TAXONOMIC POSITION AND ORIGIN OF THE ENDEMIC SICILIAN FIR *ABIES NEBRODENSIS* (LOJAC.) MATTEI BASED ON ALLOZYME ANALYSIS

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ABSTRACT

Abies nebrodensis (Lojac.) Mattei (Sicilian fir) is a forest tree species endemic to the mountainous regions of northern Sicily (the Madonie Range) that is currently represented by just one population of 29 individuals. The major questions relating to this species are its unknown origin and its uncertain taxonomic position. According to many authors *A. nebrodensis* is morphologically intermediate between the neighboring Mediterranean *Abies* species: *Abies alba* (Mill.) (silver fir), *Abies numidica* (De Lann) (Algerian fir) and *Abies cephalonica* (Loud.) (Greek fir).

In the present study we analyzed eight enzyme systems in the population of *A. nebrodensis* and in seven populations from *A. alba*, *A. cephalonica* and *A. numidica*. The aim was to clarify the taxonomic position and origin of *A. nebrodensis*.

High values of expected heterozygosity and number of polymorphic loci were found in *A. cephalonica*, while *A. alba* and *A. nebrodensis* showed intermediate levels of polymorphism and *A. numidica* was the least variable species. All values were similar to those found in other conifers. The relatively high level of diversity found in *A. nebrodensis* confirms that despite the extremely small population size, the few individuals left in this species still retain a considerable amount of the original genetic variation at the nuclear level. Results also showed that all the species were differentiated from each other, although *A. nebrodensis* showed a closer affinity to *A. alba* and in particular to the population from southern Italy.

Our results, together with results from previous studies provide support for the classification of *A. nebrodensis* as a separate taxon and suggest that this species may have originated through a past hybridization event.

Keywords: *Abies nebrodensis*, Sicilian fir, population genetics, allozymes, taxonomy.

INTRODUCTION

Abies nebrodensis (Lojac.) Mattei (Sicilian Fir) is a forest tree species endemic to the mountainous regions of northern Sicily (the Madonie Range) and it is currently represented by just one population of 29 individuals. Prior to the eighteenth century, *A. nebrodensis* was widely distributed on the higher mountains of northern Sicily, but it has declined in the last 200 years, mainly due to human activities (MORANDINI 1969, MORANDINI *et al.* 1994). The major questions relating to *A. nebrodensis* are its unknown origin and its uncertain taxonomic position. According to several authors *A. nebrodensis* is morphologically intermediate between the neighboring Mediterranean *Abies* species: *A. alba* (Mill.) (Silver fir), *A. numidica* (De Lann) (Algerian fir) and *A. cephalonica* (Loud.) (Greek fir) (NITZELIUS 1969, PIGNATTI 1982, BOTTACCI *et al.* 1990, QUEZEL & BARBERO 1990, RAIMONDO *et al.* 1990).

Abies numidica is also in a state of regression and occur on a restricted area on Mount Babors, in Northern Algeria. In contrast, the ranges of *A. alba* and *A. cephalonica* are relatively large. The range of *A. alba* extends from the mountainous regions of Central and Western Europe to Calabria (Italy), which marks the southern limit of the species. *Abies cephalonica* range extends throughout the mainland of Greece across to the islands of Cephalonia and Evia (Euboea) and according to MATTFELD (1927, 1930) only the populations occurring in southern Greece, as far north as latitude 38° 50' N,

belong to the species, while in central and northern Greece a series of intermediate *Abies* forms occur, belonging to the putative hybrid species *A. borisii-regis* (Mattfeld) of unclear origin. At the northern limit the hybrid populations mostly resembles *A. alba* and grow together with individuals of this species, while at the southern limit they mostly resemble *A. cephalonica*, and grow together with *A. cephalonica* individuals (MATTFELD 1927, 1930).

The classification of *A. nebrodensis* is a source of controversy in the current taxonomy of the genus *Abies*. According to many authors (TUTIN *et al.* 1964, LIU 1971, FARJON & RUSHFORTH, 1989) this taxon is considered a separate species, while according to others (FRANCO 1950, NITZELIUS 1969, LANDRY 1984) it represents a subspecies of *A. alba*.

VICARIO *et al.* (1995) employed allozyme, chloroplast DNA (cpDNA) and RAPD markers, to assess the genetic relationships among seven Italian populations of *A. alba* and the population of *A. nebrodensis*. Results from the allozyme and RAPD analyses showed differences between the two species, but the authors did not detect any differences in the cpDNA region they analyzed. Using restriction fragment analysis of ten different cpDNA regions amplified from ten European *Abies* species, PARDUCCI & SZMIDT (1999) found that at the haplotypic level *A. nebrodensis* shares some affinities with *A. numidica*, while it differs from the other *Abies* taxa studied. However, the unexpected high level of variation observed in this study in the *Abies* cpDNA and the limited sample size analyzed per species did not allow the authors to exclude other possible phylogenetic relationship between *A. nebrodensis* and the other taxa investigated.

