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Genetic variation of *Abies alba* in Italy

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Italy represents the southern limit of *Abies alba* (Mill.) (silver fir) distribution in Europe. In this region, populations are widely spread in different and isolated habitats. We used allozyme markers to study the genetic structure of ten natural populations of this species from the northern, central, and southern parts of Italy. Mean expected heterozygosity ranged from 0.129 to 0.180 and was similar to that found in other conifers. In contrast to other conifers, all the populations investigated in the present study showed a deficiency of heterozygotes. Partial inbreeding due to restricted pollen dispersal and Wahlund effect could be responsible for this situation. The central populations harboured more variability than the populations from other parts of Italy. The present study revealed significant differentiation among the investigated populations. The average genetic distance for all pair-wise comparisons between the ten populations was 0.014. Populations from the central Italy showed closer affinity to the southern populations than to the populations from the northern Italy. Our results suggest that the *A. alba* populations in central Italy have originated either through a postglacial blending of two different populations or through expansion of the southern populations. In addition, they confirm distinct character of populations from the southern Italy.

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Genus *Abies* belongs to the family of Pinaceae, subfamily Abietoideae. In the last century, the genus has been studied extensively from the standpoint of taxonomy and enriched by the addition of new taxa. In Europe, the genus is represented by several species, which, with the exception of *Abies alba* (Mill.) (silver fir), have rather restricted and isolated distribution ranges scattered along the Mediterranean basin. The natural range of *A. alba* extends across the Central European mountainous regions and covers the area extending approximately from 52° to 38° north latitude and from 3° to 27° east longitude (Fig. 1). Italy represents the southern limit of the species range in Europe. Here, its distribution differs between the southern and the northern parts of the country. In the central and southern parts of the Apennines chain, the individual populations occupy different and isolated habitats. In contrast, in the Alpine region of northern Italy the species is more continuously distributed from east to west. The southernmost Italian populations occurring in the Calabria region are believed to represent a glacial refugium of the species (HUNTLEY and BIRKS 1983). These southern populations are considered morphologically and genetically different from the populations occurring in the northern part of Italy and in central Europe (BERGMANN et al. 1990; DUCCI 1994; KONNERT and BERGMANN 1995; LARSEN 1986; LARSEN and FRIEDERICH 1988; LARSEN et al. 1988; PENNACCHINI and DUCCI 1994). On the other hand, the northern populations occurring in the Alps are considered to be of postglacial origin (HUNTLEY and BIRKS 1983).

So far, the genetic structure of the Italian *A. alba* populations has been little studied, and particularly little is known about the origin of populations occurring in the central part of the country. Most of the previous work on this species has focused on populations from other parts of Europe (BERGMANN 1991; BREITENBACH-DORFER et al. 1992; KONNERT 1993; KONNERT and BERGMANN 1995; KORMUTAK et al. 1982; SCHROEDER 1989a,b). BERGMANN et al. (1990) found out that the levels of allozyme variation were higher in southern Italian populations than in populations from the other areas of Europe. BERGMANN and KOWNATZKI (1988) have suggested that southern Italy represents an outstanding part of the species range, greatly differentiated from the remaining

Fig. 1. Distribution of *A. alba* in Europe.

areas, and that this region could not be origin of the western or central European *A. alba* populations. More recently, VICARIO et al. (1995) have found a higher level of allozyme and DNA variation in the southern *A. alba* populations than in populations from the other parts of Italy.

The aim of the present work was to determine the amount and distribution of genetic variability in the *A. alba* populations from the northern, central, and southern Italy by means of isozyme analysis, to provide new information about the migration history of this species in Italy and to elucidate possible origin of populations from the central part of the country.

