctica.* Monomorphic loci were Mdh-1 and Pgi-1.

Locus	Site 1M	2H	3M	4H	5F	6B	7F	8B
Adh								
(N)	50	45	48	48	38	47	49	31
1	0.020	0.056	0.250	0.448	0.329	0.021	0.102	0.306
2	0.120	0.878	0.611	0.448	0.316	0.840	0.724	0.468
3	0	0.022	0	0	0	0.096	0.041	0.032
4	0.860	0.044	0.139	0.104	0.316	0.043	0.133	0.194
5	0	0	0	0	0.039*	0	0	0
Idh-1								
(N)	50	50	50	50	50	49	50	50
2	0	0	0	0.070	0	0.071	0	0
3	1	1	1	0.920	1	0.837	1	1
4	0	0	0	0.010	0	0.092	0	0
Idh-2								
(N)	48	50	48	50	49	50	50	50
2	0.042	0	0	0	0.01	0	0	0
3	0.406	0.160	0.302	0.460	0.163	0.300	0.180	0.210
4	0	0.760	0.344	0	0.255	0.400	0.710	0.570
5	0.552	0.080	0.354	0.540	0.571	0.300	0.110	0.220
Mdh-2								
(N)	50	50	48	50	50	50	50	50
2	0.01	0	0.188	0.320	0.01	0.020	0	0.040
3	0.240	0	0.073	0.270	0.170	0.220	0.180	0.040
4	0.71	1	0.740	0.41	0.820	0.760	0.820	0.920
5	0.040*	0	0	0	0	0	0	0
Mdh-3								
(N)	50	50	49	50	50	50	50	50
2	0.060	0	0	0.190	0	0.030	0	0.030
3	0.91	1	1	0.81	1	0.930	0.980	0.970
4	0.030	0	0	0	0	0.040	0.020	0
Pgi-2								
(N)	50	50	48	50	50	50	50	50
2	0	0	0.094	0	0	0.01	0.040	0
3	0	0	0	0	0	0.01*	0	0
4	1	0.460	0.865	1	0.960	0.920	0.870	0.840
5	0	0.21	0	0	0	0.050	0.040	0.080
6	0	0.330	0.042	0	0.040	0.01	0.050	0.080
Skdh								
(N)	48	49	47	50	43	48	47	39
1	0	0	0.064*	0	0	0	0	0
2	0.844	0.643	0.681	0.850	0.779	0.781	0.564	0.692
3	0.063	0.133	0.16	0	0.198	0	0.266	0.192
4	0.094	0.224	0.149	0.150	0.023	0.219	0.170	0.115

Note: N is the number of individuals analysed per locus. Unique alleles, i.e. those occurring at only one site, are indicated with *