Recently, PARDUCCI *et al.* (2001) used the highly polymorphic chloroplast microsatellites (cpSSRs) to investigate the population genetic structure and the distribution of chloroplast haplotypic variation in *A. nebrodensis* as well as in *A. alba, A. cephalonica* and *A. numidica*. The authors found that *A. nebrodensis* differs from the other three species, which supported its classification as an independent taxon. Moreover, the authors found a lower level of cpDNA variation both in *A. nebrodensis* and *A. numidica* compared to *A. alba* and *A. cephalonica* and a high level of relatedness among the 19 *A. nebrodensis* individuals analyzed, suggesting that the latter species has experienced a genetic bottleneck at some point in its evolution. The lower level of variation found by PARDUCCI *et al.* (2001) in the cpDNA of *A. nebrodensis* however, was in contrast with results from VICARIO *et al.* (1995) and DUCCI *et al.* (1999) who found instead a high degree of diversity in this species using allozymes. In PARDUCCI *et al.* (2001) the authors suggested that the contrasting results were due to the different evolutionary dynamic of the chloroplast compared to the nuclear DNA. The chloroplast DNA is inherited paternally in conifers (NEALE *et al.* 1986, SZMIDT *et al.* 1987, WAGNER *et al.* 1989, STINE & KEATHLEY 1990), including *Abies* (ZIEGENHAGEN *et al* 1995) and its effective population size is half of that of the nuclear DNA. Therefore, the chloroplast DNA is more sensitive to reductions in the number of individuals in a population (BIRKY 1988). In PARDUCCI *et al.* (2001), the authors suggested that, after the severe reduction in size occurred in *A. nebrodensis* in the last century, there were few pollen-donating parents that successively gave rise to the extant population. Such a reduction in size had a weaker effect on the level of genetic variation at the nuclear level (allozymes).

In the present study we analyzed eight enzyme systems in the population of *A. nebrodensis* and in seven natural populations of the three neighboring *Abies* species *A. alba*, *A. cephalonica* and *A. numidica*. The aim was to investigate and clarify the taxonomic position and origin of *A. nebrodensis*.

MATERIALS AND METHODS

Material

The material analyzed included bud samples from 19 individuals from the population of *A. nebrodensis* and bud and seed samples from seven natural populations of *A. alba, A. cephalonica*, and *A. numidica*. The names, provenances and sample sizes of the eight investigated populations are given in Table 1. When seeds were used, the embryo tissues were carefully removed from the endosperm and used for the analysis.

The three *A. alba* populations ALBA-1, ALBA-2 and ALBA-3, were among the 10 Italian populations previously analyzed by PARDUCCI *et al.* (1996) and were selected as representative of the species range in the northern, central and southern parts of the Italian Peninsula. Among the three Greek populations, CEPH-1 and CEPH-2 were collected in northern and central Greece where the putative hybrid species *A. borisii-regis* grows (MATTFELD 1927, 1930; SCALTSOYIANNES *et al.* 1999). The 29 *A. numidica* individuals were collected all over the species range and can therefore be considered representative of the distribution range. Finally, we collected buds from 19 *A. nebrodensis* individuals. The material was sampled from all the three genetic clusters recently identified in the *A. nebrodensis* population by DUCCI *et al.* (1999).

METHODS

The method used for the allozyme analysis was according to the procedure described in VILLANI *et al.* (1991) modified for conifer seeds and buds (PARDUCCI unpublished). The following eight enzyme systems, encoded

Table 1. Names, provenances and plant material used for the eight *Abies* populations analyzed at 12 allozyme loci. (*N*) sample sizes, (*n*) mean number of alleles per locus, (H_o, H_e) average observed and unbiased expected heterozygosities (NEI 1978) (*F*) inbreeding coefficient and (*P*) percentage of polymorphic loci.

