

Allozyme assessment of genetic diversity within the relic Sicilian fir *Abies nebrodensis* (Lojac.) Mattei

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Abstract – Allozyme markers (11 loci, 32 alleles) have been used to estimate the genetic diversity within the unique surviving population of the relic species *Abies nebrodensis*. Results were analysed in comparison with a reference system composed of 16 Italian populations of *A. alba* and one representative provenance of *A. cephalonica*, *A. equi-trojani*, *A. bornmuelleriana* and *A. nordmanniana*. These investigations allowed us i) to show that alleles Idh-2a and Pgi-1a have contributed to the differentiation of the *A. nebrodensis* population from those of the reference system, ii) to show that the genetic diversity within *A. nebrodensis* is similar to that of dynamic silver fir populations growing in analogous isolation and progressive drifting situations, while, simultaneously, a very high excess of homozygotes is detected, iii) to identify in situ three different zones which corresponded to the diversity core of the species, one site in recolonizing phase and one site in an extinction phase. The origin of this particular situation is discussed and silvicultural interventions to relaunch the dynamics of the species are suggested. (© Inra/Elsevier, Paris.)

Abies nebrodensis / mediterranean firs / genetic diversity / allozymes

Résumé – Évaluation par analyse du polymorphisme alloenzymatique, de la diversité génétique au sein de l'espèce relique *Abies nebrodensis* (Lojac.) Mattei. Des marqueurs alloenzymatiques (11 loci, 32 allèles) ont été utilisés pour évaluer la diversité génétique au sein de la seule population existante de l'espèce relique *A. nebrodensis*. Les résultats, rapportés à un système de référence composé de 16 populations italiennes d'*A. alba* et d'une provenance représentative d'*A. cephalonica*, *A. equi-trojani*, *A. bornmuelleriana* et *A. nordmanniana*, ont permis, (i) de montrer que la fréquence des allèles Idh-2a et Idh-2b permet de différencier *A. nebrodensis* des populations du système de référence, (ii) de montrer que la diversité génétique à l'intérieur d'*A. nebrodensis* est comparable à celle des populations du système de référence présentant des situations d'isolement et de dérive génétique comparables, alors qu'en même temps on observe un fort excès d'homozygotes (iii) de mettre en évidence in situ trois zones différentes représentant respectivement, le noyau de diversité de l'espèce, un site de reconquête et un site en phase d'extinction. L'origine de cette situation particulière est discutée et des mesures de gestion susceptibles de favoriser la reprise de la dynamique de l'espèce sont proposées. (© Inra/Elsevier, Paris.)

Abies nebrodensis / sapins méditerranéens / diversité génétique / allozymes

1. Introduction

Abies nebrodensis is an endemic species of Sicily [20, 22, 26] represented by a single relic population of only 29 adult trees and about 20 small seedlings [30] growing

on the Madonie range, south of the city of Cefalù (figure 1). This species is the southernmost fir in Italy and, together with the Peloponnesus Greek fir (*A. Cephalonica*), represents the southernmost expression of the genus *Abies* in Europe.

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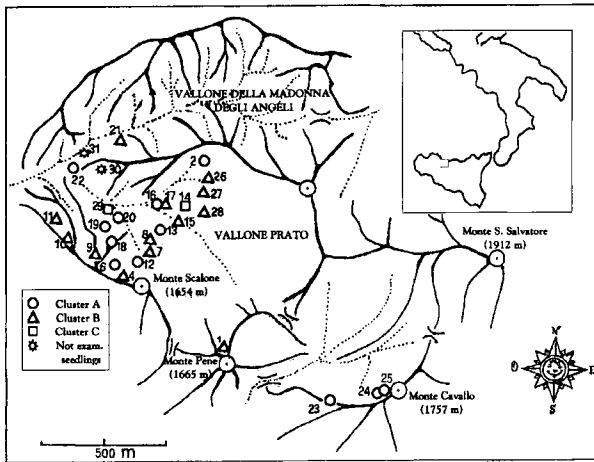


Figure 1. Natural range of *A. nebrodensis* in Sicily. Trees belonging to each similarity genetic cluster are shown with the same symbols.

The occurrence in the Madonie region of many endemic flora and fauna taxa testifies to the participation of *A. nebrodensis* in a former very ancient ecosystem, which is nowadays widely destroyed owing to intense human pressure [3, 37, 39]. However, the decline of the species seems to have occurred in relatively recent times. Indeed, it has been established that beams made from fir were still used in the XVIIth and XVIIIth centuries in roofing the churches of several villages (Polizzi Generosa, Petralia Sottana, Isnello) located within a 30–40-km circle around the Madonie range [26]. This attests to the existence at the time of quite extensive fir forest resources including *A. alba* and *A. nebrodensis* populations as confirmed by Biondi and Raimondo [4].

At present, the Sicilian fir is considered as an endangered original gene pool [36] and several international organizations such as the Council of Europe [8], IUCN [18], FAO [28] mentioned *A. nebrodensis* in their red lists. Locally, action was taken to protect this germplasm following two directions: an in situ protection of trees was ensured by the establishment, within the Natural Park of Madonie, of a strict Reserve Area covering the *A. nebrodensis* population [10] and an ex situ conservation programme is being carried out by the Forest Research Institute of Arezzo [30].

