ALLOZYME VARIATION IN NATURAL POPULATIONS OF THE ITALIAN RANGE OF *PINUS SYLVESTRIS* L.

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ABSTRACT

The genetic structure of eight populations of Scots pine (*Pinus sylvestris* L.) – seven Alpine and one Apennine, representative of the Italian range of this species – has been studied by means of electrophoretic analysis of six enzyme systems (LAP, GOT, PGM, MDH, GDH, SKDH), for a total of 12 allozyme loci. On the whole, genetic diversity parameters show rather high values; the average value of expected heterozygosity according to Hardy-Weinberg (0.283) is higher than the average value estimated for gymnosperms. Differentiation between populations is low, as revealed by parameters G_{ST} (2.6 %) and δ (4.7 %), as well as by the mean values of genetic distance by Nei and Gregorius (0.013 and 0.075, respectively), as observed in most conifers. Nevertheless, the obtained results reveal a sharp differentiation of the Apennine population. This is one of the few relict indigenous populations of this species in the Ligurian-Emilian Apennine, remnants from glacial migrations. Because of its peculiarity, this relict population, whose intrapopulation genetic variation is almost as high as in the Alpine populations, is an important genetic resource that requires specific protection measures.

Key words: *Pinus sylvestris*, relict populations, allozymes, genetic structure, genetic differentiation, genetic resources.

INTRODUCTION

Scots pine (*Pinus sylvestris* L.) occupies a larger area than any other species from the whole Pinaceae family (MIROV 1967; BORATYŃSKI 1991), extending from Europe to the Far East (Manchuria) through Siberia.

Because of such a wide geographic spreading, with very different environmental conditions, and because of the long evolutionary history of this pine, a large intraspecific variation is expected to occur. Scots pine must have developed in East Asia and then spread towards Europe, where it has been present since the Tertiary Period (MOLOTKOV & PATLAJ 1991). During the glacial and interglacial periods of the Pleistocene, its natural range underwent repeated modifications from which numerous new populations arose resulting in high differentiation and increased variability, so that it has been very difficult to create an intraspecific taxonomic system (MOLOTKOV & PATLAJ 1991).

Since this species is important to Central-European and Scandinavian silviculture, intense research activity on population genetic structure has been carried out with biochemical and, more recently, molecular genetic markers (KARVONEN & SAVOLAINEN 1993; SZMIDT & WANG 1993; SZMIDT *et al.* 1996; PROVAN *et al.* 1998; SINCLAIR *et al.* 1999). Knowledge on the European sector of Scots pine natural range is however incomplete, because Italian populations representative of the whole Italian range have never been studied from this point of view until now.

In Italy, Scots pine is present in the Alps and Northern Apennines. The Apennine range consists of scattered and relict populations which bear witness to the climatic events of the inter- and postglacial periods (CHIARUGI 1950; GIACOMINI 1958; JEDLOWSKI & MINERBI 1967; AGOSTINI 1972; WATSON 1996) and make the study of Italian populations particularly interesting.

The aim of this research work was the study of the genetic structure of eight natural Scots pine populations representative of the Italian range of this species.

MATERIALS AND METHODS

Sampled populations

Seven populations from the Alps and one from the Emilian Apennines were considered (Figure 1; Table 1). At the time of sampling, there were no significant pollution or pathogen damages. All the sampled populations are reputed to be native. Populations CAS, CER, FEN and BRE are also official seed collection areas (MORANDINI & MAGINI 1975).

Sampling was carried out in 1988 and 1989, cones from a minimum of 20 trees per population were collected, except for populations MGX and MBN

Table 1. Geographic origin of the investigated populations.

