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MORPHOLOGICAL AND ALLOZYMIC EVIDENCE OF NATURAL HYBRIDIZATION BETWEEN TWO SOUTHERN BEECHES (*NOTHOFAGUS SPP.)* AND ITS RELATION TO HETEROZYGOSITY AND HEIGHT GROWTH

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ABSTRACT

Seeds from 18 trees of *Nothofagus nervosa (= N. alpina)* were collected in the Lacar Lake basin, Neuquén Province, southwestern Argentina. During the first and second growing season, large morphological differences in the margins of the leaves from about 2000 individual seedlings were observed. Six families showed only individuals with the expected typical *N. nervosa* leaves. The other twelve families additionally showed individuals with leaves typical of the sympatric species *N. obliqua* in frequencies from 2 to 80 %. The hybridization of an isolated *N. nervosa* mother tree with pollen from the closely surrounding *N. obliqua* trees could be verified using an *Adh*-marker locus. The proportion of self-fertilization in this case was estimated as 6 %. Average degree of heterozygosity for three marker loci was higher in families with higher hybrids morphotypes frequencies. These families grew significantly faster than those without hybrids and a lower degree of heterozygosity. Putative hybrid individuals were also significantly taller within families. The possible manifestation of initial "heterosis effects" in the hybrids and of reproductive incompatibility is discussed. We concluded that interspecific hybridization can be very high in the case of isolated trees and can produce vigorous hybrid seedlings. The main direction of the hybridization is apparently *N. nervosa* × *N. obliqua.*

Keywords: hybrids, *Nothofagus*, allozyme gene markers, "heterosis effects", reproductive incompatibility, heterozygosity

INTRODUCTION

In southern South America the *Nothofagus* temperate forest lies on both sides of the Andean Mountains with a wide latitudinal distribution. In southwest Argentina, *Nothofagus nervosa* (Phil.) Dim. et Mil. (LENNON *et al.* 1987), Raulí, (= *N. alpina* (Poepp. & Endl.) Oerst.) has a small distribution area partly occurring sympatrically with *N. obliqua* (Mirb.) Oerst., Roble Pellín. Both taxa have been recognized and mantained as different species since their discovery and first classification in the middle of the last century (POOLE 1987). Both species can be clearly distinguished from another mainly on the basis of typical bark, fruit, and leaves traits (*e.g.* DIMITRI 1982).

Many of the *Nothofagus* species, also called "southern beeches", are known to cross naturally among themselves when occurring in partly sympatric distribution areas (POOLE 1987; WARDLE 1984). The occurrence of natural hybrids between the South American beeches *Nothofagus nervosa* and *N. obliqua* was first reported after observations in plantations and arboretums made in Britain (STEWART 1979; TULEY 1980). On the basis of morphological and anatomical traits of leaves and wood and of flavonoid chromatography of observed intermediate individuals, the occurrence of natural hybrids was also reported in a later work (DONOSO *et al.* 1990).

The observed material of these interesting reports consisted of few groups of individuals of unknown relationship and therefore offered no possibility of reliable inheritance inferences of the observed variation.

Additionally, interspecific hybrids are likely to have a greater proportion of heterozygous loci than the pure parental species. Average heterozygosity at quantitative traits loci (QTL) was shown to be proportional to heterosis (*e.g.* FALCONER 1981) and supposed to explain about 70–80 % of the effects on growth and developmental stability (MITTON & GRANT 1984). In southern beeches the relation between isozyme gene marker heterozygosity and growth of putative hybrids has not been reported.

In the present work the hypothesis of hybridization between the two southern beeches mentioned above is analysed on the basis of the distribution of morphological, growth and allozymic variation observed in a nursery progeny test of *Nothofagus nervosa.*

MATERIAL AND METHODS

Fruits from 18 trees of *Nothofagus nervosa* were collected in an East-West transect within the Lacar Lake basin at about 40° South Latitude and between 71° 21′ and 71° 43′ West Longitude on the eastern slope of the Andean Mountains in southwest Argentina. The mother trees were selected at random among those with low branches and visible, reachable fruits.

