ALLOZYME VARIATION IN FIVE POPULATIONS OF *PINUS HALEPENSIS* MILL. IN SOUTHERN ITALY

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ABSTRACT

This note reports the results of a study on the genetic structure of five natural *Pinus halepensis* Mill. populations from Southern Italy (two from the Gargano peninsula in Apulia, two from the Apulian Ionian coast and one from Calabria). Isozyme analyses were performed by means of horizontal starch gel electrophoresis on haploid megagametophytes and embryos of 12 seeds per mother tree, and about 10 trees per population, estimating only frequencies of the alleles of pollinic origin, in order to obtain a more representative sample. Seven enzyme systems, coded by 13 loci, were utilized: LAP, GOT, PGM, 6PGDH, MDH, GDH, SKDH; only six loci were polymorphic. The obtained results highlight the rather low genetic variation of this species (H_e is 0.119), yet higher than values reported in former researches, and the evident differentiation of the Calabrian population from the four Apulian populations, as well as the relative trend toward differentiation of the two populations from Gargano. However, the overall genetic differentiation is low (G_{st} = 3.1%; δ = 4.8%; Nei's mean genetic distance = 0.014; Gregorius' mean genetic distance = 0.057). The Calabrian population also shows the lowest genetic variation, which could result from the crossing of genetic bottlenecks, maybe in the course of a hypothetical migration from the Apulian Ionian coast. The Apulian populations, generally considered autochthonous, could be originated from seeds introduced by the ancient Greek colonizers of Southern Italy (*Magna Grecia*), as the available archaeobotanical data seem to suggest.

Key words: *Pinus halepensis*, isozymes, genetic diversity, genetic differentiation, Southern Italy.

INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) is a circum-Mediterranean species characterized by high tolerance to drought and adverse pedological conditions, hence it is particularly suitable to reforestation of arid zones. Moreover, the high adaptation of this species to Mediterranean bioclimates is confirmed by some features of its reproductive cycle – in particular the way of development of the female gametophyte (FRANCINI 1958) – as well as by the production of serotinous cones which enable the settlement of abundant natural regeneration after fire passage (SARACINO & LEONE 1993a, 1993b; SARACINO *et al.* 1997).

In Italy, the surface covered by Aleppo pine high forests stretches over about 20,000 hectares, half of which are in Apulia (especially in the Gargano peninsula and the Ionian coast) and the remnant in southern and western Sicily, in southern Sardinia, in Campania (Cilento), in Umbria (Valle Spoletina and Val Nerina), in a stretch of the Tuscan coast and along the Ligurian coast (MAGINI 1955; BERNETTI 1995). The aim of this research work was the study of the genetic structure of some typical Aleppo pine populations from southern Italy, estimated by means of isozymes.

MATERIALS AND METHODS

Five populations – reputed to be of natural origin – were considered (Figure 1), four Apulian (two from Gargano and two from the Ionian coast) and one Calabrian (Ionian coast), all of them originated from natural regeneration following wildfires. Sampling was carried out in 1995 and cones from 10 trees per population – nine in population 4, because all seeds from the tenth tree were non-vital – were collected, located at a minimum distance of 100 meters and uniformly placed on the selected area.

Isozyme analyses were performed on haploid megagametophytes and embryos of 12 seeds per mother tree (120 seeds per population, 108 only for the population 4), by means of horizontal starch gel electrophoresis, in order to distinguish between male and female gamete in each embryo. Seven enzyme systems, coded by 13 loci, were utilized: LAP (leucine aminopeptidase, E.C. 3.4.11.1), GOT (glutamate oxaloacetate transaminase, E.C. 2.6.1.1), PGM (phosphoglucomutase, E.C. 2.7.5.1), 6PGDH (6-phosphogluconate dehydro

Figure 1. Location of the studied populations. 1 – Coppa della Nuvola (Peschici, Foggia – Gargano); 2 – Monte Barone (Mattinata, Foggia – Gargano); 3 – Perronello (Riva dei Tessali, Taranto); 4 – Pineta della regina (Marina di Ginosa, Taranto); 5 – Albidona (Cosenza).