Locations	Code	Number of sampled trees	Latitude (N)	Longitude (E)	Altitude (m)	Region
Casina	CAS	20	44° 30′	10° 27′	450	Emilia Romagna
Ceriana	CER	20	43° 55′	07° 46′	1000	Liguria
Fenestrelle	FEN	20	45° 02′	07° 04′	1300–1750	Piemonte
Morgex	MGX	59	45° 46′	07° 00′	1000–1400	Valle d Aosta
Morbegno	MBN	75	46° 10′	09° 36′	250–1200	Lombardia
Bressanone	BRE	21	46° 45′	11° 39′	650–800	Trentino – Alto Adige
Borca di Cadore	BCD	20	46° 26′	12° 15′	1200	Veneto
Malborghetto	MAL	21	46° 28′	13° 27′	700–1000	Friuli–Venezia Giulia

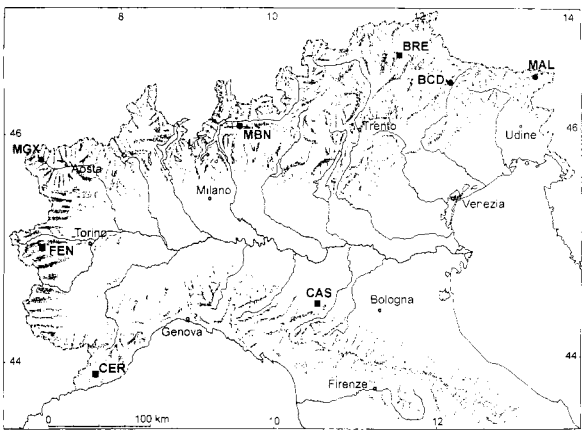

Figure 1. Location of the studied populations. Squares refer to the official seed collection areas, circles to the remaining populations.

which were sampled at different altitudes within three subpopulations (Table 1), in order to estimate the differentiation between subpopulations and clinal variations of allelic frequencies and genetic parameters, if any (PUGLISI *et al.*, 1999). Trees were located at a minimum distance of 50–100 meters and uniformly distributed on the selected areas. Seeds were dried and stored at 4–5 °C before being analyzed.

Isozyme analysis

Isozyme analyses were performed by means of horizontal starch gel electrophoresis on embryos of 12 seeds per mother tree.

Six enzyme systems, coded by 12 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), 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).

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 the former buffer system and 12 % for the latter. Staining was performed according to MÜLLER-STARCK (1998).

The genetic control of the utilized enzyme systems was previously determined by MÜLLER-STARCK (1982a, 1982b, and pers. comm.; Figure 2).

For each enzyme system, loci were designated by capital letters following the enzyme acronym, marking the most anodal zone of activity by the first letter. Within each locus, alleles were designated by numbers, starting from the fastest one.

Genetic parameters

Computations were performed with BIOSYS-1 software (SWOFFORD & SELANDER 1989) on embryo genotypes. The contingency table chi-square test (SNEDECOR & COCHRAN 1967) was used in order to estimate the heterogeneity between population distributions of allelic frequencies.

On the basis of the estimated allele frequencies, 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 most common allele has a frequency lower than 95 %; genetic diversity (υ; GREGORIUS 1978; MÜLLER-STARCK & GREGORIUS 1986), also called effective number of alleles (CROW & KIMURA 1970), whose average value per population is

