Abies nebrodensis (Lojac.) Mattei, a relevant example of a relic and highly endangered species

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Abstract

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Allozyme, DNA markers, sequence data and monoterpenes have been used to clarify the genetic and taxonomic relationships between different populations of *Abies alba* Mill. and the only existing population of *Abies nebrodensis* (Lojac.) Mattei, a relict fir restricted to a single locality in Sicily. A high level of differentiation was found using all the different approaches. The genetic distance between *A. nebrodensis* and the group of *A. alba* populations was found to be much greater than that among *A. alba* populations located more than 1000 km apart. The results provide support for the considering *A. nebrodensis* and *A. alba* as distinct taxonomic entities.

Introduction

Abies nebrodensis (Lojac.) Mattei is one of the most endangered forest tree species in the world: only 29 adult trees grow naturally in the Madonie mountain range, in Sicily (Italy) (Morandini & al. 1994). The tiny range of distribution of this species today appears to be mainly a consequence of human activities.

The evolutionary history of *A. nebrodensis* is not clearly understood and its taxonomic status is still under investigation. Accordingly to Fenaroli & Giacomini (1958) and Morandini (1968), *Abies alba* Mill. gave rise to *A. nebrodensis*. However, its geographical isolation from *A. alba*, and its Mediterranean habitat (it grows naturally amidst *Crataegus laciniata*, *Cistus incanus*, *Genista cupanii*, *Fraxinus ornus* and *Quercus ilex*), seem to indicate an independent origin with respect to *A. alba*.

This paper reports the investigations performed in our laboratories in order to shed light on genetic variability and genetic affinities between *A. nebrodensis* and various Italian populations of *A. alba*. The two firs were compared and contrasted by using isozyme

markers, DNA markers, namely chloroplast and RAPD markers, as well and terpene markers.

Materials and methods

The plant material used in the tests (buds, needles and cortical tissues) originated from 14 trees of the natural population of *A. nebrodensis* and from about 30 trees of seven natural populations of *A. alba*. The following tests were performed for the estimation of genetic variability and differentiation parameters:

(a) *Isozyme analysis*. Eight enzyme systems (Glutamate dehydrogenase, Gdh, glutamate oxalacetate transaminase, Got, isocitrate dehydrogenase, Idh, malate dehydrogenase, Mdh, malic enzyme, Me, 6-phosphogluconate dehydrogenase, 6-Pgd, phosphoglucose isomerase, Pgi, shikimate dehydrogenase, Skd), encoded by 12 gene loci, were analysed by means of starch gel electrophoresis. Genetic variability (mean number of alleles per locus, n, mean effective number of alleles per locus, n_e, and observed (H_O) and expected (H_e) heterozygosity) and differentiation parameters (G_{st} and genetic distances as proposed by Nei 1972 and 1975) were estimated using the Biosys-1 program (Swofford & Selander 1981).

(b) *RAPD analysis*. 12 decamer oligonucleotide primers, selected, from among 60 decamers tested, because of their good amplification in terms of reproducibility and clearness of the amplification patterns, were used for the amplification of genomic DNA extracted from needles following a modification of the protocols proposed by Ziegenhagen & al. (1993). Details on the RAPD amplifications are reported in Vicario & al. (1995). RAPD data was used firstly for the estimation of genetic distances betweenpopulations following the approach reported by Yu & Pauls (1993) and then for assessing that fraction of variability residing within populations and the fraction among populations following the AMOVA approach proposed by Huff & al. (1993).

(c) *PCR/RFLP of chloroplast non coding regions*. Two pairs of 20-mer primers were used for the amplification of two chloroplast intergenic spacers between tRNA genes. The amplification products were then cut with 11 restriction endonucleases. Details on amplification and restriction procedures are given in Vicario & al. (1995). The amplified intergenic spacer between the trnL and trnF genes of *Abies alba*, *A. nebrodensis* and one American fir species, *A. magnifica*, was then sequenced, using an ALF automated sequencer (Pharmacia). Alignment of the sequences was performed using MACAW software (Schuler & al. 1991).

(d) *Monoterpene composition*. Seven monoterpenes (α-pinene, canfene, β-pinene, sabinene, myrcene, limonene, cineole) were detected in cortical tissue by means of headspace gas chromatography. Variance and discriminant analysis were performed on the arcsin transformed monoterpene percentages.