genase, E.C. 1.1.1.44), MDH (malate dehydrogenase, E.C. 1.1.1.37), GDH (glutamate dehydrogenase, E.C. 1.4.1.2), SKDH (shikimate dehydrogenase, E.C. 1.1.1.25). Endosperms and embryos were separately homogenized in a buffer 0.08 M tris – 1.00 M HCl, pH 7.2 (MÜLLER-STARCK, pers. comm.). Electrophoresis was performed using the following buffer systems: A) electrode buffer: 0.06 M NaOH – 0.30 M boric acid, pH 8.2; gel buffer: 0.08 M tris – 1.00 M HCl, pH 8.7 (POULIK 1957, modified), for LAP and GOT; B) electrode buffer: 0.135 M tris – 0.047 M citric acid, pH 7.0; gel buffer: 0.034 M tris – 0.012 M citric acid, pH 7.0 (SHAW & PRASAD 1970, modified), for the remaining enzyme systems. Starch gel concentration was 11% for buffer system A and 12% for B. Staining was performed according to MÜLLER-STARCK (1998). The genetic control of the utilized enzyme systems was determined by comparing the endosperm segregation ratio of putative heterozygote mother trees with the expected 1:1 Mendelian ratio (PUGLISI *et al.*, in preparation). For each enzyme system, loci were designated by capital letters following the enzyme acronym, and the most anodal zone of activity was marked by the first letter. Within each locus, alleles were designated by numbers, starting from the fastest one.

Computations were performed with BIOSYS-1 computer program (SWOFFORD & SELANDER 1989). Only pollen allele frequencies were estimated, in order to obtain a more representative sample than the one constituted by embryo genotypes, as the number of the surveyed mother trees was small. The contingency table χ^2-test (SNEDECOR & COCHRAN 1967) was used in order to assess the significance of differences between populations. The following parameters of genetic diversity (variation within populations) were computed: average number of alleles per locus (N); percentage of polymorphic loci (P) computed on the basis of 5% criterion, i.e. the percentage of loci whose more common allele has a frequency lower than 95%; expected heterozygosity according to Hardy-Weinberg (H_e; NEI 1975); genetic diversity (v; GREGORIUS 1978; MÜLLER-STARCK & GREGORIUS 1986), whose average value per population is computed as the harmonic mean of single locus values. On the basis of pollen allele frequencies also the following parameters of genetic differentiation (variation between populations) were computed: (a) genetic diversity analysis (NEI 1973), which shows the distribution of genetic diversity: H_t (total diversity), H_s (diversity within populations), D_{st} (diversity among populations, given as the difference between the two former parameters) and G_{st} (relative degree of genetic differentiation, given as the ratio D_{st}/H_t); G_{st} values were computed for each polymorphic locus and then averaged over all loci; (b) subpopulation differentiation (d; GREGORIUS & ROBERDS 1986), which represents GREGORIUS' (1974) genetic distance between each population and the remaining ones, considered as a whole, and is regarded as more sensitive than G_{st}; (c) genetic distance, computed by means of NEI's (1972) and GREGORIUS' (1974) formulae. Values of NEI's genetic distance were used for constructing a dendrogram using the UPGMA method (SNEATH & SOKAL 1973).

RESULTS AND DISCUSSION

Only six out of the 13 loci are polymorphic (Table 1). Table 2 shows the significant χ^2- values of heterogeneity between allelic frequency distributions in the five populations, calculated for the polymorphic loci after grouping the classes whose expected absolute frequencies were less than 4.

Genetic diversity parameters (Table 3), together with the high number of monomorphic loci, confirm the results of former researches (SCHILLER *et al.* 1986; GRUNWALD *et al.* 1986; TEISSEIRE *et al.* 1995; AGÚNDEZ *et al.* 1997): genetic variation in this species is lower than variation estimated in most studied conifers, although the values obtained in the present research work are higher than those obtained in the above-mentioned publications; nevertheless, LOUKAS *et al.*

Table 1. Allele frequencies at the 13 analyzed enzyme loci.

Locus	Allele	Population				
		1	2	3	4	5
Lap–A	1	.583	.567	.558	.546	.958
	2	.417	.433	.442	.454	.042
Got–A	1	1.000	1.000	1.000	1.000	1.000
Got–B	1	1.000	1.000	1.000	1.000	1.000
Got–C	1	1.000	1.000	1.000	1.000	1.000
Pgm–A	1	1.000	1.000	1.000	1.000	1.000
6pgdh–A	1	.900	.975	1.000	1.000	1.000
	2	.100	.025	.000	.000	.000
6pgdh–B	1	.000	.000	.000	.009	.000
	2	.825	.925	.683	.731	.533
	3	.175	.075	.317	.259	.467
Mdh–A	1	.000	.000	.058	.028	.017
	2	1.000	1.000	.942	.972	.983
Mdh–B	1	1.000	1.000	1.000	1.000	1.000
Mdh–C	1	1.000	1.000	1.000	1.000	1.000
Mdh–D	1	.558	.325	.400	.361	.575
	2	.442	.675	.600	.639	.425
Gdh–A	1	1.000	1.000	1.000	1.000	1.000
Skdh–A	1	.192	.025	.000	.009	.008
	2	.000	.000	.025	.083	.067
	3	.717	.825	.975	.898	.925
	4	.092	.150	.000	.009	.000

Table 2. Heterogeneity χ^2 values computed on allelic frequence distributions of the studied populations (d. f. – degrees of freedom; the asterisks represent the significance level: three asterisks correspond to $p < 0.005$).