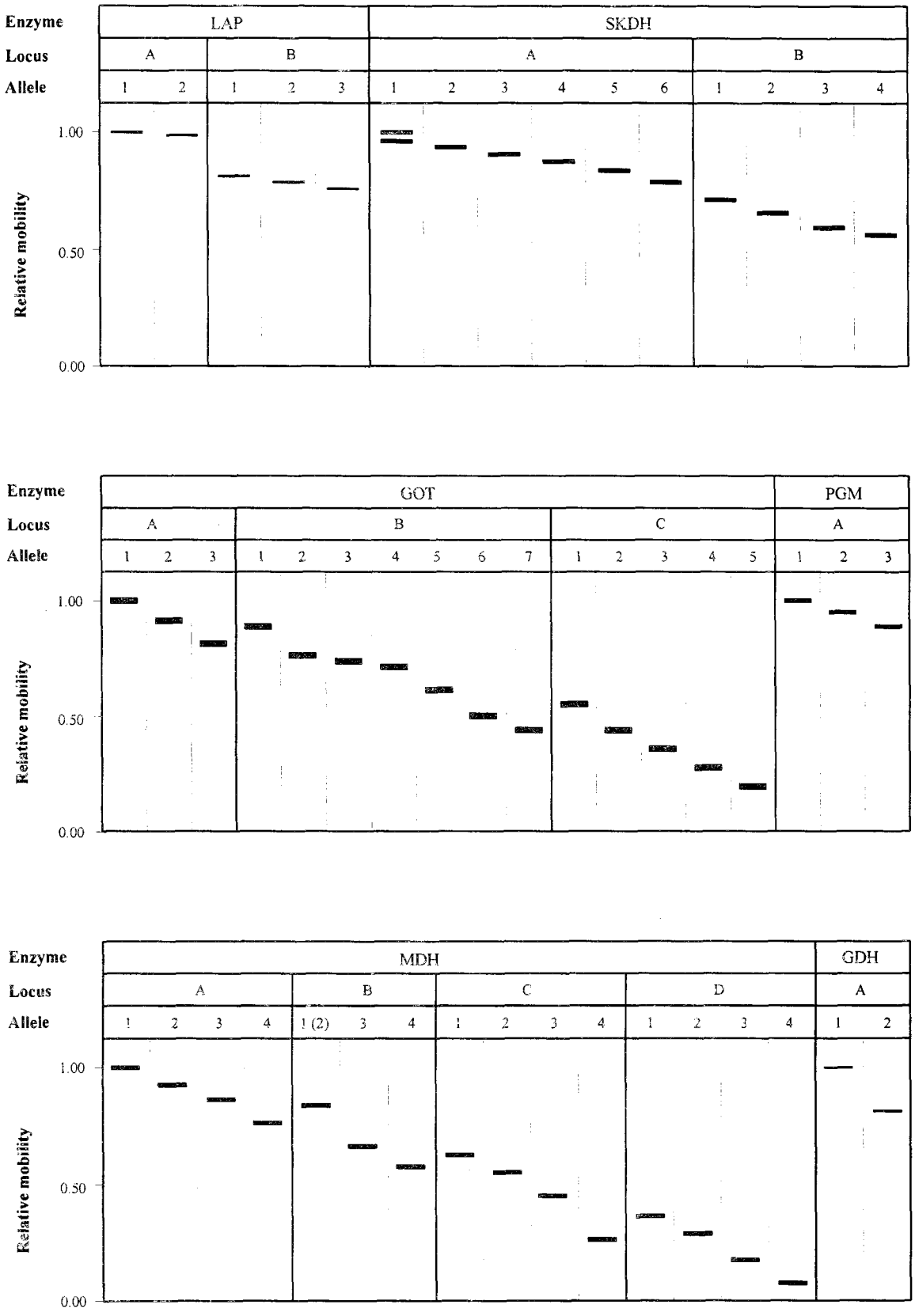

Figure 2. Banding patterns of isozymes from 12 enzyme loci in *Pinus sylvestris* L., according to the genetic interpretation by MÜLLER-STARCK (pers. comm.), with modifications. LAP, PGM and SKDH have a monomeric structure; GOT and MDH have a dimeric structure (MDH presents also inter-locus hybrid bands); GDH has a tetrameric structure.

computed as the harmonic mean of single locus values; expected heterozygosity according to Hardy-Weinberg (H_e; NEI 1978); observed heterozygosity (H_o). Wright's fixation index (F; WRIGHT 1922) was computed in order to compare observed heterozygosities with panmictic expectations.

On the basis of allele frequencies also the following parameters of genetic differentiation (variation between populations) were computed:

- genetic diversity analysis (NEI 1973), which show the distribution of genetic diversity: H_T (total diversity), H_s (diversity within populations), D_{ST} (diversity among populations) and G_{ST} (relative degree of genetic differentiation);
- subpopulation differentiation (δ; GREGORIUS & ROBERDS 1986), which represents Gregorius' genetic distance between each population and the remaining ones, considered as a whole, and is regarded as more sensitive than G_{ST};
- genetic distance, computed by means of NEI's (1978) 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 the loci *Got-A* and *Mdh-B* can be considered to be monomorphic in all populations on the basis of the 5% criterion (Table 2). Several rare alleles are present; two of them were found only in one population ($Mdh\text{-}A_2$ in population MAL and $Mdh\text{-}A_4$ in population MGX).