After the first inventories made by Morandini in 1964 and 1968 [26, 27] and a field survey carried out in 1992, an updated list of *A. nebrodensis* trees growing in the Madonie range was drawn up and, for each tree, topographical, morphological and phytoecological data were

recorded [30, 37]. Two clonal grafted collections including copies of 27 of the 29 compiled trees were established in 1992–1993 at the Forest Research Institute of Arezzo. Two trees were too small to endure scion removal without damage.

In this paper we investigated the genetic diversity within this material which represents an almost exhaustive collection of the species, using allozyme markers which have proved to be accurate in several genetic and phylogenetic studies on *Abies* species [1, 5, 11, 12, 17, 21, 32, 33, 41, 42, 44]. This information is essential to assess the genetic potential of the species in order to re-establish a biological dynamics and decide on appropriate conservatory actions.

A. nebrodensis was compared to a group of dynamic populations of silver fir (*A. alba*) ranging from northern to southern Italy and one representative provenance of four fir species originating in the eastern Mediterranean region (*A. nordmanniana*, *A. bornmuelleriana*, *A. equitrojani*, *A. cephalonica*).

2. Material and methods

2.1. Plant material

The 29 Sicilian adult fir trees are distributed over an area of about 150 ha (figure 1).

This zone can be divided into four main sub-zones according to the site morphology and phytoecological parameters [23, 24, 37].

1) The central sub-zone of the lower part of Vallone Prato is phytoecologically variable. Depending on orientation and altitude, the Sicilian fir trees occur in three situations:

- in the middle part (trees 18–20, 29) beech (*Fagion*) with *Luzula sicula* is dominant;
- on the western side, *Quercus petraea* and *Q. pubescens* are present with *Brachypodium sylvaticum* and *Juniperus hemispherica* (trees 2, 14–17, 26–28);
- in the south-eastern part, fir trees (nos 7, 8, 12, 13) are scattered over a wide moving slope area.

2) The peripheral sub-zone of Vallone della Madonna degli Angeli which mainly includes northern–north-eastern slopes, belongs to the *Quercion ilicis* (trees: 21, 22, 30, 31).

3) The peripheral sub-zone of Monte Cavallo which suffers from very hard site conditions can be connected with the *Brachypodietalia phenicoides*, but also includes truncated soils or lithosoils (trees: 23–25).

4) The peripheral sub-zone of Monte Pene and Monte Scalone ridges characterized by very windy positions with exposure to the north-east, is covered by mixed patches of *Geranio-versicoloris-Fagion* and *Cisto-erictalia* (trees: 1, 4, 6, 9–11);

Only 18 out of the 29 adult fir trees produce pollen and/or cones. For this reason we observed two distinctive populations in the analyses:

– Nebr 1, representing the total population of the 27 grafted trees;

– Nebr 2, representing that part of the population which is potentially capable of contributing to stand regeneration. This second population is composed of tree nos 1, 2, 6–13, 17–23, 27.

Nebr 1 and Nebr 2 have been compared to a reference system composed of 16 *A. alba* populations from Italy (several have been selected as seed stands by Morandini and Magini [29]) and one representative provenance [11, 12, 25, 41] of each of the following Mediterranean fir species: *A. nordmanniana*, *A. bornmuelleriana*, *A. equi-trojani* and *A. cephalonica* (table I). All these populations have been described as dynamic, with good natural regeneration.

2.2. Allozyme analysis

Allozyme analysis was performed on samples of about 30–40 buds per tree, collected during winter.

The sample extraction was carried out after centrifugation of the homogenated tissues for 10 min at 10 000 g. The electrophoretic and staining procedures were performed according to Conkle et al. [7] and Santi [40]. Eight enzyme systems coded for by 12 loci were analysed: glutamic-dehydrogenase (Gdh, EC 1.4.1.2), glutamic-oxaloacetate-transaminase (Got, EC 2.6.1.1), isocitric-dehydrogenase (Idh, EC 1.1.1.42), leucine-amino-peptidase (Lap, EC 3.4.11.1), malate-dehydrogenase (Mdh, EC 1.1.1.37), 6-phosphogluconic-dehydrogenase (6-Pgdh, EC 1.1.1.44), phosphogluconic-isomerase (Pgi, EC 5.3.1.9) and shikimate-dehydrogenase (Skdh, EC 1.1.1.25). Due to insufficient availability of samples, this last enzyme system has only been analysed in the Nebr 1 and Nebr 2 populations.

The inheritance models of isozyme variants were described for *Abies* species by Schroeder [42], Bergmann et al. [1], Fady and Conkle [11], Pascual et al. [33], Hussendorfer et al. [17] and Longauer [21].

Table I. List of the examined populations and main geographical parameters.