Materials and methods

Winter buds were collected from ten natural populations of *A. alba* throughout the Alps and the Apennines chain. The geographical origin of the investigated populations and their coordinates are presented in Fig. 2 and in Table 1. The collections included three populations from the Alps (Trentino Alto Adige region), one population from the northern part of the Apennines chain (Toscana region), three populations from the central part of

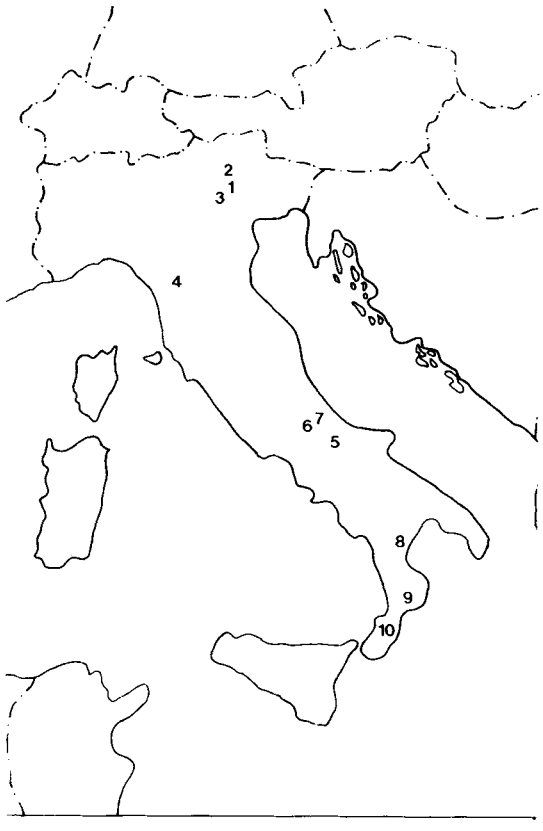

Fig. 2. Location of the investigated *A. alba* populations in Italy.

the Apennines chain (Molise region), and three populations from the southern part of Apennines chain (Basilicata and Calabria regions). The number of individuals per population analysed ranged from 24 to 30.

The material was analysed for allozyme variation by means of horizontal starch gel electrophoresis as described by VILLANI et al. (1991). The following ten enzyme systems were studied: leucine aminopeptidase (*Lap*, Enzyme Commission (E.C.) No. 3.4.11.1), peroxidase (*Pex*, E.C. 1.11.1.7), glutamate dehydrogenase (*GDh*, E.C. 1.4.1.2), isocitrate dehydrogenase (*IDh*, E.C. 1.1.1.42), aspartate aminotransferase (*Got*, E.C. 2.6.1.1), 6-phospogluconate dehydrogenase (*6-PgDh*, E.C. 1.1.1.43), glucosephosphate isomerase (*Pgi*, E.C. 5.3.1.9), phosphoglucomutase (*Pgm*, E.C. 2.7.5.1), shikimate dehydrogenase (*SkDh*, E.C. 1.1.1.25), and diaphorase (*Dia*, E.C. 1.6.4.3). Inheritance of the isozyme loci used in the present

Table 1. Geographic origin of the investigated populations of *A. alba*

Locations	Sample size	Latitude (N)	Longitude (E)	Altitude (m)	Region
1 Val Canali	27	46°09′	11°50′	1160	Trentino Alto Adige
2 Val Noana	27	46°12′	11°50′	1100	Trentino Alto Adige
3 Valsugana	27	45°56′	11°18′	1050–1400	Trentino Alto Adige
4 Campolino	28	44°05′	10°42′	1200–1400	Toscana
5 Pescolanciano	27	41°43′	14°21′	800–950	Molise
6 Pescopennataro	27	41°52′	14°17′	1100–1300	Molise
7 Vallazzuna	24	41°54′	14°19′	900	Molise
8 M. Pollino	30	39°55′	16°10′	1400	Basilicata
9 M. Gariglione	27	39°08′	16°39′	1400–1700	Calabria
10 Archiforo	27	38°33′	16°19′	900–1300	Calabria

study was determined by HUSSENDÖRFER et al. (1995), SCHROEDER (1989c), and PARDUCCI (unpublished).