Taxa	Provenances	Coordinates	Sample Material	*N*	*n*	H_o	H_e	*F*	*P**
ALBA-1 *A. alba*	Val Canali, northern Italy	46° 09′ N 11° 50′ E	buds from single adult trees	27**	1.8	0.124	0.142	0.127	41.7
ALBA-2 *A. alba*	Pescolanciano, central Italy	41° 43′ N 14° 21′ E	buds from single adult trees	27**	1.8	0.103	0.131	0.214	33.3
ALBA-3 *A. alba*	Archiforo, Calabria, southern Italy	38° 33′ N 16° 19′ E	buds from single adult trees	27**	1.9	0.135	0.140	0.037	58.3
Mean					1.8	0.121	0.138	0.126	44.4
CEPH-1 *A. borisii-regis*	Aridea, northern Greece	41° 06′ N 22° 30′ E	bulked seed collection	60	2.3	0.236	0.254	0.070	58.3
CEPH-2 *A. borisii-regis*	Agios Dimitrios, north-central Greece	40° 08′ N 22° 14′ E	bulked seed collection	60	2.2	0.161	0.199	0.191	58.3
CEPH-3 *A. cephalonica*	Taygetos, southern Greece	37° 16′ N 22° 18′ E	bulked seed collection	60	2.3	0.180	0.200	0.100	75
Mean					2.3	0.194	0.228	0.120	63.8
NEBR *A. nebrodensis*	Madonie, Sicily southern Italy	37° 51′ N 14° 20′ E	buds from single adult trees	19	1.5	0.161	0.150	–0.070	33.3
NUM *A. numidica*	M. Babors, northern Algeria	36° 30′ N 5° 51′ E	seeds from single adult trees	29	1.6	0.098	0.126	0.222	33.3

* A locus is considered polymorphic when the frequencies of the most common allele does not exceed 0.95.

** Group of populations previously analyzed at 15 loci in PARDUCCI *et al.* (1996).

by 12 loci (PARDUCCI *et al.* 1996) were analyzed: leucine aminopeptidase (*Lap*, Enzyme Commission (E.C.) No. 3.4.11.1), glutamate dehydrogenase (*GDh*, E.C. 1.4.1.2), isocitrate dehydrogenase (*IDh*, E.C. 1.1.1.42), aspartate aminotransferase (AAT, *Got*, E.C. 2.6.1.1), 6-phosphogluconate dehydrogenase (*6PgDh*, E.C. 1.1.1. 44), glucosephosphate isomerase (*Pgi*, E.C. 5.3.1.9), phosphoglucomutase (*Pgm*, E.C. 2.7.5.1) and shikimate dehydrogenase (*SkDh*, E.C. 1.1.1.25). The inheritance modes of the allozyme variants were described for *Abies* species in SCHROEDER (1989), FADY and CONKLE (1993) PARDUCCI (unpublished), PASCAUL *et al.* (1993) and HUSSENDÖRFER *et al.* (1995).

Observed (H_o) and unbiased expected (H_e) heterozygosities (NEI 1978), percentage of polymorphic loci (*P*) as well as the mean number of alleles per locus (*n*) were used to estimate the amount of genetic variability within populations. Conformation of the investigated populations to Hardy-Weinberg equilibrium was estimated for each locus using the exact test (HALDANE 1954), employing the Markov Chain method (GUO & THOMPSON 1992), using a dememorization number of 1000, with 10 batches and 2000 permutations.

Unbiased genetic distances were calculated according to NEI (1978). The exact test of population differentiation of RAYMOND & ROUSSET (1995) was conducted to determine if significant differences in allele frequencies existed among species and populations within species, again using a dememorization number of 1000, with 100 batches and 2000 permutations. Cluster analysis based on genetic distance matrices was performed using the UPGMA method (SNEATH & SOKAL 1973).

All calculations were carried out using the TFPGA program (Mark P. Miller, Northern Arizona University, USA, URL: http://www.public.asu.edu/~mmille8/tfpga.htm) and the BIOSYS program (SWOFFORD & SELANDER 1981).

RESULTS

We observed allozyme variation at 11 of the 12 loci

examined; for the *GDh* locus only one single invariant zone of activity was found in all the *Abies* populations analyzed. Allozyme frequency data for individual loci are presented in Table 2. Among the 12 loci examined *IDh*, 6*PgDh*-1 and 6*PgDh*-2 were polymorphic in all the populations analyzed, while *Lap*, *IDh*, 6*PgDh*-1, *Pgi*-1, and *Pgi*-2 showed the highest polymorphism. The loci that contributed most to the differentiation among species and populations were: *IDh*, 6*PgDh*-1, *Pgi*-1 and *SkDh*.

Measures of genetic variability per population are presented in Table 1. Values of expected heterozygosity ranged from 0.126 in the *A. numidica* population to 0.254 in population CEPH-1. The average inbreeding coefficients calculated over all loci showed a positive value in all populations, except *A. nebrodensis*. Moreover, single-locus *P* values from exact test showed that deviation was not statistically significant at all loci analyzed in both *A. nebrodensis* and population ALBA-1 (results not shown). The loci that significantly deviated from equilibrium ($p < 0.05$) in the other populations were: *Got*-2 (CEPH-2), 6*PgDh*-1 (ALBA-2), *Pgi*-1 (ALBA-3, CEPH-1, CEPH-2 and CEPH-3) and *Pgm*-2 (ALBA-2 and NUM).