The seeds (nuts) were extracted, dryied and storaged at 4 °C for four months. After 35 days of cold/humid stratification the seeds were sown in a greenhouse according to a completely randomized block design with each spontaneous pollinated family (assumed halb-sib) as an experimental unit and five replications. A nursery trial with four replications was then established in late winter. An additional plot with pure *N. obliqua* seedlings obtained from a commercial nursery was also included

At the end of the first growing season unexpected differences in the leaf margin morphology were observed in about 2,000 seedlings. Three different morphotypes were then identified: "typical *nervosa*" (individuals with undulated-crenate margins on all leaves) , "typical *obliqua*" (individuals with crenate-dentate margins on all leaves; this means with a marked split between two secondary veins) and "*obliqua*-like" (similar to the previous type but with a moderate split) (Figure 1). During the second growing period the two *obliqua*-morphotypes were also observed but they were scored jointly as "*obliqua*". In both years the frequencies of the two different morphotypes (*nervosa* and *obliqua*) were determined within each family. The individuals with the *obliqua* morphotype will be called in the following „putative hybrids".

The height of the seedlings was measured at the end of both growing periods. The variation in this trait was then analysed through an ANOVA. The Tukey test was used to compare family averages. Only those families with more than 50 individuals in the nursery trials were used for all kind of comparisons. In four families (1, 2, 7 and 12) with putative hybrids frequencies higher than 30 % and more than 60 individuals, comparisons between the height of the different morphotypes within families were performed. Growth termination was scored on the basis of already formed buds before all the families finished the second growing season. Another height measure was taken at this time. All statistical analysis was done with the same significance level established *a priori* ($P < 0.05$).

Seed tissues of the same harvest used to produce the seedlings were employed in horizontal starch gel electrophoresis. Staining solutions were prepared according to CHELIAK and PITEL (1984) with some small modifications. Gene markers determined in *N. nervosa* by GODOY (1994) in seeds from the same harvest as that used for producing the seedlings were identified for ADH (E.C. 1.1.1.1), IDH (E.C. 1.1.1.42) and MDH (E.C. 1.1.1.37) systems. Part of the data presented in that work were added to ours to increase the sample size in the families comparison. Our data were based on a seed sample from the same harvest. Only those families with a number of analysed seeds between 60 and 315 were used. An exception to this was family 13 with just 45 analysed seeds. Qualitative and quantitative analysis of the observed distribution of homozygotes and heterozygotes were performed among families. Chi square test ($P < 0,05$) was used to compare distribution homogeneity. The average degree of heterozygosity (H_a), described elsewhere, (*e.g.* HATTEMER 1991) was estimated for each family with the proper sample size, for the three analysed loci.

The metric traits measured in the seedlings and the isozyme traits estimated in the seed samples from the same harvest were jointly compared between families.

Seeds from four of the six *N. obliqua* trees that closely and completely surrounded the isolated and planted *N. nervosa* mother tree number 7, were additionally collected two years later. For this special case the interspecific hybridization hypothesis was additionally tested.

RESULTS

Interspecific hybridization

In Figure 1 the three different leaf types found in one of the families at the end of the first growing season are shown compared with the typical leaf shapes of both species. The appearance of the intervein split was considered with sufficient discriminating capacity to identify morphologically putative interspecies hybrids as those with this trait. Within each family the frequency of thusly defined putative interspecies hybrids varied from 0 to 80 % at the end of the first growing period. In six of the 18 families no hybrids were found, while in the other 12, five presented frequencies over 40 %. The families with higher putative hybrid frequencies resulted from mother trees rather isolated in the forest and partially surrounded by *N. obliqua* trees. The family with the highest frequency originated from the special case of isolated and planted mother tree number 7. During the second growing period the *obliqua* morphotype could be found again in the same families with similar proportions. Also some additional marked trees in the first year presented the same morphotypes in the second.

Figure 1. Different leaf morphotypes found in a nursery progeny test of *Nothofagus nervosa*. The leaf on the left belongs to *N. obliqua* and that on the right to *N. nervosa*. In the middle from the left to right the three different morphotypes found in one-year-old seedlings within one of the open pollinated families of *N. nervosa* are shown: "typical *obliqua*", "similar *obliqua*", and "typical *nervosa*". In the second growing season the first two morphotypes were considered jointly as "*obliqua*"

Table 1. Genotypic frequencies for loci *Adh*, *Idh*, and *Mdh-C* in the analyzed families.