The following measures of genetic variability were calculated for each population: observed and an unbiased expected heterozygosity (NEI 1978). A locus was considered polymorphic when the frequency of the most common allele did not exceed 0.95. The single-locus and mean inbreeding coefficients (F) were computed as described by CURIE-COHEN (1982) using the GENEISO program (SZMIDT, unpublished). This method yields better estimates of F than other methods when alleles have different frequencies and the true inbreeding coefficient is suspected to be low, which was the case in our study.

The conformance of the investigated populations to the Hardy-Weinberg equilibrium was estimated using the exact test (GUO and THOMPSON 1992; WEIR 1991) employing the complete enumeration method (LOUIS and DEMPSTER 1987). In addition, we have performed a global multi-score test for heterozygote deficiency across loci and across populations (ROUSSET and RAYMOND 1995) employing the Markov chain method (GUO and THOMPSON 1992) using dememorization number 1000 with 100 batches and 2000 iterations. These calculations were made using the GENEPOP ver. 2.0 program (RAYMOND and ROUSSET 1995b).

Unbiased measures of the apportionment of genetic diversity within and among populations (NEI 1987), were calculated for polymorphic loci (0.95 criterion) using the GENEISO program (SZMIDT, unpublished). The statistical significance of the observed gene differentiation among populations was tested by the probability test (RAYMOND and ROUSSET 1995a) employing the Markov chain method (GUO and THOMPSON 1992) using the GENEPOP ver. 2.0 program (RAYMOND and ROUSSET 1995b), dememorization number 1000 with 100 batches and 2000 iterations. The test for among population differentiation is based on the assumption that there is Hardy-Weinberg equilibrium within populations. If this is not the case, then the exact test for differentiation will lead to erroneous results. We therefore performed an additional test using WEIR and COCKERHAM (1984) estimators of *F*-statistics proposed by GUODET (1995) which eliminates this difficulty. The test was made using the FSTAT ver. 1.2 program (GUODET 1995).

Unbiased genetic distances (NEI 1978) separating the investigated populations, were calculated using all the loci and used for cluster analysis employing the UPGMA method (SNEATH and SOKAL 1973). These calculations were carried out using the BIOSYS package (SWOFFORD and SELANDER 1981).

Results

Genetic variation

The following 15 isozyme loci were detected using the ten enzyme systems analysed: *Lap*, *Pex*, *GDh*, *IDh*, *Got-1*, *Got-2*, *Got-3*, *6PgDh-1*, *6PgDh-2*, *Pgi-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *SkDh*, and *Dia*. Allozyme frequency data for individual loci can be obtained upon request from the senior author or retrieved *via anonymous ftp* at the following address: *linne.genfys.slu.se* (directory: */pub/data*).

We found a single invariant zone of activity for the *GDh* locus similar to that reported by HUSSENDÖRFER et al. (1995). However, other authors have found two alleles at this locus in some European populations of *A. alba* (LONGAUER 1994; SCHROEDER 1988; VICARIO et al. 1995). For the *IDh* enzyme system, we observed a single locus

Table 2. Average observed (H_O) and unbiased expected (H_E) (NEI 1978) heterozygosities, fixation indices (F) (CURIE-COHEN 1982), and results of analysis of significance of deviations from HW proportions in the investigated populations of *A. alba*. Standard deviations in parentheses

Pop. No.	Location	Mean sample size	H_O	H_E	F	P[1]	P[2]
1	Val Canali	26.7	0.127 (0.044)	0.148 (0.051)	0.042	ns	ns
2	Val Noana	26.7	0.140 (0.057)	0.138 (0.049)	0.016	ns	ns
3	Valsugana	26.7	0.129 (0.049)	0.142 (0.056)	0.009	ns	ns
4	Campolino	27.8	0.112 (0.045)	0.129 (0.052)	0.044	ns	*
5	Pescolanciano	26.3	0.162 (0.052)	0.180 (0.055)	0.041	*	*
6	Pescopennataro	26.7	0.134 (0.047)	0.165 (0.055)	0.077	**	*
7	Vallazzuna	22.9	0.131 (0.043)	0.144 (0.045)	0.047	ns	ns
8	M. Pollino	28.3	0.130 (0.048)	0.140 (0.043)	0.036	ns	ns
9	M. Gariglione	26.1	0.123 (0.032)	0.145 (0.038)	0.052	ns	ns
10	Archiforo	26.7	0.143 (0.042)	0.149 (0.038)	0.038	ns	ns