Species	Region	Population	Mean altitude (m)	Latitude (°N)	Longitude (°E)	Number of trees Examined	
<i>A. nordmanniana</i>	eastern Turkey	1 Ardanug	1 925	41° 34'	42° 11'	36	
<i>A. bornmuelleriana</i>	central northern Turkey	2 Uludag	1 300	40° 08'	26° 43'	36	
<i>A. equi-trojani</i>	western Turkey	3 Kazdag	1 280	39° 50'	26° 43'	36	
<i>A. cephalonica</i>	southern Greece	4 Vetyna	1 300	37° 35'	22° 15'	36	
<i>A. alba</i>	Carnia	5 Paularo	1 000	46° 36'	12° 38'	20	
		6 Chiusa Pesio	1 400	44° 13'	7° 48'	30	
	Maritim Alps eastern Tuscany	7 Campigna	1 000	43° 51'	13° 09'	36	
		8 Vallombrosa	1 000	43° 49'	13° 21'	36	
		9 Camaldoli	1 100	43° 48'	13° 05'	36	
		10 La Verna	1 000	43° 44'	13° 10'	29	
		11 Abeti Soprani	1 220	41° 52'	14° 17'	36	
		12 San Francesco	1 500	39° 54'	16° 11'	36	
		13 Macchia di Pietra	1 265	39° 15'	16° 19'	43	
	Sila Grande Sila Piccola Serra San Bruno	14 Gariglione	1 550	39° 06'	16° 27'	35	
		15 Monte Pecoraro	1 400	38° 33'	16° 20'	35	
		16 Archiforo	1 150	38° 33'	16° 20'	36	
		17 Santa Maria	900	38° 33'	16° 20'	36	
	Aspromonte	18 Fossa Nardello	1 690	38° 10'	15° 47'	35	
		19 Listi alto	1 560	38° 10'	15° 47'	36	
		20 Listi basso	1 370	38° 10'	15° 47'	35	
	<i>A. nebrodensis</i>	Sicilia	21 Madonie	1 865	38° 50'	14° 20'	27

2.3. Statistical analysis

In order to assess genetic variation within the populations, the following parameters were used: allelic frequencies, mean number of alleles per locus, percentage of polymorphic loci, deviation from Hardy-Weinberg equilibrium, observed (H_o) and expected (H_e) heterozygosity and the fixation index (F_{is}), which were calculated using Biosys-1 [9, 43].

The Levene's [43] correction for small size samples was used to carry out the Chi square test for deviation from the Hardy-Weinberg equilibrium.

For the analysis of the genetic variation within the Sicilian fir population, the genotype pattern of each tree was transformed into binary language (each allele at each locus was scored 1 for presence and 0 for absence). Data were then processed using the NTSYS statistic software [38] to carry out correspondence analysis and UPGMA clustering.

2.4. Topographical distribution of the genotypes

In order to visualize in situ the genetic differentiation within the *A. nebrodensis* population, the clusters established after the NTSYS analysis were plotted on the map, tree by tree.

3. Results

3.1. Genetic variation within the populations of the *Abies* reference system

The 11 loci analysed were polymorphic in at least one of the 20 reference populations. Thirty-two alleles were observed (*table II*). In *A. alba*, the mean number of alleles per locus estimated using pooled data without considering the population sub-divisions, was 2.8 (*table III*). Among the populations it ranged from 2.5 to 1.5 and the percentage of polymorphic loci varied from 36.4 % (La Verna) to 90.9 % (San Francesco, Santa Maria and Listì basso). Lowest values of these parameters were recorded in the northern Alpine provenances (Paularo and Chiusa Pesio).

Values for eastern fir species populations were globally similar, though varying within a narrower range.

The observed heterozygosity (H_o) ranged from 0.108 to 0.248 in the *A. alba* reference populations and from 0.157 to 0.264 among the eastern *Abies* species. Positive values of estimated F_{is} in all populations (*table III*) indicated a general heterozygote deficiency within the reference system. The lower deficiencies were observed in the southern populations (Monte Pecoraro, Archiforo, Fossa Nardello, Listì alto).

Loci that deviated less frequently from Hardy-Weinberg equilibrium were Idh-2, 6, Pgd-2, Gdh-1, Pgi-1 and Pgi-2 (*table IV*). Idh-2, 6, Pgd-2 and Gdh-1 were characterized by an excess of heterozygosity among the examined *A. alba* populations.

3.2. *A. nebrodensis* compared to the reference system

Results of *table III* clearly show specific traits of genetic structure in the Nebr 1 population. Compared to the reference system, the mean number of alleles per locus, % of polymorphic loci and H_o were inferior. Higher value of F_{is} indicated an increased heterozygote deficiency. These observations were particularly evident when Nebr 1 was referred to the *A. alba* pooled population. However, when the comparison was made individually with each of the 16 *A. alba* populations included in the reference system, some variations could be observed. The Nebr 1 mean number of alleles per locus and % of polymorphic loci were very similar to that measured in the *A. alba* extensive populations of northern and central Italy (Chiusa Pesio, La Verna), while wider divergences were found with the southern populations (Fossa Nardello, San Francesco, Macchia di Pietra and Listì alto).

H_e was close to that of several *A. alba* populations (Chiusa Pesio, Abeti Soprani) and in some cases superior to northern (Paularo) or small and relatively isolated populations (La Verna, Gariglione). Nevertheless, H_o in Nebr 1 was lower than in all the silver fir analysed populations.