The Apennine population (CAS) is clearly different at loci *Gdh-A* and *Mdh-D*. At the former locus the more common allele is $Gdh\text{-}A_1$, instead of $Gdh\text{-}A_2$ as in the remaining populations; moreover, a marked frequency difference can be observed, while in the other populations differences between the two allele frequencies are lower. At the locus *Mdh-D* the frequencies of the two more common alleles (no. 2 and no. 4) are almost balanced, while in the other populations $Mdh\text{-}D_4$ is prevailing.

It is also worth noting that some other alleles show peculiar frequencies in population CAS, for instance at loci *Lap-B* (alleles 1 and 3 present low frequencies); *Got-B* (allele 4 frequency is higher than elsewhere) and *Got-C* (frequencies of alleles 2 and 3 are very different from the remnant); *Pgm-A* (lower frequency of allele 3); *Skdh-A* (higher frequency of allele 2); and, especially, *Mdh-A*, where the more common allele (no. 3) is fixed while the remaining populations have other low-frequency alleles.

Table 3 shows the significant chi-square values of heterogeneity between allelic frequency distributions in all populations, calculated for the polymorphic loci after grouping the alleles whose expected absolute frequencies were less than 4. The allele frequencies of 10 loci out of 12 are significantly heterogeneous, so lending statistical importance to some of the above-mentioned differences.

Average values of the expected (H_e) and observed heterozygosity (H_o) are high (0.283 and 0.242, respectively; Table 4); as a matter of fact, the former is higher than the average value of expected heterozygosity within populations estimated for gymnosperms (0.151; HAMRICK *et al.* 1992). The other estimated parameters show rather high values too, including genetic diversity (υ) whose values are all higher than 1, which is the minimum value (Table 4). Our results reveal that the analyzed populations are as variable as the Scots pine natural populations studied by other authors in several European and Asiatic countries (MEJNARTOWICZ 1979; GULLBERG *et al.* 1982; MEJNARTOWICZ & BERGMANN 1985; KINLOCH *et al.* 1986; MUONA & HARJU 1989; SAVOLAINEN & YAZDANI 1991; WANG *et al.* 1991; GONCHARENKO *et al.* 1994; NEET-SARQUEDA 1994; PRUS-GŁOWACKI & BERNARD 1994; PRUS-GŁOWACKI & STEPHAN 1994; ZHELEV *et al.* 1994; SAVOLAINEN & HEDRICK 1995; SZMIDT *et al.* 1996). Populations BRE and MGX show the highest values of the estimated parameters. The lowest values belong to the Apennine population (CAS), but, despite its isolation, they are equally high since there are no strong differences between populations. GIANNINI *et al.* (1991) and MORGANTE & VENDRAMIN (1991) observed the same phenomenon in an Apennine relict and isolated population of Norway spruce (*Picea abies* (L.) Karst).

Values of fixation index (Table 4) are positive in all the studied populations, showing a deficiency of heterozygotes relative to expected frequencies in panmictic equilibrium. The small and isolated Apennine population (CAS) presents the highest value (0.207), but the Alpine population FEN shows a similar value and other ones are characterized by slightly lower values. As observed in many other conifers, which – contrary to angiosperms – lack any prezygotic incompatibility mechanisms (MÜLLER-STARCK & GREGORIUS 1988), Scots pine is a partially self-pollinating species (MÜLLER-STARCK 1977, 1979, 1982a, 1982b; YAZDANI *et al.* 1985; RUDIN *et al.* 1986; KÄRKKÄINEN & SAVOLAINEN 1993; KÄRKKÄINEN *et al.* 1996). Plants originating from self-pollinated seeds are usually characterized by reduced viability (inbreeding depression), due to lethal and semi-lethal alleles, and are consequently subjected to natural selection (MÜLLER-STARCK 1982b; YAZDANI *et al.* 1985; LUNDKVIST *et al.* 1987; MUONA *et al.* 1987; KÄRKKÄINEN & SAVOLAINEN 1993; SAVOLAINEN & HEDRICK 1995; KÄRKKÄINEN

Table 2. Allele frequencies at the 12 analyzed enzyme loci.