Family	*Adh*-genotypes			Number of seeds	*Idh*-genotypes			Number of seeds	*Mdh-C*-genotypes				Number of seeds
	11	12	22		11	12	22		33	13	23	34	
1	0.000	0.520	0.480	159	0.550	0.450	0.000	108	0.506	0.070	0.411	0.012	170
2	0.000	0.270	0.730	70	0.360	0.510	0.130	53	0.747	0.080	0.160	0.013	75
3	0.000	0.021	0.979	289	0.990	0.010	0.000	313	1.000	0.000	0.000	0.000	291
4	0.000	0.000	1.000	61	0.880	0.120	0.000	122	1.000	0.000	0.000	0.000	160
5	0.000	0.000	1.000	72	0.830	0.170	0.000	82	1.000	0.000	0.000	0.000	89
7	0.000	0.940	0.060	117	0.340	0.660	0.000	177	0.204	0.065	0.731	0.000	93
8	0.000	0.042	0.958	214	0.929	0.071	0.000	184	0.951	0.019	0.024	0.005	206
12	0.000	0.590	0.410	68	0.420	0.580	0.000	65	0.865	0.000	0.135	0.000	89
13	0.000	0.020	0.980	45	0.930	0.070	0.000	45	0.980	0.000	0.020	0.000	50
14	0.000	0.060	0.940	71	1.000	0.000	0.000	52	1.000	0.000	0.000	0.000	124

Three marker loci: *Mdh–C*, *Adh* and *Idh*, resulted discriminant among families. Their genotypic frequencies are presented in Table 1. The fourth analysed locus: *Mdh–B*, did not show significant differences. For locus *Adh* no progeny in the analysed families presented the homozygote genotype *11*, and for *Idh* only seven individuals of family 2 were homozygotes for allele *2* (Table 1). The average degree of heterozygosity was then estimated for each family for these discriminant three loci. The distribution of the heterozygosity in the families agreed with that of the frequencies of hybrids determined through the number of "*obliqua*" morphotypes (Figure 2).

The analysed seeds from the *N. obliqua* trees showed isozymic variation that could be considered as belonging to the same gene loci found in *N. nervosa*

Table 2. ***Adh*-genotypes found in the 1996 seed samples from *N. nervosa* isolated mother tree number 7 and from surrounding *N. obliqua* trees.**

Mother tree	Nr. of seeds analyzed	Genotype frequencies			Putative mother tree genotype
		11	12	22	
nervosa 7	107	0.00	0.94	0.06	2/2*
obliqua 1	41	1.00	0.00	0.00	1/1
obliqua 2	59	0.97	0.03	0.00	1/1
obliqua 3	31	1.00	0.00	0.00	1/1
obliqua 4	15	0.93	0.07	0.00	1/1

- Confirmed with bud tissues by GODOY (1994). In the seed sample analysed in 1994 mother tree 7 showed the same genotype frequencies

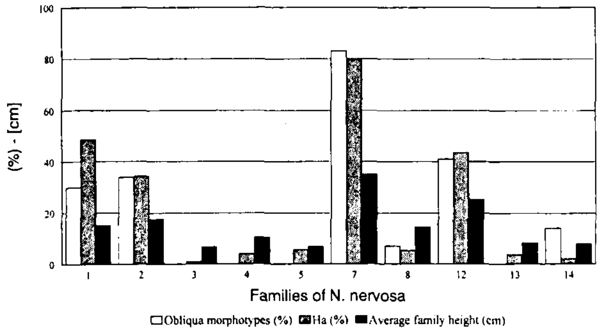

Figure 2. Relation between *N. obliqua* morphotypes within open pollinated families of *N. nervosa*, family average degree of heterozygosity (H_a), and family average height at the second year.

progenies and trees. Due to the simplicity of the ADH-system and the occurrence of exclusive species alleles, only zymograms of an *Adh-* marker gene locus were used to analyse the occurrence of interspecific crossing with mother tree number 7. The data surveyed in the seed samples from this tree and four surrounding *N. obliqua* trees are presented in Table 2. The genotype frequencies in the two analysed harvests were the same. No genotype *Adh–1/1* was found in the progeny of mother tree 7, while genotype *Adh–2/2* was not found in the progeny from the surrounding *N. obliqua* trees. The genotype of mother tree 7 was previously determined as *2/2* (GODOY 1994) and those of the four surrounding trees are assumed to be *1/1*.