Allele frequencies also showed Nebr 1-specific traits (*table II*). Idh-2a for instance exhibited a higher frequency in Nebr 1 than in the reference system while, conversely, Idh-2b was rare. A similar situation was observed for allele Pgi-1a versus alleles Pgi-1b and c.

The number of rare or absent alleles in Nebr 1 (15) was higher than in the *A. alba* pooled population (9) although the wider sample size in this species. However, the number of absent alleles observed in the silver fir was about twice as high. Significant deviations from the Hardy-Weinberg equilibrium were found in five of the 11 examined alleles.

The main characteristics of the genetic structure observed in Nebr 1 were also found in the Nebr 2 restricted population. The observed differences concerned principally the percentage of polymorphic loci and the estimated F_{is} which were inferior in Nebr 2 (*table III*). However, among the 11 analysed loci, seven exhibited slight excess of heterozygotes (*table IV*). Nevertheless, the mean value of estimated F_{is} remained positive (*table III*). Results were similar for Skdh-2 (F_{is} : -0.048).

Table II. Estimated allele frequencies for 11 allozyme loci (12 loci for *A. nebrodensis*) in the six examined fir species. Values for *A. alba* were estimated using pooled data without considering the population sub-divisions.

Alleles	<i>A. alba</i>	<i>Abies nordmanniana</i>	<i>Abies equi-trojani</i>	<i>Abies bornmuelleriana</i>	<i>Abies cephalonica</i>	<i>Abies nebrodensis</i> NEBR 1	<i>Abies nebrodensis</i> NEBR 2
Idh-2							
a	0.595	0.074	0.280	0.391	0.556	0.981	0.972
b	0.405	0.926	0.720	0.609	0.444	0.019	0.028
6,PGD-1							
a	0.282	0.185	0.087	0.125	0.355	0.463	0.500
b	0.705	0.815	0.913	0.875	0.645	0.537	0.500
c	0.013	0.0	0.0	0.0	0.0	0.0	0.0
6,Pgd-2							
a	0.917	1.0	0.978	1.0	0.891	0.907	0.972
b	0.083	0.0	0.022	0.0	0.109	0.093	0.028
Gdh-1							
a	0.070	0.019	0.080	0.033	0.016	0.0	0.0
b	0.923	0.981	0.920	0.967	0.984	1.0	1.0
c	0.007	0.0	0.0	0.0	0.0	0.0	0.0
Mdh-2							
a	0.862	0.407	0.560	0.483	0.766	0.630	0.556
b	0.138	0.556	0.440	0.500	0.234	0.370	0.444
c	0.0	0.037	0.0	0.017	0.0	0.0	0.0
Lap-2							
a	0.055	0.259	0.080	0.150	0.031	0.042	0.063
b	0.865	0.741	0.840	0.800	0.844	0.792	0.937
c	0.080	0.0	0.080	0.050	0.125	0.166	0.0
Pgi-1							
a	0.109	0.0	0.0	0.0	0.0	0.778	0.778
b	0.887	0.935	0.978	1.0	0.865	0.222	0.222
c	0.004	0.065	0.022	0.0	0.135	0.0	0.0
Pgi-2							
a	0.002	0.021	0.043	0.017	0.081	0.0	0.0
b	0.010	0.0	0.0	0.0	0.0	0.037	0.028
c	0.984	0.979	0.957	0.983	0.919	0.963	0.972
d	0.004	0.0	0.0	0.0	0.0	0.0	0.0
Got-1							
a	0.040	0.037	0.0	0.067	0.031	0.0	0.0
b	0.920	0.963	1.0	0.867	0.969	1.0	1.0
c	0.040	0.0	0.0	0.066	0.0	0.0	0.0
Got-2							
a	0.135	0.037	0.083	0.0	0.266	0.111	0.028
b	0.786	0.963	0.917	1.0	0.531	0.889	0.972
c	0.079	0.0	0.0	0.0	0.203	0.0	0.0
Got-3							
a	0.150	0.167	0.083	0.078	0.182	0.019	0.028
b	0.793	0.722	0.875	0.766	0.636	0.981	0.972
c	0.057	0.111	0.042	0.156	0.182	0.0	0.0
Skdh-2							
a	n.e.	n.e.	n.e.	n.e.	n.e.	0.719	0.682
b						0.281	0.318

n.e.: not examined.

Table III. Genetic variation at 11 allozyme loci (12 loci for *A. nebrodensis*) in all the examined populations. Standard errors in brackets.