Locus	Allele	CAS	CER	FEN	MGX	MBN	BRE	BCD	MAL
Lap–B	1	.004	.013	.051	.033	.016	.020	.004	.013
	2	.991	.968	.902	.845	.959	.904	.936	.901
	3	.004	.019	.047	.122	.025	.075	.060	.086
Got–A	1		.006		.006				
	2	.998	.994	.996	.994	.999	.988	.998	.996
	3	.002		.004		.001	.012	.002	.004
Got–B	1	.006		.004	.001	.027	.004	.004	.004
	2	.105	.186	.064	.095	.019	.056	.126	.108
	3	.275	.238	.256	.138	.198	.152	.141	.147
	4	.216	.084	.150	.149	.135	.156	.137	.147
	5	.397	.477	.521	.618	.618	.632	.590	.592
	6		.002	.002					.002
	7		.013	.002		.003		.002	
Got–C	1					.001		.009	
	2	.071	.320	.419	.257	.315	.270	.258	.232
	3	.059	.002		.007	.020	.026	.042	.008
	4	.870	.678	.578	.709	.651	.700	.692	.760
	5			.002	.026	.013	.004		
Pgm–A	1	.057	.021	.055	.058	.044	.033	.059	.016
	2	.934	.960	.902	.886	.898	.881	.910	.932
	3	.008	.019	.043	.056	.057	.086	.032	.052
Mdh–A	1		.019	.013	.063	.063	.034	.024	.038
	2								.002
	3	1.000	.981	.987	.936	.937	.966	.976	.960
	4				.001				
Mdh–B	1	1.000	.979	.996	1.000	.999	1.000	1.000	1.000
	3		.021	.004		.001			
Mdh–C	1		.019	.013	.013	.014	.016		.006
	2		.002				.006		.058
	3	.679	.729	.795	.725	.660	.604	.714	.706
	4	.321	.250	.192	.262	.326	.375	.286	.230
Mdh–D	1	.091	.179	.115	.173	.223	.167	.167	.146
	2	.468	.296	.342	.327	.283	.327	.240	.258
	3	.002	.013	.026	.008	.008	.010	.112	.008
	4	.439	.512	.517	.492	.486	.496	.481	.588
Gdh–A	1	.808	.230	.390	.410	.419	.392	.308	.474
	2	.192	.770	.610	.590	.581	.608	.692	.526
Skdh–A	2	.096	.052	.061	.030	.040	.034	.017	.042
	3	.713	.700	.804	.785	.730	.792	.810	.755
	4	.176	.162	.118	.168	.196	.132	.133	.163
	5	.013	.071	.017	.013	.034	.042	.011	.040
	6	.002	.015		.004			.030	
Skdh–B	1		.013			.002	.003		
	2	.017	.085	.032	.074	.066	.182	.011	.029
	3	.983	.902	962	.924	.931	.813	.989	.966
	4			.006	.002	.002	.003		.004

Table 3. Heterogeneity chi-square values computed on allelic frequency distributions of the studied populations.

Locus	Chi-square	D.F.	*P*
Lap–B	236.415	14	0.00000
Got–B	403.092	28	0.00000
Got–C	301.570	21	0.00000
Pgm–A	76.730	14	0.00000
Mdh–A	75.578	7	0.00000
Mdh–C	62.767	7	0.00000
Mdh–D	392.772	21	0.00000
Gdh–A	400.764	7	0.00000
Skdh–A	145.169	21	0.00000
Skdh–B	155.086	7	0.00000
Total	2249.943	147	0.00000

et al. 1996; HEDRICK *et al.* 1999). Our values refer to embryo genotypic frequencies, which normally include a certain amount of inbred progeny. A detailed comparison between embryonic and adult phases will be published in a separate paper (PUGLISI *et al.*, in preparation).