The average degree of heterozygosity in the seed samples from isolated mother tree number 7 varied significantly with the consecutive harvests. The decrease in the average degree of heterozygosity in 1995 coincided when considering three or two loci together as well as for each locus (Table 3). It coincides also with the decrease in the frequency of "*obliqua*" morphotypes observed in few plants from this harvest.

Pure *N. obliqua* individuals and, in the second term, morphological putative hybrids finished their growth up to one and half months later than the "typical *nervosa*" individuals. Naturally both deciduous species change leaf colour during the fall. In the case of *N. obliqua* it turns to yellowish or brownish colours while *N. nervosa* turns to reddish. All the "typical *nervosa*" morphotypes in our nursery progeny test turned to reddish colour during the fall of the second year while some of the putative hybrids turned to yellowish.

Vigorous putative hybrids

Significant height differences among families were measured for the first year as well as for the two measurements made in the second year. Average family height comparisons using Tukey test showed significant differences between family 7, the one with the highest proportion of putative interspecies hybrids, and the rest. The second tallest family (12) also presented the second largest frequency of hybrids. The Spearman rank correlation coefficient between individual second year height and individual morphotypes reached a significant value of $r = 0.60^*$ whereas the average values of the families between these two variables covariated with $r = 0.88^*$. The average degree of hetereozygosity correlated also significantly with the average second year height of the families ($r = 0.79^*$) and with the proportion of putative morphological hybrids ($r = 0.75^*$). A similar distribution of both variables and of the average degree of heterozygosity estimated from loci *Mdh–C*, *Adh* and *Idh*, determined in seed samples from the same harvest, can be observed clearly in Figure 2. The average height of the families with putative hybrids was twice as high as the average height of the families without them.

The grand average height value of the second year measurement (16.47 cm) was quite larger than the median of the distribution (11 cm). The *nervosa*

Table 3. Average degree of heterozygosity (H_a) in three consecutive harvests from isolated *N. nervosa* mother tree number 7 surrounded by *N. obliqua* trees.

Year	Proportion of heterozygotes			H_a
	Adh	*Idh*	*Mdh-C*	
1994	0.94 (117)	0.66 (177)	0.80 (93)	0.80
1995	-	0.31 (35)	0.47 (71)	0.39
1996	0.94 (108)	0.39 (113)	0.88 (107)	0.74

Numbers of analyzed seeds in brackets

individuals presented an average height of 10.47 cm with a median of 8 cm, while the putative hybrids had an average of 32.76 cm with a median of 29 cm. The difference in height growth between the two morphotypes was statistically significant. A distribution plot of these height data confirmed the bimodal distribution. The frequency distribution of *nervosa* morphotype concentrates around the lower values with just a few individuals with greater heights. The height distribution of the *obliqua* morphotypes is flatter (larger variance) and lies shifted to the larger values. Both distributions tend to normality.

At the end of the first growing period within each of the four "halb-sib" families with more than 30 % putative hybrids (1, 2, 7 and 12) the morphotypes "typical" or "*obliqua*-like" were significantly taller than those "typical *nervosa*". The bulked data from the four families also presented this significant difference. The intermediate hybrid morphotype "*obliqua*-like" was also significantly taller than the "typical *nervosa*" in two families and significant shorter than the "typical *obliqua*". At the end of the second year the average height of the putative hybrids was significantly twice as high as that of the "typical *nervosa*" (Figure 3). All but one individual from the 23 that grew more than 20 cm in the last month of the second growing period belonged to the hybrid type, and all of them but one belonged to these four families with the higher frequencies of hybrids.

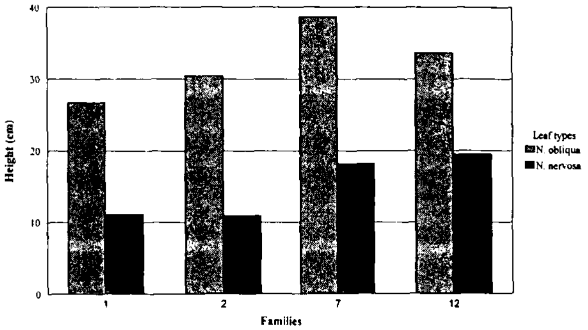

Figure 3. Average heights of two year old seedlings from two different leaf types found within four open pollinated families of *Nothofagus nervosa*.