Results from the exact test of population differentiation based on allele frequencies are shown in Table 3a, 3b and 3c. Loci *IDh*, 6*PgDh*-1, *Pgi*-1 and *SkDh* contributed mostly to the differentiation of *A. nebrodensis* from the other *Abies* populations investigated (Table 3a). Single-locus *P* values calculated for pair-wise comparisons between species presented in Table 3b, revealed that the two most differentiated species were *A. alba* and *A. cephalonica*. Finally, single-locus *P* values revealed that also within species existed significant differences in allele frequencies, in particular in the group of Greek populations (Table 3c).

Nei's genetic distance values among populations and species are shown in Tables 4 and 5, respectively. Values from Table 5 showed that all the species differed considerably from each other. *Abies nebrodensis* showed the highest distance with the group of Greek populations ($D = 0.245$) while the smallest was found with *A. alba* ($D = 0.094$). In particular, *A. nebrodensis* was closest to population ALBA-3 ($D = 0.078$) (Table 4).

The UPGMA dendrogram based on Nei's genetic - distances revealed a distinct differentiation among the four investigated species (Figure 1). The eight populations were separated into two groups. *Abies alba* populations formed a cluster together with *A. nebrodensis*, while the second cluster comprised *A. numidica* and the three Greek populations.

DISCUSSION

The highest values of expected heterozygosity and percentage of polymorphic loci were found in the three *Abies* populations from Greece, while the population of *A. numidica* showed the lowest level of polymorphism. These results are in accordance with SCALTSOYIANNES *et al.* (1999) who recently used allozymes to investigate eight *Abies* species from the Mediterranean region. The authors found that the highest values of heterozygosity were concentrated in the *Abies* populations occurring in Greece, while the lowest values were observed in the North African *Abies* species, including *A. numidica*. They attributed the low heterozygosity of the North African taxa to their prolonged isolation and small population size. Our present results agree with this suggestion, however we found the highest value of heterozygosity in the *Abies* population from northern Greece, while the highest levels SCALTSOYIANNES *et al.* (1999) found, were in populations from the central and southern regions of the Greek peninsula. SCALTSOYIANNES *et al.* (1999) rejected the hypothesis of a post-glacial contact occurred in central Greece between *A. alba* and *A. cephalonica* that originated *A. borisii-regis* (MATTFELD 1930), and suggested instead that populations of the ancient *Abies* progenitor are still present in this region and grow and hybridize with *A. alba* (TURRIL1937). Our results do not allow us to confirm any of the hypotheses proposed so far on the origin of the *Abies* populations occurring in Greece. Further analyses employing specific diagnostic markers and larger sample sizes should be carried out to clarify the relationships among the Greek *Abies* populations.

We found a relatively high level of diversity in the *A. nebrodensis* population: an unexpected result if we assume that the species has experienced a severe reduction in population size in the last two centuries (MORANDINI 1969, MORANDINI *et al.* 1994) with a consequent genetic bottleneck (PARDUCCI *et al.* 2001). Similarly, VICARIO *et al.* (1995) and DUCCI *et al.* (1999) using allozymes found a relatively high genetic variation in this species. This result confirms that despite the extremely small population size of *A. nebrodensis*, the few individuals left in the population still retain a considerable amount of the original genetic variation at the nuclear level. Therefore, special attention should be given to the preservation as well as the propagation of material from this population for *ex situ* preservation of the species.

Generally, conifers show an excess of homozygotes over panmictic expectations at the embryo stage, which later disappears at the adult stage (SZMIDT & MUONA 1985, PLESSAS & STRAUSS 1986). This is in agreement with results from our study, since we found positive values of the inbreeding coefficient in all the *Abies*

Table 2. Estimated allele frequencies for 12 allozyme loci in the eight analyzed *Abies* populations.