[1] Hardy-Weinberg exact probability test (WEIR 1991) with complete enumeration (LOUIS and DEMPSTER 1987)
[2] Unbiased estimate of Hardy-Weinberg exact P-values using Markov chain method (GUO and THOMPSON 1992) when there is a heterozygote deficit (ROUSSET and RAYMOND 1995)
ns — not significant, * — $P < 0.05$; ** — $P < 0.01$

while some authors found two different loci (BERGMANN and KOWNATZKI 1988; BREITENBACH-DORFER et al. 1992; HUSSENDÖRFER et al. 1995; KONNERT 1993; SCHROEDER 1989c). This discrepancy was probably due to a different separation protocol used in the present study. Five loci, were monomorphic in most of the populations but exhibited polymorphism in some parts of the Italian *A. alba* range (results not shown). Namely, the *Got-1* locus was polymorphic only in the northern populations, but it was fixed in all the other populations from the Apennines chain, while the *Pgi-1* locus was polymorphic only in the southern populations. On the other hand the *Pgi-2*, *SkDh*, and *Dia* loci were polymorphic both in the central and southern populations. The most polymorphic loci were: *Lap*, *Pex*, *IDh*, *Got-3*, and *6-PgDh-1* (results not shown).

Measures of genetic variability in the ten investigated *A. alba* populations are given in Table 2. Mean observed and unbiased expected heterozygosity (NEI 1978) ranged from 0.112 to 0.162 and from 0.129 to 0.180, respectively. The two central populations (Pescolanciano and Pescopennataro) harboured more variability than the populations from the northern and southern parts of Italy. Inbreeding coefficients were positive in all investigated populations, indicating a deficiency of heterozygotes relative to the panmictic expectations. The exact test (GUO and THOMPSON 1992; WEIR 1991) revealed that this deficiency was statistically significant ($p < 0.05$) in the two central populations: Pescolanciano and Pescopennataro (Table 2). When the populations were analysed using the heterozygote deficiency test (ROUSSET and RAYMOND 1995), one additional population Campolino also showed significant deficiency of heterozygotes.

Population differentiation

The results of the present analysis of the apportionment of gene diversity within and among populations are presented in Table 3. Except for one locus (*Got-2*) the gene differentiation among populations for the remaining 13 polymorphic loci was statistically significant. The test proposed by GUODET (1995) gave identical results and therefore is not presented.

Table 3. Unbiased measures of the apportionment of gene diversity within and among populations (NEI 1987) and the results of the probability test (RAYMOND and ROUSSET 1995a)

Locus	H_t	D_{st}	G_{st}	P
Lap	0.180	0.012	0.067	**
Pex	0.212	0.020	0.095	**
IDh	0.497	0.044	0.088	**
Got-1	0.029	0.001	0.046	**
Got-2	0.030	0.001	0.033	ns
Got-3	0.478	0.033	0.068	**
6PgDh-1	0.493	0.081	0.164	**
6PgDh-2	0.108	0.004	0.034	**
Pgi-1	0.033	0.003	0.104	**
Pgi-2	0.062	0.003	0.042	**
Pgm-1	0.077	0.002	0.027	*
Pgm-2	0.044	0.002	0.052	**
SkDh	0.104	0.015	0.146	**
Dia	0.058	0.006	0.106	**
Average	0.172	0.015	0.088	**

ns — not significant; * — $P < 0.05$; ** — $P < 0.01$

Table 4. Unbiased genetic distance (NEI 1978) for all pair-wise comparisons among the investigated populations of *A. alba*