DISCUSSION

Interspecific hybridization

WARDLE (1984) mentions the leaf as the main diagnostic feature for recognising hybrid individuals in *Nothofagus* species and emphasizes the incidence and shape of teeth on the leaf margin as a feature of special importance. He also adds that hybridization is most apparent where the tooth-leaved species have crossed with the entire-leaved ones. This would be the case in a putative intercross between *N. obliqua* ("tooth" leave species) and *N. nervosa* ("entire"-leaved species) (Fig. 1). In interspecific crosses between these two species, individuals with the expected *N. nervosa* leaves could theoretically also be interspecific hybrids in which the intervein split is not expressed. However, the distribution of our chosen morphological trait was also significantly correlated with the distribution of the growth and allozyme traits. Therefore, according to our results, this possibility seems less probable and we assume the occurrence of the chosen trait (intervein split) to be dominant and highly discriminating between both species. In addition, the intervein split appeared to be a quite stable trait in the first two years, since the observed family frequencies of interspecies hybrids were kept very similar and also some extra marked individuals in the first year presented the same morphotype in the second. Putative hybrid individuals of more than 100 years age seen in the native forest presented a *nervosa* bark type but with the typical *obliqua* leaf shape. This supports the assumption of the stability of our diagnostic trait.

"Halb-sib" families with the highest frequencies of interspecies hybrids proceed from such mother trees of *N. nervosa* which are to a certain degree isolated from other individuals of the same species. The extreme case is represented by tree number 7, planted in complete isolation out of the forest of *N.nervosa* and surrounded within a distance of a couple of meters by six planted trees of *N. obliqua*. In this case there is a high probability of natural intercross with these trees.

According to our results of gene marker variation both species intercross. Apparently the hybridization occurs mainly in the direction *N. nervosa* × *N. obliqua*.

The latter could be inferred with the gene marker locus *Adh* in the special case of the progeny analysis from the isolated mother tree number 7 (Table 2). This was possible because of the simplicity of the zymograms within the progenies, the genotyping of tree 7 as *Adh–2/2* by Godoy (1994), and the strong agreement with the postulated hypothesis that all possible pollen contributors have the same genotype and do not share a common allele with the mother tree. Through the marker locus *Adh,* only a very small proportion (0.03 and 0.07) of intercross in the other direction (*N. obliqua* × *N. nervosa*) can be inferred in two trees (Table 2). The morphological leaf trait of the hybrids also resembles that of the pure *N. obliqua* type. This supports the hypothesis presented by DONOSO *et al.* (1990) of a possible introgression of *N. obliqua* within the gene pool of *N. nervosa.* This also seems to be the case with "*N. leoni",* an interspecific hybrid between *N.obliqua* × *N. glauca* (DONOSO & LANDRUM 1979), where the former seems to introgress in the latter.
In both cases the confirmation with genetic markers in more study cases is necessary.

Also in other species of the *Fagaceae* family, a unidirectional intercross seems to be more compatible. Interspecific controlled crosses between *Quercus robur* × *Q. petraea* had a higher reproduction rate in this direction than when *Q. robur* pollen was used (STEINHOF 1993). Nevertheless, natural hybridization between these closely related taxa is suspected but could not yet be proven.

BACILIERI *et al.* (1995) found no specific allozyme marker and a lack of interspecific gene flow in a mixed stand of these two sympatric *Quercus* species. In our case, we did find a specific allozyme marker, at least in the analysed case of the *Adh* genotype from isolated mother tree 7 compared with the surrounding *N. obliqua* trees (Table 2). This was also confirmed in the analysis of the progeny of all families. No homozygotes *Adh–11* were found in the 1166 seeds analysed, although the mother trees cover a big area of the Lacar-Lake basin and in the progeny of many of them the presence of hybrids was inferred. The marker allele *Adh–1* seems therefore to be present only in *N. obliqua* whereas the marker allele *Adh–2* could be present only in *N. nervosa.* If this were really so the locus *Adh* would have a very important value to be used in the study of evolutive and population genetics questions of these two species.

The *Quercus* species mentioned above seem to share the same gene loci, at least in the studied isozyme systems (MÜLLER-STARCK *et al.* 1993). We can assume the same for the *Nothofagus* species studied here, although this needs to be confirmed for more isozyme systems and more material.