The parameters listed in Table 5 show the distribution of genetic diversity between and within populations. The average G_{ST} value is 2.6 %, similar to many other wide-ranged conifers, thus showing that the overwhelming majority of the measured genetic diversity is within populations. As shown in the table, G_{ST} values are not uniform so that for some loci the proportion of diversity due to population differentiation is higher (10.4 % for the locus *Gdh-A*). In most of the studied conifers, the same pattern of genetic diversity distribution has been observed; GULLBERG *et al.* (1985), KINLOCH *et al.* (1986), MUONA & HARJU (1989), SZMIDT & WANG (1993), GONCHARENKO *et al.* (1994), PRUS-GŁOWACKI & STEPHAN (1994), ZHELEV *et al.* (1994) found G_{ST} values among Scots pine populations of several origins similar to the values we found for the Italian populations. Nevertheless, GULLBERG *et al.* (1985) compared G_{ST} values from various studies on conifers and found that populations which occupy areas not concerned with glaciations had a higher differentiation: Swedish populations of Scots pine had low G_{ST} values while populations from areas not covered by ice in glacial periods had G_{ST} = 16 %. A similar comparison was made by PRUS-GŁOWACKI & BERNARD (1994), who found G_{ST} = 7.60 % for populations from areas not covered by ice and G_{ST} = 3.52 % for populations from areas covered by ice. The Alps, like the Scandinavian countries, were recolonized in the post-glacial period (CHIARUGI 1950; GIACOMINI 1958; PRAVDIN 1969), and our low G_{ST} mean value could result from a relatively recent diffusion of this species, which would not have had sufficient time for significant population differentiation.

The mean value of the subpopulation differentiation parameter δ over all loci and populations is 4.7 % (Table 6), which is low but almost twice the G_{ST} mean value. An analysis of the differentiation values of each population against the others considered as a whole, reveals a sharp difference of the Apennine population CAS (D_{j1} = 11.3 %). The parameter δ is fit for a graphic representation called "differentiation snails" (Figure 3). From this picture the sharp differentiation of the Apennine population (CAS) appears evident, as well as the trend to differentiation of populations CER, FEN and BCD. The loci which better make it possible to distinguish among populations are *Got-B, Got-C, Mdh-D* and *Gdh-A.*

The observed low level of average differentiation between populations is corroborated by the rather low Nei's and Gregorius' genetic distance average values

Table 4. Parameters of genetic diversity. *N*: mean number of alleles per locus; *P*: percentage of polymorphic loci at the 5% criterion; *v*: genetic diversity; H_e: expected heterozygosity according to Hardy-Weinberg; H_o: observed heterozygosity; F: fixation index. '

Population	*N*	*P*	*v*	H_e	H_o	*F*
CAS	2.8	58.3	1.319	.242	.192	.207
CER	3.3	58.3	1.391	.282	.248	.121
FEN	3.2	66.7	1.387	.280	.223	.204
MGX	3.3	83.3	1.431	.301	.266	.116
MBN	3.3	75.0	1.422	.297	.253	.148
BRE	3.2	75.0	1.449	.310	.265	.145
BCD	3.0	66.7	1.370	.271	.230	.151
MAL	3.2	66.7	1.385	.278	.255	.083
Mean	3.2	68.8	1.394	.283	.242	.147

Table 5. Genetic diversity analysis. H_t: total diversity; H_s: diversity within populations; D_{st}: diversity among populations ($H_t - H_s$); G_{st}: relative degree of genetic differentiation (D_{st}/H_t).