Results and discussion

A. nebrodensis can be easily distinguished from *A. alba* by isozyme analysis, DNA markers, sequence data and monoterpenes. Genetic distance values estimated by using Nei's equation, G_{st}, AMOVA analysis, all indicated a high level of genetic differentiation

between the two groups of populations, much higher than those observed among the various silver fir populations.

A. nebrodensis population showed a high value of heterozygosity estimated using isozyme data (Fig. 1), higher than expected considering its tiny distribution and population size. Such genetic variation can be considered an efficient strategy of survival for this population.

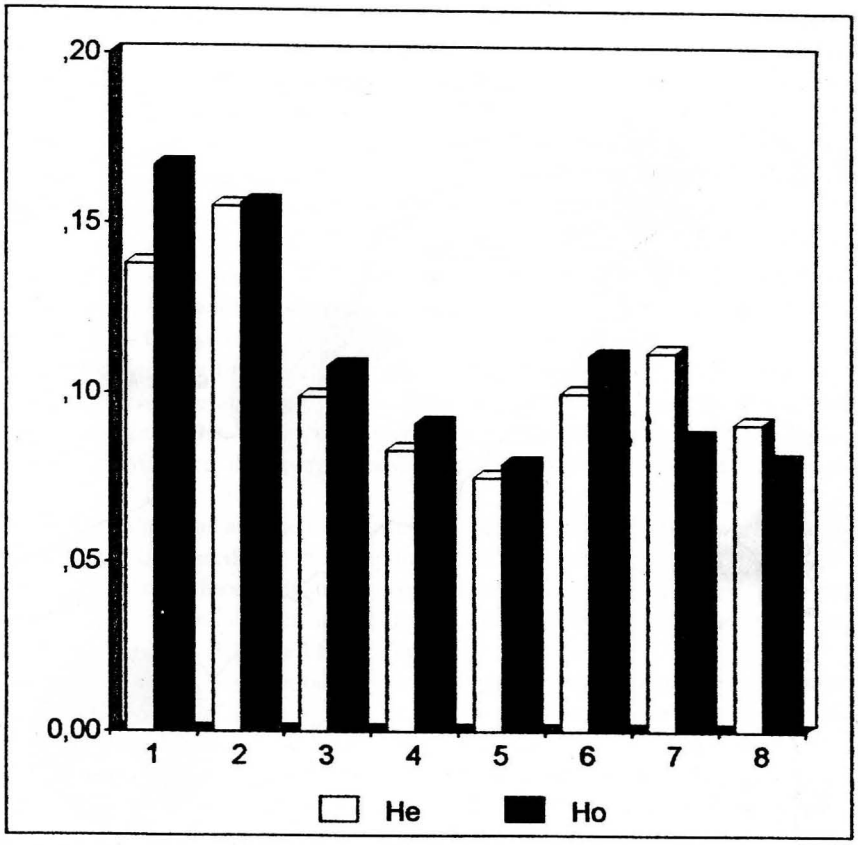

Fig. 1. Mean expected (H_e) and observed (H_O) heterozygosity in the 8 sampled fir populations. Population numbers: **1**, *A. nebrodensis* Madonie population; **2-8**, Italian *A. alba* Populations: **2**, Aspromonte; **3**, Serra San Bruno; **4**, Gariglione; **5**, Abeti Soprani; **6**, Collemeluccio; **7**, Abetone; **8**, Lavarone.

Sequence data of the non-coding chloroplast DNA regions confirmed the presence of a high genetic divergence among the two species, as already evidenced by isozyme and RAPD data (Vicario & al., 1995).

In comparison to *A. alba* populations, *A. nebrodensis* possesses a characteristic monoterpene pattern (Fig. 2). Quantitative differences in α-pinene, limonene, β-pinene and β–myrcene appear between silver fir populations and/or population groups.