The phenological, morphological, growth and allozymic traits observed and analysed in this work give good evidence that intercrossing mainly in the direction *N. nervosa* × *N. obliqua* not only occurs but can also be very frequent, especially in such situations where individuals of the former species are rather isolated and phenological flowering conditions coincide.

Reproductive incompatiblity

The reproduction rate of the intercrosses between the two *Quercus* species mentioned above seemed to depend on individual incompatibilities (STEINHOF 1993). In our case some isolated *N. nervosa* mother trees, like tree number 13, did not produce morphological hybrids within its progeny, despite ist location very near to the isolated tree number 12 that produced over 30 % putative hybrids. Differential individual phenology could be responsible for this. Phenological differences in the flowering time between the two studied *Nothofagus* species (DONOSO *et al.* 1990) could also be the cause for the different proportions of heterozygous individuals found in mother tree 7 in seeds from different harvests (Table 3). This values agreed with those of the frequencies of hybrid morphotypes analysed in the seedlings from the first harvest year and observed in a few plants obtained from the second harvest year.

Isolation of *N. nervosa* mother trees seems to play a big role in the occurrence and amount of intercrossing. The high isolation of mother tree number 7 could be also used to estimate the effective rate of self-fertilization in this tree. The frequency of the seedlings with typical expected *N. nervosa* leaves (20 %) could be assumed in this special case to equal the rate of self-fertilization. This estimated value coincides with a moderate selfing rate found in other forest tree species (HATTEMER *et al.* 1993) and also coincides with the proportion of homozygotes found by locus *Mdh–C.* However, this value resulted higher than the amount of *Adh 2/2* homozygotes (0.06) which in the special case of this mother tree could be a good estimate of selfing rate, since at this locus it shares no common alleles with the surrounding *N. obliqua* trees. This value was obtained from the same seed lot from which the seedlings in the nursery originated. It also coincided with the proportion of *Adh* homozygotes found in the seed from this tree collected two years later. This lower rate of selfing agrees with that found by other authors in *Fagus sylvatica* (MERZEAU *et al.* 1994; MÜLLER-STARCK 1996).

However, if the chosen morphological leaf trait is considered dominant and highly discriminant between the species, as pointed out above, the proportion of the observed „*obliqua*" morphotypes should coincide with the proportion of *Adh*-heterozygotes. The difference found in our material could be the manifestation of a postzygotic incompatibility between the two species. This would mean that the speciation process probably initiated with a prezygotic incompatibility (*e.g.* a phenological difference in the flowering) evolving in the sympatric areas in the presence of postzygotic incompatibility. As pointed out by GREGORIUS (1992), prezygotic incompatibility seems to be the only stable pathway of speciation, and its chances to evolve are increased in the presence of postzygotic incompatibility after secondary reproductive contact, especially in parapatry. The gene exchange between the species seems in our case to be limited to those special cases where the *N. nervosa* individuals were rather isolated and surrounded by many *N. obliqua* trees and to depend strongly on the climatic conditions of the year. As shown in Table 3, in spite of high isolation, the production of hybrids can be highly reduced in some years, perhaps due to shortness of overlap in flowering time in both species.

The presence of specific and individual pre- and postzygotic incompatibilities could be some of the probable causes for the rather low frequency of hybrids found in the natural forest. If this were so, the vigorous growth of the hybrids, if considered as an adaptive trait, should decline with time, unless the artificial environmental conditions of our trials provide a suitable „hybrid habitat“ for their adaptive potential. This interesting question will be pursued with our material.

Vigorous putative hybrids

The phenomenon of heterosis, often viewed as the "direct opposite of inbreeding depression", has been found in many interspecific crosses of forest trees and it was also exploited in many hybrid selection programs (NAMKOONG 1981). Interspecific heterotic hybrids between some related species are the commercial material normally used in massive afforestations. Such is the case of some interspecific hybrids of genus *Populus, Pinus, Eucalyptus* and *Larix*, among the most important (WRIGHT 1976; ZOBEL & TALBERT 1988). Heterosis effects in putative hybrids between *Pinus halepensis* and *P. brutia* subsp. *brutia* have recently been reported (KOROL *et al.* 1995). In other cases, such as the interspecific hybrids between *Quercus robur* and *Q. petraea*, no significant indication for heterosis has been found (STEINHOF 1993). Heterosis effects, at least in the juvenile phases, are described for the known case of *Larix eurolepis* (*L. decidua* × *L. leptolepis*), although this can be expected only for certain individual combinations (WEISSGERBER & ŠINDELÁŘ 1992). The dependence of hybrid superiority on specific combinations has also been found in other forest tree species (GALLO 1991), suggesting that heterosis would be detected more as an individual phenomena where specific gene interactions, mainly alellic, would be one of its main causes.