Species/Population	Mean sample size per locus	n. ^a	$P_{5\%}$ ^b	H_o	H_e	F_{is}
<i>A. nordmanniana</i>						
1 Ardanug	26.4 (.4)	2.1 (.2)	54.5	.184 (.052)	.197 (.057)	.066
<i>A. equi-trojani</i>						
2 Kazdag	24.0 (.3)	2.1 (.2)	63.6	.163 (.045)	.188 (.048)	.133
<i>A. bornmuelleriana</i>						
3 Uludag	27.6 (1.5)	2.1 (.3)	54.5	.157 (.055)	.188 (.061)	.165
<i>A. cephalonica</i>						
4 Vetyna	31.3 (.5)	2.3 (.1)	81.8	.264 (.057)	.298 (.056)	.114
<i>A. alba</i> Pooled population ^c	514.8 (13.9)	2.8 (.2)	90.9	.200 (.032)	.252 (.041)	.206
5 Paularo	17.3 (1.4)	1.5 (.2)	54.5	.108 (.035)	.129 (.049)	.163
6 Chiusa Pesio	21.0 (2.4)	1.7 (.2)	45.5	.163 (.063)	.204 (.078)	.201
7 Campigna	33.2 (1.3)	2.3 (.1)	72.7	.211 (.045)	.251 (.051)	.159
8 Vallombrosa	31.8 (2.4)	2.3 (.1)	81.8	.248 (.038)	.302 (.051)	.179
9 Camaldoli	35.8 (.1)	2.1 (.3)	63.6	.176 (.054)	.223 (.056)	.211
10 La Verna	29.1 (2.6)	1.8 (.2)	36.4	.132 (.048)	.180 (.061)	.267
11 Abeti Soprani	32.5 (1.9)	2.1 (.2)	63.6	.181 (.049)	.209 (.052)	.134
12 San Francesco	34.4 (.8)	2.4 (.2)	90.9	.221 (.053)	.269 (.043)	.178
13 Macchia di Pietra	42.3 (.4)	2.4 (.2)	81.8	.210 (.043)	.229 (.045)	.083
14 Gariglione	32.8 (2.1)	2.1 (.2)	72.7	.159 (.035)	.182 (.035)	.126
15 Monte Pecoraro	34.5 (.6)	2.0 (.1)	72.7	.247 (.052)	.257 (.053)	.039
16 Archiforo	34.0 (1.4)	2.2 (.1)	81.8	.202 (.038)	.213 (.039)	.052
17 Santa Maria	32.3 (2.1)	2.1 (.1)	90.9	.200 (.036)	.223 (.030)	.103
18 Fossa Nardello	33.5 (.7)	2.5 (.2)	81.8	.237 (.051)	.259 (.048)	.085
19 Listi alto	34.4 (.9)	2.3 (.2)	63.6	.199 (.050)	.214 (.056)	.070
20 Listi basso	36.0 (.0)	2.1 (.2)	90.9	.197 (.047)	.222 (.043)	.113
<i>A. nebrodensis</i>						
21 Nebr 1	25.6 (1.4)	1.9 (.2)	54.5	.102 (.029)	.201 (.058)	.493
including Skdh-2	24.8 (1.5)	1.9 (.1)	58.3	.119 (.032)	.219 (.056)	.457
22 Nebr 2	17.1 (.9)	1.8 (.2)	36.4	.107 (.036)	.162 (.057)	.340
including Skdh-2	16.6 (1.0)	1.8 (.1)	41.7	.136 (.046)	.186 (.060)	.269

^a Mean number of alleles per locus; ^b percentage of polymorphic loci (a locus is considered polymorphic if more than one allele was detected); ^c estimate using pooled data without considering population sub-divisions.

3.3. Genetic differentiation within the *A. nebrodensis* population and in situ structuration of diversity

The 27 *A. nebrodensis* trees showed different genotype in at least one locus. Seventy-three per cent of the total variance were explained by the first five factors of the correspondence analysis. Seven alleles were significantly correlated to these factors: Idh-2b, 6, Pgd-1a and 1b, Pgi-1b, Got-2a and 2b and Got-3a.

The UPGMA dendrogram built using these alleles is given in *figure 2*. Taking into account the small size of the population, we accepted a differentiation into three main clusters. Cluster A included 12 trees (nos 2, 6, 12, 13, 16, 18–20, 22–25), cluster B grouped 13 trees (nos 1, 4, 7–11, 15, 17, 21, 26–28) and cluster C, two trees (nos 14 and 29).

The Nebr 2 population was represented in clusters A and B, each with 50 % of total trees.

Trees of the different clusters were then plotted on the map (*figure 1*). It appeared that all of the three clusters were represented in the central sub-zone (bottom part of Vallone Prato). In contrast, in the peripheral sub-zones trees belonged to only one of the main clusters, namely cluster A in the peripheral sub-zone of Monte Cavallo, and cluster B in the peripheral sub-zone of Monte Pene-Monte Scalone. Tree 6 on the Monte Scalone crest was the unique exception.

The peripheral sub-zone of Vallone della Madonna degli Angeli was characterized by the presence of trees nos 21 and 22, belonging to clusters A and B, respectively.

The in situ localization of the rare alleles identified in *table II*, confirmed this unequal distribution of the genotypes within the range of the species (*table V*). Indeed, all the rare alleles were located in the central sub-zone of Vallone Prato.