Alleles		ALBA-1	ALBA-2	ALBA-3	CEPH-1	CEPH-2	CEPH-3	NUM	NEBR
Lap	1	0.827	0.907	1.000	0.992	0.950	0.956	1.000	1.000
	2	–	0.037	–	0.008	0.033	–	–	–
	3	0.154	–	–	–	0.017	0.044	–	–
	4	0.019	–	–	–	–	–	–	–
	5	–	0.056	–	–	–	–	–	–
Gdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh	1	0.593	0.426	0.352	0.819	0.482	0.268	0.034	0.026
	2	0.407	0.574	0.648	0.181	0.518	0.723	0.966	0.974
	3	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	0.009	–	–
Got-1	1	0.963	1.000	1.000	0.973	0.977	0.941	1.000	1.000
	2	–	–	–	0.027	0.023	0.042	–	–
	3	0.037	–	–	–	–	0.017	–	–
Got-2	1	0.981	1.000	0.926	0.451	0.659	0.255	0.963	1.000
	2	0.019	–	0.037	–	–	–	–	–
	3	–	–	0.037	0.069	–	0.745	0.037	–
	4	–	–	–	0.480	0.341	–	–	–
6PgDh-1	1	0.380	0.596	0.944	0.845	0.800	0.855	0.655	0.395
	2	0.580	0.231	0.056	0.129	0.200	0.145	0.345	0.447
	3	0.040	0.173	–	0.026	–	–	–	0.158
6 PgDh-2	1	0.907	0.907	0.926	0.966	0.892	0.810	0.966	0.895
	2	0.093	0.019	–	–	–	–	–	–
	3	–	0.074	0.074	0.034	0.108	0.190	0.034	0.105
Pgi-1	1	1.000	1.000	0.870	0.483	0.232	0.192	0.310	0.289
	2	–	–	0.130	0.225	0.018	–	0.690	0.711
	3	–	–	–	0.292	0.750	0.808	–	–
Pgi-2	1	1.000	0.980	0.944	0.169	0.045	0.117	0.052	1.000
	2	–	–	0.019	0.093	–	–	–	–
	3	–	0.020	0.037	0.738	0.955	0.883	0.948	–
Pgm-1	1	0.942	1.000	0.963	0.850	0.966	0.967	1.000	1.000
	2	0.058	–	0.037	0.050	0.017	0.025	–	–
	3	–	–	–	0.100	0.017	0.008	–	–
Pgm-2	1	1.000	0.963	0.981	0.714	0.880	0.925	0.810	1.000
	2	–	–	0.019	0.286	0.120	0.075	0.190	–
	3	–	0.037	–	–	–	–	–	–
Skdh	1	1.000	0.981	0.760	0.983	0.992	0.933	1.000	0.417
	2	–	–	–	0.017	–	0.011	–	–
	3	–	0.019	0.240	–	0.008	0.056	–	0.583

populations analyzed except *A. nebrodensis*. Single-locus *P* values indicated that deviation from panmixia was not statistically significant at all of the loci analyzed in *A. nebrodensis* as well as in population ALBA-1. The excess of homozygotes found at the adult stage in populations ALBA-2 and ALBA-3 confirmed the general trend of heterozygote deficiency recently observed in *Abies* (DUCCI *et al.* 1999 and references therein). Single-locus *P* values also showed that deviation from panmixia was particularly high at some of the loci studied (*Got*-2, 6*PgDh*-1, *Pgi*-1 and *Pgm*-2), suggesting that selection may have been more pronounced at this loci, however further study should be carried out in order to confirm this hypothesis. Self-

Table 3a. Single-locus *P* values for the exact test of population differentiation (RAYMOND & ROUSSET 1995) for all pairwise comparisons between A. nebro*densis* and the seven *Abies populations* investigated.

Locus	NEBR/CEPH-1	NEBR/CEPH-2	NEBR/CEPH-3	NEBR/ALBA-1	NEBR/ALBA-2	NEBR/ALBA-3	NEBR/NUM
Lap	1.000	0.758	0.317	0.015	0.212	1.000	1.000
Idh	*	*	*	*	*	*	1.000
Got-1	0.573	1.000	0.477	0.514	1.000	1.000	1.000
Got-2	*	*	*	1.000	1.000	0.394	0.550
6PgDh-1	*	*	*	0.131	0.093	*	*
6PgDh-2	0.097	1.000	0.315	*	0.836	0.707	0.205
Pgi-1	*	*	*	*	*	*	1.000
Pgi-2	*	*	*	1.000	1.000	0.509	*
Pgm-1	*	1.000	1.000	0.263	1.000	0.509	1.000
Pgm-2	*	*	0.112	1.000	0.510	1.000	*
SkDh	*	*	*	*	*	*	*

* – $P < 0.05$

Table 3b. Single-locus *P* values for the exact test for population differentiation (RAYMOND & ROUSSET 1995) for all pairwise comparisons among the *Abies* species investigated.

Locus	NEBR/CEPH	NEBR/ALBA	NEBR/NUM	CEPH/ALBA	CEPH/NUM	ALBA/NUM
Lap	1.000	0.657	1.000	*	0.813	0.314
Idh	*	*	1.000	0.202	*	*
Got-1	0.688	1.000	1.000	*	0.554	1.000
Got-2	*	1.000	0.549	*	*	0.286
6PgDh-1	*	*	*	*	*	0.076
6PgDh-2	1.000	0.264	0.211	*	0.096	0.402
Pgi-1	*	*	1.000	*	*	*
Pgi-2	*	1.000	*	*	0.177	*
Pgm-1	0.407	0.588	1.000	*	0.149	0.332
Pgm-2	*	1.000	*	*	0.427	*
SkDh	*	*	*	*	0.762	0.021

* – $P < 0.05$

Table 3c. Single-locus *P* values for the exact test of population differentiation (RAYMOND & ROUSSET 1995) for all pairwise comparisons within *A. alba* and *A. cephalonica*.