Locus	H_t	H_s	D_{st}	G_{st}
Lap–B	.140	.136	.003	.025
Got–A	.009	.009	.000	.003
Got–B	.623	.611	.012	.020
Got–C	.431	.416	.015	.035
Pgm–A	.163	.161	.001	.009
Mdh–A	.062	.061	.001	.015
Mdh–B	.006	.006	.000	.014
Mdh–C	.429	.423	.006	.014
Mdh–D	.622	.614	.009	.014
Gdh–A	.490	.439	.051	.104
Skdh–A	.393	.390	.003	.008
Skdh–B	.124	.119	.006	.045
Mean	.291	.282	.009	.026

(0.013 and 0.075, respectively; Table 7); the latter, unlike the former, can only range between 0 and 1. In most studies carried out until now on Scots pine, values of genetic distance are low, even between very distant populations such as some Swedish and Chinese ones (WANG *et al.* 1991). Yet both measures show the strong differentiation of the Apennine relict population (CAS) from the remnant; mean values of Nei's and Gregorius' genetic distance between population CAS and the remaining ones are 0.036 and 0.118, respectively.

Similar and even higher values of Gregorius' distance were observed by MEJNARTOWICZ & BERGMANN (1985) between some Polish populations of Scots pine from areas not covered by ice in the past, and similar values of Nei's distance were recorded by GONCHARENKO *et al.* (1994) between isolated populations in Eastern Europe and Siberia. On the basis of similar values of Nei's genetic distance from other geographic races, GONCHARENKO *et al.* (1995) recognized *P. sylvestris* var. *hamata* as a distinct taxon. PRUS-GŁOWACKI & STEPHAN (1994) observed a comparable pattern of differentiation between the Scots pine population representing the southern border of the species' natural range (Sierra Nevada) and the other surveyed Spanish populations.

Comparable results were obtained by SZMIDT (1982) between populations of *Pinus cembra* L., a species characterized by a discontinuous geographic range separated into relatively small and isolated zones, and by FINESCHI (1983), NIKOLIĆ & TUCIĆ (1983) and SCALTSOYIANNES *et al.* (1994) between some subspecies of *Pinus nigra* Arn., whose range is also discontinuous and where populations belonging to different subspecies are often located a long distance away from one another.

The high differentiation of the Apennine population (CAS) is clearly shown by the dendrogram constructed on the basis of Nei's genetic distance values (Figure 4). On the contrary, the Alpine populations are all alike.

According to some morphological features, several authors hypothesized for Scots pine in the Emilian Apennine the status of "race" or "variety" (AGOSTINI 1972). At this level, more research work is needed on

Table 6. Genetic differentiation between populations (δ values). D_{ji}: differentiation values of single populations.

Locus	Population								δ
	CAS	CER	FEN	MGX	MBN	BRE	BCD	MAL	
	D_{j1}	D_{j2}	D_{j3}	D_{j4}	D_{j5}	D_{j6}	D_{j7}	D_{j8}	
Lap–B	.077	.052	.033	.098	.054	.018	.018	.030	.056
Got–A	.002	.005	.002	.005	.004	.011	.002	.002	.004
Got–B	.204	.187	.084	.077	.099	.071	.064	.051	.099
Got–C	.235	.048	.156	.040	.061	.014	.034	.071	.071
Pgm–A	.044	.059	.010	.026	.012	.040	.019	.032	.026
Mdh–A	.047	.027	.033	.027	.028	.010	.021	.006	.026
Mdh–B	.002	.020	.002	.003	.002	.002	.002	.002	.004
Mdh–C	.037	.044	.109	.040	.058	.101	.020	.068	.056
Mdh–D	.170	.022	.064	.020	.069	.017	.102	.099	.062
Gdh–A	.417	.211	.037	.018	.007	.035	.126	.054	.076
Skdh–A	.069	.068	.070	.037	.050	.052	.083	.013	.051
Skdh–B	.056	.034	.038	.012	.001	.129	.063	.041	.032
Gene pool	.113	.065	.053	.034	.037	.042	.046	.039	.047