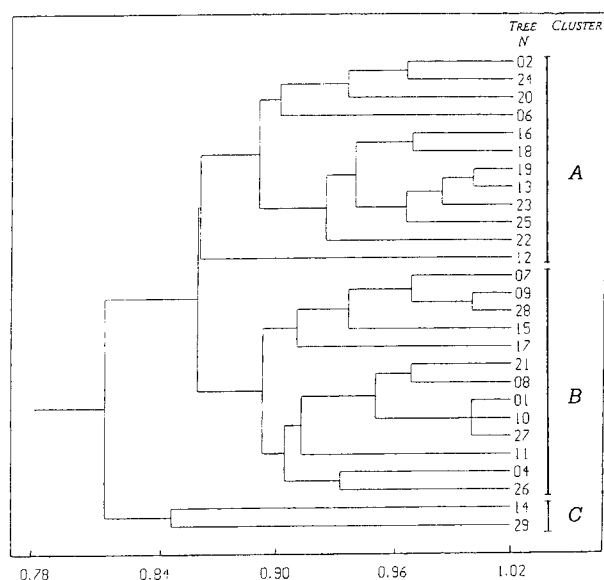


Figure 2. UPGMA dendrogram based on similarity matrix [38] obtained for all the examined trees.

4. Discussion

1) Many of the previous allozyme studies carried out on *Abies* species reported a deficiency of heterozygotes regardless of the examined enzyme loci, the number of analysed populations or the sample size. This was shown in several populations of *A. alba* as well as in other Mediterranean fir species such as *A. cephalonica*, *A. equi-trojani*, *A. bornmuelleriana* and *A. borisii regis* [1, 10, 11, 21, 32, 41, 42, 44]. In *A. alba* for instance, estimated *Fis* values ranging from 0.140 to 0.280 have been reported [44: northern Italian populations] indicating clear excess of homozygotes. However, in some populations, lower *Fis* values showing no significant differences from the Hardy-Weinberg equilibrium have been observed. This is notably the case of the Calabrian population of Serra San Bruno in which the reported *Fis* values range from -0.080 [44] to 0.050 [32]. For a given population, differences among authors could be high. In Abeti Soprani (central Apennines) for instance, *Fis* values ranged from 0.050 [44] to 0.188 (calculated from Parducci et al. [32]), but the analysed loci, the revealed alleles and the number of sampled trees were different.

The results obtained in this study confirmed the general trend to heterozygote deficiency observed in *Abies* species, especially when referring to small and isolated stands. The occurrence of self pollination in *Abies*

species [11, 41], confirmed in *A. alba* [13, 32], together with the Wahlund effect resulting from the ancient fractioning of the natural range of *Abies* around the Mediterranean basin, have probably contributed to the maintenance, such high homozygosity levels. The artificial origin of the *A. alba* Tuscan populations can also have determined higher deficiency of heterozygotes than in the natural populations of this species.

In addition to low heterozygosity, some authors ([1] and to some extent, [44]) confirmed by Parducci et al. [32] also observed an increasing variation in genetic parameters from the northern Alpine to the southern Italian *A. alba* populations. Our results also confirmed such a clinal northern-southern gradient within the Italian range of this species, more evidently when the artificial populations of Camaldoli, Campigna and Vallombrosa were excluded.

In conclusion, the overall consistency of our results with the previously collected data, also evident for genetic parameters such as the number of alleles per locus, percentage of polymorphic loci and *He*, shows that the 16 *A. alba* and four eastern fir species populations analysed can be considered as representative of the general situation in the Mediterranean *Abies* species, and thus validates the choice of these populations as a reference system in assessing the genetic diversity within *A. nebrodensis*.

2) In a general way, the genetic diversity within both the Nebr 1 and Nebr 2 populations was lower than in the reference system. However, a detailed examination of the main genetic parameters showed similarities with several *A. alba* populations (Paularo, Chiusa Pesio, La Verna and Gariglione) which share with *A. nebrodensis* common traits such as ecological conditions, altitudinal and/or geographical position, long time isolation and progressive reduction in tree density.

The northern geographic position, where genetic diversity is known to be low [1] and exposure to extreme climatic conditions owing to altitude and relative isolation for instance, can explain the results obtained in Paularo and Chiusa Pesio, respectively. The isolating effects of altitude and local topography can also account for the similarity of genetic parameters in the small-sized population of La Verna and *A. nebrodensis*. The case of the wider population of Gariglione which surprisingly showed lower genetic diversity (*He*) than *A. nebrodensis* and all the other analysed Calabrian populations, could be considered as a result of long time geographic isolation as already indicated by Parducci et al. [32].

A. nebrodensis collects together the specific traits of these four geographically distant dynamic *A. alba* populations of the reference system: geographic and topo-

Table IV. Mean fixation index (*Fis*) and probability of deviation from Hardy-Weinberg equilibrium estimated using χ^2 test between observed and expected alleles frequencies at polymorphic loci (* indicates significant deviation at $P = 0.05$).