Locus	χ^2	d.f.	Significance level
Lap–A	65.156	4	***
6pgdh–B	54.507	4	***
Mdh–D	25.503	4	***
Skdh–A	134.890	12	***
Total	280.056	24	***

(1983), KOROL *et al.* (1995) and KOROL & SCHILLER (1996) found values similar or higher than ours in Greek, Israeli and Jordanian populations. Mean H_e is 0.119, while the average value of H_e within populations

Table 3. Parameters of genetic diversity. N – mean number of alleles per locus; P – percentage of polymorphic loci at the 5% criterion; H_e – expected heterozygosity according to Hardy-Weinberg; v – genetic diversity.

Population	N	P	H_e	v
1	1.5	38.5	0.145	1.170
2	1.5	30.8	0.109	1.122
3	1.4	30.8	0.120	1.137
4	1.6	30.8	0.123	1.140
5	1.5	23.1	0.095	1.105
Mean	1.5	30.8	0.119	1.134

(H_{ep}) estimated for gymnosperms by HAMRICK *et al.* (1992) is 0.151. Population 1 (Gargano) is characterized by the highest diversity values, while the lowest values are found in population 5 (Calabria). Also in SCHILLER *et al.* (1986) a population from Gargano was characterized by the highest H_e value found in the whole range of this species.

The parameters listed in Table 4 show the distribution of genetic diversity: the average G_{st} value is 3.1%, similar to many other wide-ranged conifers, thus showing that the overwhelming majority of the measured genetic diversity is within populations. For some loci the proportion of diversity due to population differentiation is higher (10.9% for the locus *Lap–A*, 9% for the locus *6Pgdh–B*). Table 5 shows values of the parameter d (subpopulation differentiation): the mean value over all loci and populations is 4.8%, just a little higher than G_{st} average. "Differentiation snails" (Figure 2) evidently show the loci better enabling to distinguish between populations: *Lap–A*, *Skdh–A*, *6Pgdh–B* and *Mdh–D*.

NEI's and GREGORIUS' genetic distance average values are rather low (0.014 and 0.057). Yet, both measures (Table 6), together with the dendrogram constructed on the basis of Nei's genetic distance values (Figure 3), efficaciously show the sharp differentiation of the Calabrian population (no. 5) from the four Apulian ones and the trend toward differentiation of the two populations from the Gargano peninsula (no. 1 and 2).

The Calabrian population stands out also for its lowest genetic variation (Table 3). Since this population is accepted as spontaneous, it can be hypothesized that its differentiation could be the result of the crossing, occurred in the past, of *genetic bottlenecks* which considerably reduced variation, probably because of wildfires occurred in immature stands, before seed production age (6–7 years). This could have taken place in the course of a hypothetical migration and coloniza-

Table 4. Genetic diversity analysis.

Locus	H_t	H_s	H_{st}	D_{st}
Lap–A	0.459	0.409	0.050	0.109
Got–A	0.000	0.000	0.000	–
Got–B	0.000	0.000	0.000	–
Got–C	0.000	0.000	0.000	–
Pgm–A	0.000	0.000	0.000	–
6pgdh–A	0.049	0.046	0.003	0.062
6pgdh–B	0.386	0.351	0.035	0.091
Mdh–A	0.040	0.039	0.001	0.023
Mdh–B	0.000	0.000	0.000	–
Mdh–C	0.000	0.000	0.000	–
Mdh–D	0.494	0.472	0.021	0.043
Gdh–A	0.000	0.000	0.000	–
Skdh–A	0.241	0.222	0.018	0.076
Mean	0.128	0.118	0.010	0.031

tion of the Calabrian Ionian belt from the Apulian coast, since Aleppo pine is generally considered autochthonous in Apulia but not in Calabria (FRANCINI 1953; BERNETTI 1995).