Population	1	2	3	4	5	6	7	8	9
1									
2	0.002								
3	0.001	0.004							
4	0.000	0.006	0.005						
5	0.014	0.015	0.009	0.013					
6	0.024	0.026	0.019	0.020	0.003				
7	0.019	0.016	0.010	0.020	0.003	0.003			
8	0.025	0.020	0.014	0.027	0.007	0.009	0.001		
9	0.022	0.015	0.011	0.027	0.010	0.023	0.008	0.004	
10	0.040	0.029	0.031	0.041	0.015	0.017	0.009	0.005	0.011

Table 5. Average unbiased genetic distance (NEI 1978) among the three main geographic regions of *A. alba* distribution in Italy

Region	No. of pops	North	Centre	South
North	4	0.003 (0.000–0.006)		
Centre	3	0.017 (0.009–0.026)	0.003 (0.003–0.003)	
South	3	0.025 (0.011–0.041)	0.011 (0.001–0.023)	0.007 (0.004–0.011)

The average unbiased genetic distance for all pair-wise comparisons between the ten *A. alba* populations was 0.014, with values ranging from 0.000 to 0.041 (Table 4). The average distances within each of the three main groups of populations (north, centre, and south) are presented in Table 5. The greatest distance was found in comparisons between the northern and southern groups of populations, while the central populations showed closer affinity to the southern group of populations. Distances separating southern populations were generally larger than distances among populations from the central and northern groups.

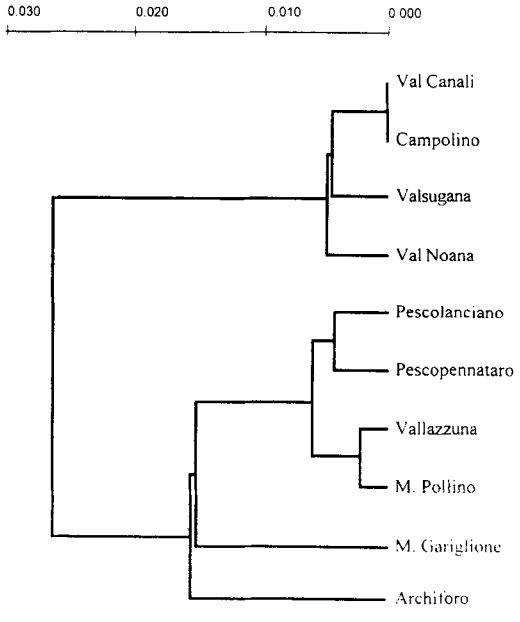

Fig. 3. UPGMA dendrogram based on an unbiased genetic distance (NEI 1978) for the investigated populations of *A. alba*.

The UPGMA dendrogram based on the unbiased genetic distances (NEI 1978), revealed a weak, but distinct, differentiation among the investigated *A. alba* populations (Fig. 3). The northern group of populations formed a separate and weakly differentiated cluster. The second, more heterogeneous cluster comprised populations from the central and from the southern parts of Italy.

Discussion

The relatively high polymorphism found for the *Lap*, *Pex-2*, *IDh*, *Got-3*, and *6PgDh-1* loci is consistent with the results obtained in other studies (BERGMANN and KOWNATZKI 1988; BREITENBACH-DORFER et al. 1992; HUSSENDÖRFER et al. 1995; KONNERT 1993; SCHROEDER 1989c). Some loci showed polymorphism exclusively in some parts of the Italian *A. alba* range. A similar result

was obtained by BERGMANN and KOWNATZKI (1988).

In general, our results revealed a lower level of genetic variability in *A. alba* than that observed by other authors in the same species (BREITENBACH-DORFER et al. 1992; FADY and CONKLE 1993; KORMUTAK et al. 1982; SCALTSOYIANNES et al. 1991). However, most of these earlier results were based on a few polymorphic loci, or on a limited number of populations and therefore are not well comparable with our present results. The level of allozyme polymorphism found in our study was in fact similar to that reported for a similar number of loci, in *A. alba*, by VICARIO et al. (1995), and in other conifers (e.g., CHELIAK 1988; LI and ADAMS 1989; SZMIDT and WANG 1993).