Species/Population	Locus IDH-2	6Pgd-1	6Pgd-2	Gdh-1	Mdh-2	Lap-2	Pgi-1	Pgi-2	Got-1	Got-2	Got-3
<i>A. nordmanniana</i>											
1 Ardanung	-.080	.264	m	-.019	.010	.036*	-.070	-.021	-.038	-.038*	.070
<i>A. equi-trojani</i>											
2 Kazdag	-.190	-.095*	-.022	-.087	.188	.432*	-.022	-.045	m	.455*	.262
<i>A. bornmuelleriana</i>											
3 Uludag	-.053	.429*	-.034*	m	-.096	.104	m	-.017	.444*	m	.044
<i>A. cephalonica</i>											
4 Vetyna	.015	-.127	-.123	-.016	.042	.770*	.175	.347*	-.032	.175*	-.060
<i>A. alba</i>											
Pooled population ^a	.177*	.249*	.057	.155*	.101*	.183*	.289*	.226*	.178*	.257*	.271*
5 Paularo	-.091	.367	m	.297	-.056	m	m	m	m	-.086	-.152
6 Chiusa Pesio	.406*	.067	-.021	m	.064	m	m	m	m	-.083	.316
7 Campigna	.153	.022	-.031	.471*	.354*	.171*	-.043	-.029	.107	.222*	.152
8 Vallombrosa	.010	.159	.196	-.180	.291	.164	-.043	-.014	-.043	.232	.313
9 Camaldoli	-.277	.167	-.043	m	-.094	.237*	m	m	.450*	.786*	.172
10 La Verna	-.097	.187	-.016	.318	-.049*	m	m	m	.650*	1.00*	.434*
11 Abeti Soprani	.134	-.011	.195	-.014	.355*	1.00*	m	m	-.118	.030	.227
12 San Francesco	-.412*	.163	.044	.074	.209	.176	-.085	m	.106*	.825*	.718*
13 Macchia di Pietra	.184	.048	-.111	.236	-.099	-.057	-.024	m	.264*	.070	.187
14 Gariglione	.150	.067	-.077	-.029	.103	-.094	-.099	m	-.029	.067	1.00*
15 Monte Pecoraro	-.042	-.014	-.167	-.043	.153	-.074	.291	m	-.030	.014	.051
16 Archiforo	.0001	-.001	.156	-.029	-.125	-.107	-.075	-.043	-.056	.121	.447
17 Santa Maria	-.125	.438*	-.108	-.091	-.108	.270	.156	-.043	.636*	-.113	.179
18 Fossa Nardello	-.202	.264	-.048	-.061	.006	.072	.150	.449*	-.034	.355*	.040*
19 Listi alto	.0001	.351*	-.043	-.059	-.043	.129	.026	m	-.029	.113	-.110
20 Listi basso	-.155	.156	-.091	-.059	-.037	-.125	1.00*	m	-.079	.056	-.059
<i>A. nebrodensis</i>											
21 Nebr 1	-.019	.777*	-.102	m	.365*	.758*	.357*	-.038	m	.625*	-.019
22 Nebr 2	-.029	.778*	-.029	m	.100	-.067	.357	-.029	-.029	-.029	-.048

^a Estimate using pooled data without considering population sub-divisions.
m: monomorphic locus in the population.

graphic isolation, extreme climatic and edaphic growth conditions, anthropic pressure, and a dramatically reduced number of trees. The main difference concerned the genetic structure, characterized by a really high excess of homozygotes. *Fis* values recorded in both the Nebr 1 and Nebr 2 populations were about twice as high as in the pooled *A. alba* reference population and even more in the Paularo, Chiusa Pesio, La Verna and Gariglione populations.

These very high values of *Fis* could be attributed to several causes probably joint in their results, i.e. too great a distance between the trees, increased rate of self pollination due to high scattering of the trees, genetic drift, stochastic selection effect of position of the living trees and of their earlier parent trees [14, 15].

In contrast with the estimated *Fis* the other genetic parameters (especially the number of alleles per locus, percentage of polymorphic loci and *He*) exhibited close

values in the Nebr 1 and Nebr 2 populations compared to the four comparable *A. alba* populations of the reference system. This suggests that despite the relic conditions and very small size of population (27 trees), *A. nebrodensis* still retains a representative sampling of the genetic potential of the former tree generations, when the stand was more extended. The fact that the allele pattern found in Nebr 1 and in Nebr 2 was relatively similar to those of populations of other species confirmed this conclusion. Indeed, among the 32 alleles detected within 11 enzyme systems only two (Idh-2a versus Idh-2b and Pgi-1a versus Pgi-1b) turned to higher or lower frequency in *A. nebrodensis*, whereas they were conversely rare or frequent in the reference system.

The case of locus Idh-2 was especially interesting to consider. Frequency of allele Idh-2a was two to ten times higher in *A. nebrodensis* than in the *A. alba*, *A. cephalonica* and *A. nordmanniana* populations. Bergman

Table V. Repartition of *A. nebrodensis* rare (frequency < 0.05) and fairly rare (frequency 0.1 ~ 0.05) alleles among the clusters and distribution within the geographical sub-zones.