Locus	ALBA-1/ALBA-2	ALBA-1/ALBA-3	ALBA-2/ALBA-3	CEPH-1/CEPH-2	CEPH-1/CEPH-3	CEPH-2/CEPH-3
Lap	*	*	0.058	0.128	*	0.149
Idh	0.109	*	0.565	*	*	*
Got-1	0.498	0.498	1.000	1.000	0.566	0.534
Got-2	1.000	0.428	0.234	*	*	*
6PgDh-1	*	*	*	0.088	0.342	0.288
6PgDh-2	*	*	1.000	*	*	0.101
Pgi-1	1.000	*	*	*	*	0.315
Pgi-2	0.479	0.256	1.000	*	*	0.057
Pgm-1	0.112	0.671	0.498	*	*	1.000
Pgm-2	0.498	1.000	0.495	*	*	0.364
SkDh	1.000	*	*	0.449	*	0.066

* – $P < 0.05$

Table 4. Unbiased genetic distances (NEI 1978) for all pair-wise comparisons among the eight investigated *Abies* populations.

	ALBA-1	ALBA-2	ALBA-3	CEPH-1	CEPH-2	CEPH-3	NEBR	NUM
ALBA-1	***							
ALBA-2	0.012	***						
ALBA-3	0.043	0.014	***					
CEPH-1	0.160	0.146	0.135	***				
CEPH-2	0.196	0.171	0.160	0.039	***			
CEPH-3	0.263	0.226	0.197	0.102	0.048	***		
NEBR	0.123	0.102	0.078	0.268	0.249	0.279	***	
NUM	0.190	0.158	0.142	0.118	0.084	0.127	0.134	***

Table 5. Unbiased genetic distances (NEI 1978) for all pair-wise comparisons among the four investigated *Abies* species.

	ALBA	CEPH	NEBR	NUM
ALBA	***			
CEPH	0.155			
NEBR	0.094	0.245	***	
NUM	0.155	0.090	0.134	***

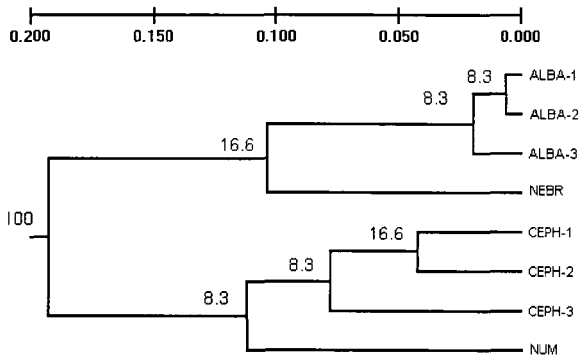

Figure 1. UPGMA dendrogram based on the unbiased genetic distances (NEI 1978) for the eight *Abies* populations investigated. Bootstrap values are indicated at each node.

pollination and other forms of inbreeding, discontinuous distribution, together with the Wahlund effect caused by population subdivision due to restricted gene flow, may also have contributed to the maintenance of heterozygote deficiency in these populations.

Taxonomy and origin of *Abies nebrodensis*

Based on Nei's genetic distance values, we found that *A. nebrodensis* differed from the other *Abies* populations investigated, although it showed some closer affinity to population ALBA-3 from southern Italy. Together with results from previous studies (VICARIO *et al.* 1995, PARDUCCI & SZMIDT 1999, PARDUCCI *et al.* 2001) the present results provide support for the classification of *A. nebrodensis* as a separate taxon and suggest that this species may have originated through a past hybridization event.

Although we do not have sufficient evidence to present more than a tentative explanation for the origin of *A. nebrodensis*, based on our present and previous results (PARDUCCI & SZMIDT 1999, PARDUCCI *et al.* 2001) we propose the following hypothesis. At the beginning of the Miocene (26–5 My BP) an ancient *Abies* progenitor existed in southern Europe that became widely distributed in the Northern Hemisphere (HUNTLEY & BIRKS 1983). During the climatic crises of the Miocene the *Abies* range became more and more fragmented and several species differentiated. At that time *A. alba* was probably confined to the Apennines chain and to central and northern Europe while the other *Abies* species were restricted to the mountainous regions of the Balkans, Northern Africa and the Middle East. During the successive Messinian salinity crises of the latest parts of the Miocene, when the Mediterranean became a hyper-saline land-locked sea, the European and African continents were connected. This connection probably offered a possibility for the North African *A. numidica* to come into contact with *A. alba* in the region today occupied by Sicily, resulting in the appearance of *A. nebrodensis* through hybridization. During the successive ice ages of the Pleistocene (1.6–0.01 My BP) the land was subjected to considerable climatic fluctuations as the polar icecap successively expanded and retreated. One major consequence of this was a series of sea-level changes in the Mediterranean area with the establishment of new land-links (HALLAM 1994), which may have facilitated additional contacts among *A. nebrodensis, A. alba* and *A. numidica*. In the warmer period of the Holocene, *A. nebrodensis* became isolated from both *A. numidica* and *A. alba*, which promoted further divergence. The decline of *A. nebrodensis* occurred in recent times, mainly due to human activities. Indeed, several authors attested to the existence of