In contrast to other conifers, which usually display a slight excess of heterozygotes over panmictic expectations at the adult stage (PLESSAS and STRAUSS 1986; SZMIDT and MUONA 1985), all the populations investigated in the present study showed positive fixation indices indicating a deficiency of heterozygotes. In three populations, this deficiency was statistically significant. *A. alba* is considered to be a partially self-pollinating species (KIELLANDER 1962). In comparison with other conifers, the *A. alba* pollen is rather big and heavy and its sinking speed is approximately six times more than the sinking speed of *Picea abies* pollen (ROHMEDER and SCHÖNBACH 1959). These features of the taxon combined with discontinuous distribution of individual populations are likely to promote various forms of inbreeding which could be responsible for the heterozygote deficiency found in this study. An additional factor that could have contributed to this deficiency, could be Wahlund effect (WAHLUND 1928) caused by population subdivision due to restricted gene flow. In fact, analysis of the apportionment of gene diversity revealed statistically significant differentiation among the investigated populations at most loci. Surprisingly, the two available estimates of outcrossing rates in *A. alba* populations do not indicate an increased level of selfing (GIANNINI et al. 1994; SCHROEDER 1989d). Additional studies are therefore necessary in order to determine the exact causes of the deficiency of heterozygotes found in the present study.

Palynological studies suggest that *A. alba* has been present in Europe throughout the temperate stages of the Pleistocene (HUNTLEY and BIRKS 1983). The interglacial occurrences of *Abies* pollen indicate a much wider distribution of the genus than in the late Holocene. In the most southern part of the Apennines chain (Calabria region), *A. alba* was abundant already at about 37,000 BP (HUNTLEY and BIRKS 1983). After the last glaciation the species range was confined to three refugia: southern Balkans, southern Italy (Calabria region), and probably Iberia (HUNTLEY and BIRKS 1983). The post-glacial migration from these refugia was probably triggered by the early Holocene climatic warming. By 10,000 BP, *Abies* was present in the Alps in northern Italy, and it subsequently expanded steadily in this area. According to pollen analysis, *A. alba* spread into the central part of Italy from the Calabrian refugium mainly in the period of passage from the Boreal to the Atlanticum (7000-5000 BP). This expansion ended by 5000 BP; by that time *A. alba* was well established in the Apennines chain, in the Pyrenees, in the western and central Alps, and in the former Yugoslavia (KRAL 1989). Therefore, it is likely that the *A. alba* populations in central Italy had a dual postglacial origin. The postglacial species' expansion could have occurred from the north (Balkan refugium), as well as from the south (Calabrian refugium), and the two populations eventually met in the central Italy. This is in accordance with the relatively intermediate position of the central populations found in this study. The increased genetic diversity found in some of these populations may be the effect of blending of the two genetically different populations.

MAYER (1973) has suggested that *A. alba* spread into the Alpine regions mostly from the Calabrian refugium, because the dissemination of the taxon from the Balkans into the southeastern Alps was influenced by the presence of the Boreal-Atlantic barrier of *Fagus sylvatica* (beech). Therefore, the northward migration of *A. alba* from the Calabrian refugium was probably faster than that from the Balkans. This could explain the smaller genetic distance between the central and southern groups of populations relative to the northern populations found in the present study. It is also possible that the central populations have been solely derived from the southern group and become isolated from their ancestor as a result of human activities. In fact, in the last centuries, the forest area in Italy was strongly reduced by human activity and substituted by anthropogenous vegetation types (HUNTLEY and BIRKS 1983; KRAL 1989).

The most pronounced divergence among populations occupying individual regions was found in the southern group of populations. This finding

gives additional support to the previous results suggesting genetically distinct character and considerable differentiation of the *A. alba* occurring in the southern part of Italy (BERGMANN et al. 1990; BERGMANN and KOWNATZKI 1988; DUCCI 1994; KONNERT and BERGMANN 1995; LARSEN 1986; LARSEN and FRIEDERICH 1988; LARSEN et al. 1988; PENNACCHINI and DUCCI 1994; SVOBODA 1953; VICARIO et al. 1995; and others). Prolonged isolation of this region and restricted gene flow among individual populations are the likely causes of this situation.

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