Rare allele	Frequency within Nebr 1	Frequency within Nebr 2	Tree no.	Cluster	Sub-zone
Idh-2b 6,Pgd-2b	0.019 0.093	0.028 0.028	12*	A	Vallone Prato
			4	B	Monte Pene
			11*	B	Monte Pene
			14	C	Vallone Prato
			16	A	Vallone Prato
Lap-2b Pgi-2b	0.042 0.037	0.063 0.028	26	B	Vallone Prato
			20*	A	Vallone Prato
			15	B	Vallone Prato
			20*	A	Vallone Prato
Got-2a	0.111	0.028	4	B	Monte Pene
			14	C	Vallone Prato
			22*	B	Vallone M. Angeli
			29	C	Vallone Prato
Got-3a	0.019	0.028	17*	B	Vallone Prato

* Trees belonging to both the Nebr 1 and Nebr 2 populations.

and Gregorius [2] studying 45 European populations of *A. alba* including six from Calabria, showed inverse variation from north to south of Idh-2a and Idh-2b frequencies, the former being lowest in the south. They interpreted this result as a consequence of a lower thermostability of this allele. Thus, the maintenance within *A. nebrodensis* of a high frequency of Idh-2a (present in all trees but one) could be due to the cold climatic conditions (altitude, northern slopes) of the Madonie site, which reduce the selection against this negatively thermo-influenced allele. The same explanation can be given to account for the results of Longauer [21] who also found high Idh-2a frequencies (0.70) in Calabrian populations of *A. alba* close to *A. nebrodensis*.

Finally, compared to the reference system, the relic population of *A. nebrodensis* appeared to be characterized by both relatively normal genetic diversity (i.e. number of alleles per locus, number of polymorphic loci, expected heterozygosity) and increased deficiency of heterozygotes. According to Gregorius and Bergmann [14] the higher frequency of some alleles, as well as homozygosity, could be explained by the adaptation to peculiar local environmental conditions, indicating thus that the genetic diversity within *A. nebrodensis* is compatible with a possible restoring of dynamics in the population.

3) The in situ identification of trees according to the UPGMA clustering of the *A. nebrodensis* population showed a heterogeneous distribution of genetic variation in connection with the high microenvironmental diversity

of the species area. Such an explicative relationship between genetic variation and microenvironmental diversity has also been suggested by Müller-Starck [31] for altitude Alpine spruce stands.

Within the Sicilian fir range, three different situations can be described. On both the southern peripheral crest zones of Monte Cavallo and Monte Pene–Monte Scalone, which are subjected to extreme environmental conditions, especially freezing cold winds, trees belonged to only one of the three identified clusters. The future long-term survival of these trees is doubtful, and although some seedlings are present around tree 1 on the Monte Pene summit, an efficient recolonizing process is very unlikely. These sites can be therefore considered as in an extinction phase.

The situation in the central zone of Vallone Prato is quite different. The three genotype clusters, as well as the five rare alleles observed, were represented among the trees present on this site. Vallone Prato can be thus considered as the diversity core of the population, containing the main part of the gene pool and hence constitutes the priority zone for in situ conservation.

It is important, however, to note that this central zone also conceals high microenvironmental and phytoecological diversity which can represent, on the one hand suitable conditions to maintain genetic diversity, but on the other adverse conditions for natural regeneration and population increase. Along the upper limits of the Vallone, as on the surrounding ridges of Monte Scalone, trees are exposed to drastic edaphic conditions (deep intensively eroded rocky slopes) which are unsuitable for regeneration. In lower locations the soil conditions are better, but trees are subjected to increasing beech coppice competition. The evolution of the beech coppice into high forest formation was predicted as early as 1960 by Hoffmann [16] and recently confirmed by Raimondo et al. [37]. The environmental conditions, especially the light conditions, created by the expansion of the coppice, today aged 40–45 years [16] are unsuitable for cone production, germination and development of the fir seedlings. In some cases the fir trees are in danger of being surpassed by the coppice. Examples of such a regression and sometimes complete substitution of *Abies* by beech have been observed in several Calabrian forests [6].

This evolution within the diversity core of the population poses a threat to the future of the fir trees. Silvicultural interventions should be rapidly realized in order to stimulate the fir reproductive maturation and regeneration. Selective thinning of the beech coppice, for instance, would open patches favourable to seed germination and growth of seedlings, relaunching the dynamics of the fir population as recommended by Ciancio et

al. [6] and Lovino and Menguzzato [19] to preserve the *A. alba* populations of the Calabrian forest.

The northern zone of Vallone della Madonna degli Angeli harbours the only two adult trees at present able to regenerate living seedlings with wide survival potential (trees 21 and 22). They represent the unique part of the population which can be considered in expansion. They belong to clusters A and B, respectively. Tree 22 possess the Got-2a rare allele. These trees possibly derive from Vallone Prato and find in this new site favourable ecological conditions. Indeed, in the opening of this small valley, phytoecological conditions are different from those of Vallone Prato. As in the typical Mediterranean oak/fir succession [34, 35], *A. nebrodensis* is associated there with *Quercus ilex* and a mixture of mountain and sub-mountain hardwoods and shrubs such as *Fraxinus ornus*, *Arbutus unedo*, *Ilex aquifolium*, *Acer campestre*, etc. [16, 26] and finds appropriate conditions to re-start an expansion phase.

Therefore, in contrast with Vallone Prato, any silvicultural intervention should be avoided in this zone. Only the establishment of small diffusion cores composed of propagation material issued from the three clusters could possibly be considered. Special interest could also be given to the diffusion of seedlings born from rare allele carrier mother trees.

In this way, the genetic diversity patterns within this potential expansion zone of the species could be improved.

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