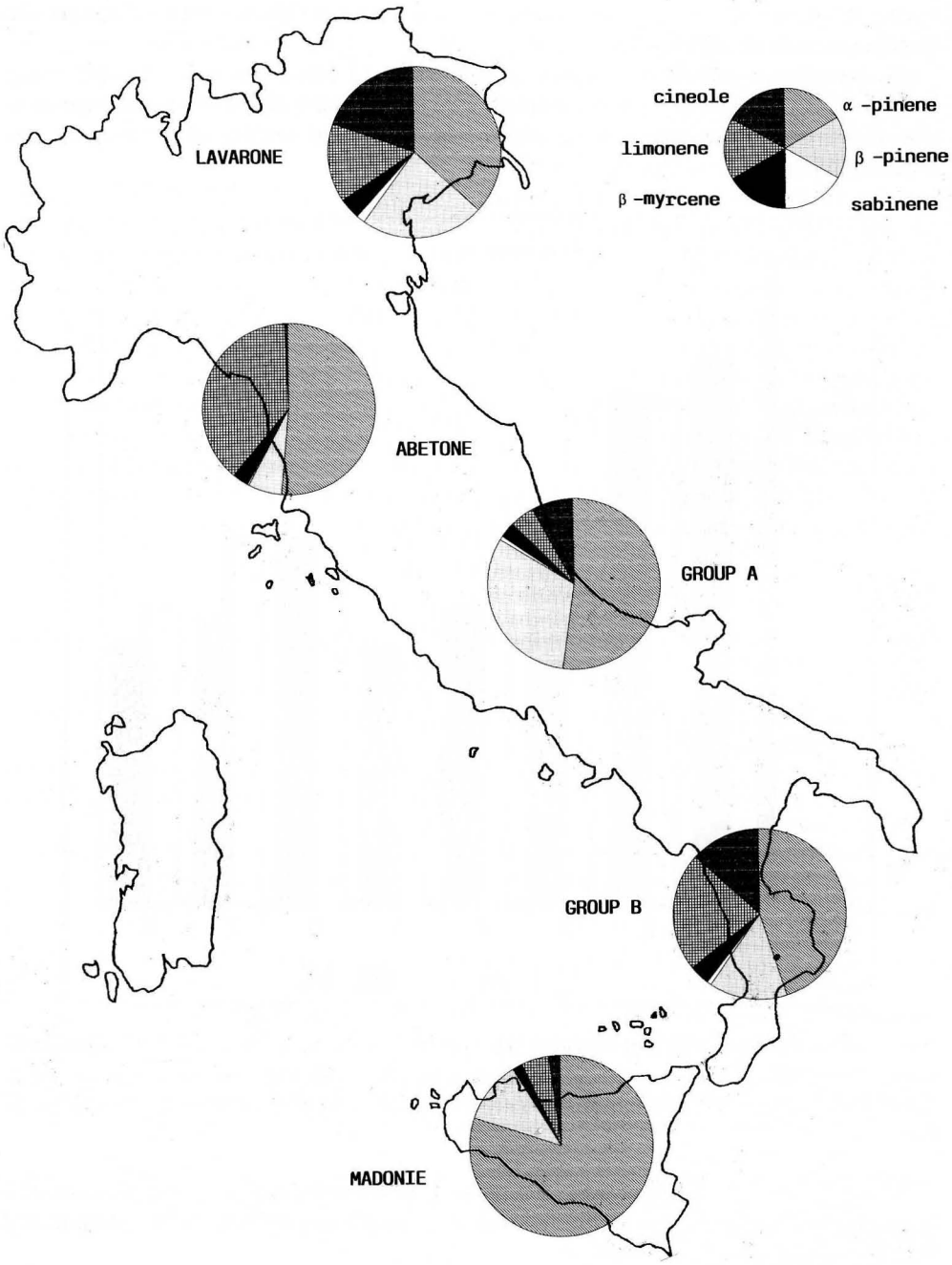

Fig. 2. Monoterpene composition in the eight populations tested.

On the basis of uni- and multivariate analyses Abeti Soprani and Collemeluccio populations, which showed the same terpene profile, can be grouped together (group A), while Aspromonte, Gariglione and Serra San Bruno populations belong to the same group of provenances (group B). These findings are in substantial agreement with monoterpene data on geographic variability of *A. alba* obtained by Lang (1994) and with the results based on allozyme and DNA markers (Vicario & al. 1995).

In conclusion, *A. nebrodensis* is clearly differentiated from *A. alba* populations on the basis of DNA, isozyme and terpene markers. All the different approaches used to clarify relationships between *A. nebrodensis* and *A. alba* validate their classification as two distinct taxonomic entities. The information obtained on *A. nebrodensis*, using the different kinds of markers may be very usefull for developing appropriate strategies of gene conservation of this highly endangered species, following both *in situ* and *ex situ* approaches. The most efficient strategy of *in situ* conservation, in addition to the recent institution of a natural reserve on the Madonie mountain, could be the creation of reproduction communities around the single and isolated existing trees. The *ex situ* conservation, namely the creation of artificial stands outside the distributional area of this species and the storage of material (as suggested by Mazzola & al. 1993), might further contribute to the preservation of the genetic resources of *A. nebrodensis*.

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