In our case, we cannot confidently speak of "heterosis effects", since we could not relate the individual genotypes to their individual superiority nor do we know the average performance of the parents (RIEGER *et al.* 1991). Besides this, all our analysis relies on the discrimating capacity of the morphological trait chosen. Nevertheless, the height comparisons among families with and without morphological hybrids and the observed correspondence with the average degree of heterozygosity for the three analysed loci allows us the inference that the so-called "heterosis effects" are likely to be manifested in this material at the studied age.

A higher degree of heterozygosity was reported to be related with other adaptive traits in forest trees, such as tolerance to air pollution in *Fagus sylvatica* (ZIEHE & MÜLLER-STARCK 1991). Under the hypothesis of proportionality to heterozygosity in QTLs, average heterozygosity in gene marker loci is a good predictor of heterosis (CHARCOSSET & ESSIOUX 1994). The close agreement between the distribution of the average degree of heterozygosity and that of the average family height growth shown in Figure 2 indicates that, in this special case of interspecific hybrids, isozyme gene marker heterozygosity could be a good predictor for vigor. If we consider that the distribution of the proportion of „*obliqua*“ morphotypes was very similar to that of H_a for the three analysed loci, then we could assume that this parameter would also be suitable to infer „heterosis“ even within families (Fig.3).

However, the gene markers, when neutral, must be in linkage disequilibrium with QTLs to have a predictive value (CHARCOSSET & ESSIOUX 1994). The discrimination of the intermediate morphotype "*obliqua*-like" in the first year, significant different in growth from the „typical *obliqua*“, suggests that the depth of the intervein split could be considered to be a continuous trait, probably positively correlated with the growth of the seedlings. The manifestation of the „heterosis effects“ should then be seen as a polygenic expression. A wide variation in the distribution of the height value of the putative hybrids could be expected, and therefore an overlapping with part of the pure *nervosa* type distribution would be probable.. This was observed in the height distribution of the seedlings with the *obliqua* heights shifted to the larger values. After this consideration, the presence of interspecific hybrids in material proceeding from pure *N. nervosa* mother trees could be inferred by the occurrence of the intervein split and the vigor of their growth through its depth intensity. The latter trait indeed, should be then considered related to the degree of heterozygosity.

The taller growth of the putative hybrids could be due not only to a higher growth rate but also to a longer growing period than most of the "*nervosa* morphotype" seedlings analysed. The significant correlation observed between termination of growth, morphotypes and height values gives support to this hypothesis. A longer growing period can be advantageous under particular environmental conditions and was considered one of the possible causes of the so-called "heterosis effect" found in other forest trees species, such as in hybrid aspen (GALLO 1991).

The interspecific hybridization of these southern beeches is not only important in relation to the evolutionary history of this very ancient genus and the actual speciation process but has also interesting practical consequences for the conservation and utilization of their genetic resources. Anthropogenic influences through silvicultural management of this mixed forest could create the "hybrid habitats" conditions that supposedly could favour the establishment and recruitment of interspecific hybrids. The combination of desirable specific traits, such adaptation and wood properties, would be expected in some of the hybrids, as pointed out by DONOSO *et al.* (1990). The present finding of more vigorous growth in the hybrids could be added to their probable advantages in the case that this phenomenon retained its expression at older ages. A breeding strategy like multiple breeding population (NAMKOONG *et al.* 1988), handling separate populations of pure species and hybrids, should then be advantageous. Finally, the confirmed fact of the hybridization between both species increases the risk of lack of fulfilment of the many necessary assumptions that allow a conventional quantitative genetic analysis. The occurrence of linkage disequilibrium is perhaps the most important bias, but others such as sexual asymmetry are likely to happen also (GALLO 1991). In such cases, another kind of analysis like the Response Function Analysis method (GALLO *et al.* 1995) should be considered to infer genetic effects.

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