extensive fir forests in the Madonie Range until approximately 200 years ago (MORANDINI *et al.* 1994 and references therein). By the beginning of the 19th century *A. nebrodensis* was considered lost by the scientific community, although later investigations led to the discovery of a few individuals in a restricted area in Sicily. These individuals, together with those discovered in the following years, constitute the extant *A. nebrodensis* population, all of which are at least 70-80 years old. It is likely that due to the severe reduction in size occurred in the last two centuries, many alleles went lost in this species and consequently, only a fraction of the original genetic variation was present in the small number of founders that gave rise to the extant population. At the same time the few available mates and the lack of contact with other heterogeneous source of variation increased the level of relatedness among the individuals (PARDUCCI *et al.* 2001), although a considerable amount of genetic variation was retained at the nuclear level (VICARIO *et al.*1995, DUCCI *et al.* 1999, results from this study).

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REFERENCES

BIRKY, C. JR. 1988: Evolution and variation in plant chloroplast and mitochondrial genomes. *In*: Plant Evolutionary Biology (ed. L. Gottlieb, S. Jain). pp. 23–53. Chapman & Hall, London.

BOTTACCI, A., GELLINI, R. & GROSSONI, P. 1990: Morphological and anatomical aspects of *Abies nebrodensis* (Lojac.) Mattei. *In*: International EEC Workshop on Mediterranean Firs. Avignon, France. pp. 117–124.

DUCCI, F., PROIETTI, R. & FAVRE, J. 1999: Allozyme assessment of genetic diversity within the relic Sicilian fir *Abies nebrodensis* (Lojac.) Mattei. *Annals of Forest Science* **56**: 345–355.

FADY, B. & CONKLE, M. T. 1993: Allozyme variation and possible phylogenetic implications in *Abies cephalonica* Loudon and some related Eastern Mediterranean firs. *Silvae Genetica* **42**: 351–359.

FARJON, A. & RUSHFORTH, K. D. 1989: A classification of *Abies* Miller (*Pinaceae*). *Notes Royal Botanical Garden Edinburgh* **46**: 59–79.

FRANCO, J. A. 1950: Abetos. Universidade Técnica de Lisboa, Lisbon.

GUO, S. W. & THOMPSON, E. A. 1992: Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* **48**: 361–372.

HALDANE, J. B. S. 1954: An exact test for randomness of mating. *Journal of Genetics* **52**: 631–635.

HALLAM, A. 1994: Neogene. *In*: An outline of phanerozoic biogeography (ed. A. Hallam, B. R. Rosen and T.C. Whitmore) pp. 178–203. Oxford University Press, Oxford.

HUNTLEY, B. & BIRKS, H. J. B. 1983: An atlas of past and present pollen maps for Europe – *Abies*– Fir. Cambridge University Press, Cambridge. pp. 73–90.

HUSSENDÖRFER E., KONNERT M. & BERGMANN F. 1995: Inheritance and linkage of isozyme variants of silver fir (*Abies alba* Mill.). *Forest Genetics* **2** (1): 29–40.

LANDRY, P. 1984: Synopsis du genre *Abies*. *Bulletin Societe Botanique France* **3**: 223–229.

LIU, T. S. 1971: A monograph of the genus *Abies*. Forestry College of Agriculture National Taiwan University, Taipei, Taiwan.

MATTFELD, J. 1927: A botanical Journey in Greece in the summer of 1926. *Journal of the Arnold Arboretum* **8** (3): 133–149; 205–233.

MATTFELD, J. 1930: Uber hybridogene Sippen der Tanne. *Bibliotheca Botanika* **100**: 1–84.

MORANDINI, R. 1969: *Abies nebrodensis* (Lojac). Mattei. – Inventario. *Annali Istituto Sperimentale Selvicoltura d'Arezzo* **18**: 1–93.

MORANDINI, R., DUCCI, F. & MENGUZZATO, G. 1994: *Abies nebrodensis* (Lojac.) Mattei. – Inventario1992. *Annali Istituto Sperimentale Selvicoltura d'Arezzo* **22**: 5–51.

NEALE, D. B., WHEELER, N. C. & ALLARD, R. W. 1986: Paternal inheritance of chloroplast DNA in Douglas fir. *Canadian Journal of Forest Research* **16**: 1152 –1154.

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

NITZELIUS, T. 1969: A review of the firs in the Mediterranean. *Lustgården* **49**: 178–189.