Nevertheless, the available archaeobotanical data would dismiss the hypothesis of a natural origin of Apulian (FOLLIERI 1968; FIORENTINO 1995a, 1995b, 1995c, 1995d, 1998a, 1998b, 1999a, 1999b and pers. comm.; FIORENTINO & RADINA 1998) and Calabrian populations (VALLINO & VENTURA 1984). PIGNATTI (1979) hypothesized the anthropic origin of the present Apulian populations.

As a consequence, also the anthropic origin of the Calabrian population cannot be excluded, or the above-mentioned migration could have occurred in a more recent period. The high genetic variation of the Apulian populations could have originated from Greek provenance of the propagation material introduced by Greek colonizers present in southern Italy in historical times (*Magna Grecia*), since Greek and Apulian populations show the highest variation values (LOUKAS *et al.* 1983; SCHILLER *et al.* 1986; MORGANTE & VENDRAMIN, pers. comm.). According to SCHILLER & BRUNORI (1992) and SCHILLER & MENDEL (1995), Aleppo pine diffusion in its present natural range could have been quite affected by human activity.

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Table 5. Genetic differentiation between populations (δ values).

Locus	Population 1	2	3	4	5	δ
	D_{i1}	D_{i2}	D_{i3}	D_{i4}	D_{i5}	
Lap–A	0.077	0.097	0.109	0.121	0.394	0.160
Got–A	0.000	0.000	0.000	0.000	0.000	0.000
Got–B	0.000	0.000	0.000	0.000	0.000	0.000
Got–C	0.000	0.000	0.000	0.000	0.000	0.000
Pgm–A	0.000	0.000	0.000	0.000	0.000	0.000
6pgdh–A	0.094	0.001	0.0.32	0.031	0.032	0.038
6pgdh–B	0.107	0.233	0.073	0.010	0.262	0.140
Mdh–A	0.026	0.026	0.047	0.009	0.004	0.023
Mdh–B	0.000	0.000	0.000	0.000	0.000	0.000
Mdh–C	0.000	0.000	0.000	0.000	0.000	0.000
Mdh–D	0.141	0.151	0.057	0.104	0.063	0.124
Gdh–A	0.000	0.000	0.000	0.000	0.000	0.000
Skdh–A	0.232	0.124	0.135	0.098	0.114	0.142
Gene pool	0.052	0.049	0.035	0.029	0.075	0.048

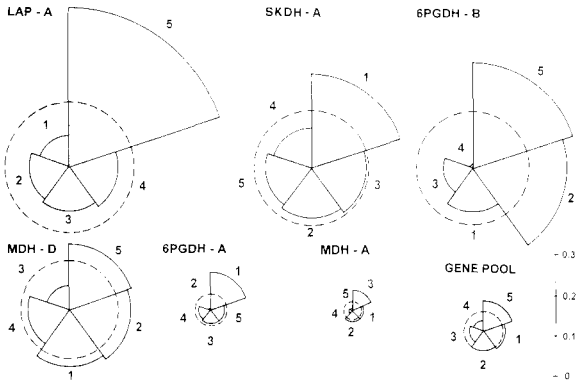

Figure 2. Graphic representation ("differentiation snails") of the values listed in Table 5. Lengths of radii of the dotted circles correspond to the total differentiation level (δ), and the length of each sector radius corresponds to the differentiation value of one population.

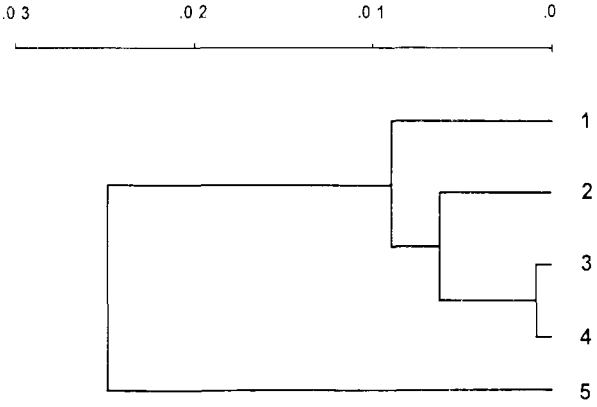

Figure 3. Dendrogram of Nei's genetic distance obtained with UPGMA method.

Table 6. Genetic distance calculated following Gregorius (above the diagonal) and Nei (below the diagonal).

Population	1	2	3	4	5
1 – Coppa della nuvola	–	.045	.059	.055	.083
2 – Monte Barone	.008	–	.045	.035	.096
3 – Perronello	.010	.008	–	.017	.063
4 – Pineta della regina	.009	.005	.001	–	.067
5 – Albidona	.024	.034	.019	.022	–

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