PARDUCCI, L., SZMIDT, A. E., VILLANI, F., WANG, X. R. & CHERUBINI, M. 1996: Genetic variation of *Abies alba* in Italy. *Hereditas* **125** (1): 11–18.

PARDUCCI, L. & SZMIDT A. E. 1999: PCR-RFLP analysis in the cpDNA of the genus *Abies*. *Theoretical and Applied Genetics* **98** (5): 802–808.

PARDUCCI, L., SZMIDT, A. E, MADAGHIELE, A., ANZIDEI, M. & VENDRAMIN, G. G. 2001: Genetic variation at chloroplast microsatellites (cpSSR) in *Abies nebrodensis* (Lojac.) Mattei and three neighboring *Abies* species. *Theoretical and Applied Genetics.* In press.

PASCUAL, L., GARCIA, F. J. & PERFECTTI, F. 1993: Inheritance of isozyme variations in seed tissues of *Abies pinsapo* Boiss. *Silvae Genetica* **42**: 335–340.

PIGNATTI, S. 1982: Flora d'Italia. (ed. Edagricole). Bologna, Italy. pp. 74–75.

PLESSAS, M. E. & STRAUSS, S. H. 1986: Allozyme differentiation among populations, stands, and cohorts in Monterey pine. *Canadian Journal of Forest* Research **16**:1155–1164

QUEZEL, P. & BARBERO, M 1990: Caracteristiques ecologiques, dynamiques et structurales des populations naturelles de sapins sur le pourtour mediteranneen. *In*: International EEC Workshop on Mediterranean Firs. Avignon, France. pp. 23–25.

RAIMONDO, F. M., VENTURELLA, G. & DI GANGI, F. 1990: Variazioni fenotipiche in *Abies nebrodensis* (Lojac.) Mattei e comportamento vegetativo nella sua discendenza. *Quaderni di Botanica Ambientale Applicata* **1**: 183–210.

RAYMOND, M. & ROUSSET, F. 1995: An exact test for population differentiation. *Evolution* **49**: 1280–1283.

SCALTSOYIANNES, A., TSAKTSIRA, M. & DROUZAS, A. D. 1999: Allozyme differentiation in the Mediterranean firs (*Abies*, *Pinaceae*). A first comparative study with phylogenetic implications. *Plant Systematics and Evolution* **216**: 289–307.

SCHROEDER, S. 1989: Outcrossing rates and seed characteristics in damaged natural populations of *Abies alba* (Mill.). *Silvae Genetica* **38**: 185–189.

SNEATH, P. H. & SOKAL, R. R. 1973: Numerical Taxonomy. Freeman W.H. & Co., San Francisco.

STINE, M. & KEATHLEY, D. E. 1990: Paternal inheritance of plastids in Engelmann spruce × blue spruce hybrids. *Journal of Heredity* **81**: 443–446.

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

SZMIDT, A. E., ALDEN, T. & HÄLLGREN, J. E. 1987: Paternal inheritance of chloroplast DNA in *Larix*. *Plant Molecular Biology* **9**: 59–64.

SZMIDT, A. E. & MUONA, O. 1985: Genetic effects of Scots pine (*Pinus sylvestris* L.) domestication. *Lecture Notes in Biomathematics* **60**: 241–252.

TURRILL W. B. 1937: XI–On the flora of the near east: XVIII. New species, new records and notes. *Kew Bulletin* 1937 (2): 79–86.

TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., VALENTINE, D. H., WALTERS, S. M. & WEBB, D. A. 1964: Flora Europaea. Cambridge University Press, Cambridge, U.K.

VICARIO, F., VENDRAMIN, G. G., ROSSI, P., LIÒ, P. & GIANNINI, R. 1995: Allozyme, chloroplast DNA and RAPD markers for determining genetic relationships between *Abies alba* and the relic population of *Abies nebrodensis*. *Theoretical and Applied Genetics* **90**: 1012–1018.

VILLANI, F., BENEDETTELLI, S., PACIUCCI, M., CHERUBINI, M. & PIGLIUCCI, M. 1991: Genetic variation and differentiation between natural populations of chestnut (*Castanea sativa* Mill.) from Italy. *In*: Biochemical markers in the population genetics of forest trees (ed. H.H. Hattemer, S. Fineschi, F. Cannata, M.E. Malvolti). SPB Academic Publishing bv, The Hague. pp. 91–103.

WAGNER, D. B., GOVINDARAJU, D. R., YEATMAN, C. W. & PITEL J. A. 1989: Paternal chloroplast DNA inheritance in a diallel cross of jack pine (*Pinus banksiana* Lamb). *Journal of Heredity* **80**: 483–485.

ZIEGENHAGEN B., KORMUTÁK A., SCHAUERTE M. & SCHOLZ F. 1995: Restriction site polymorphism in chloroplast DNA of silver fir (*Abies alba* Mill.). *Forest Genetics* **2**: 99–107.