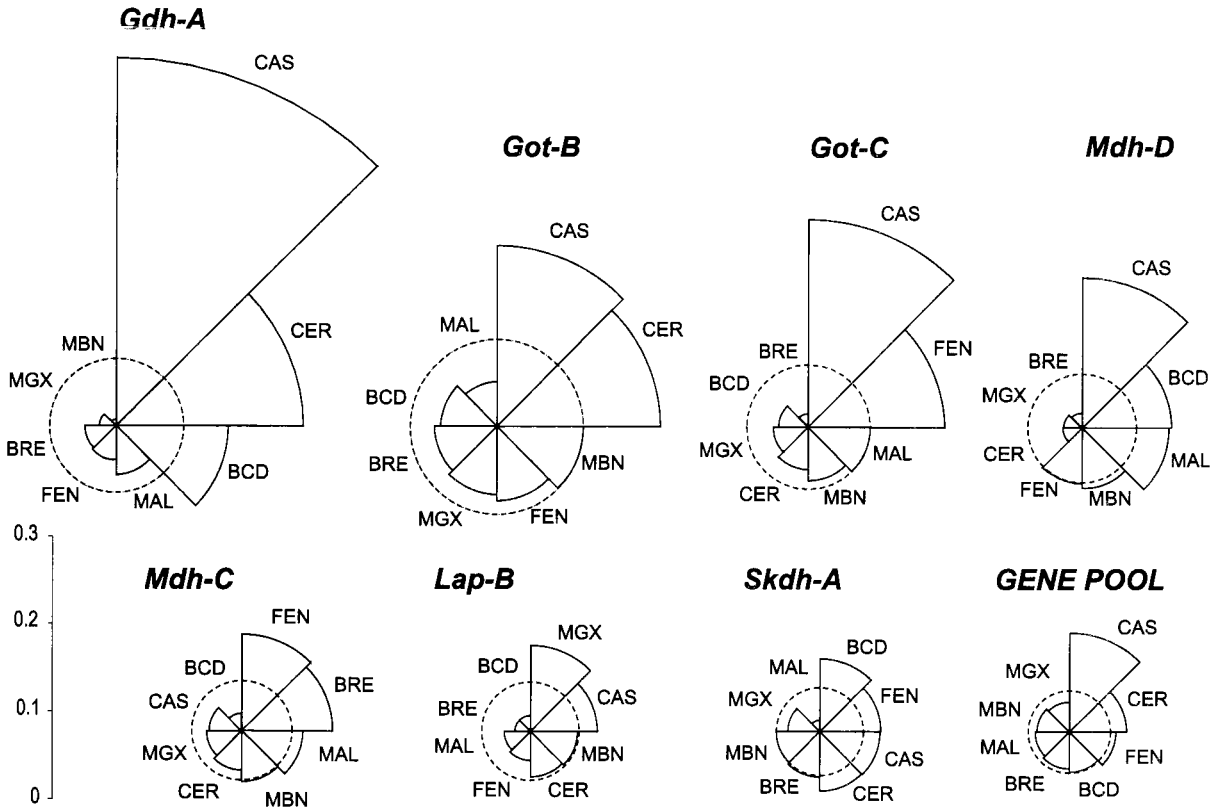

Figure 3. Graphic representation ("differentiation snails") of the values listed in Table 6. Lengths of radii of the dotted circles correspond to the total differentiation level (δ), and the length of each sector radius corresponds to the differentiation value of a single population.

Table 7. Genetic distances calculated following Gregorius (above the diagonal) and Nei (below the diagonal).

Population	CAS	CER	FEN	MGX	MBN	BRE	BCD	MAL
CAS	–	.125	.118	.119	.113	.130	.117	.105
CER	.050	–	.071	.073	.066	.082	.065	.077
FEN	.037	.007	–	.057	.063	.067	.063	.064
MGX	.031	.009	.006	–	.041	.042	.049	.043
MBN	.031	.009	.006	.003	–	.048	.060	.056
BRE	.035	.010	.010	.003	.003	–	.060	.059
BCD	.041	.005	.006	.003	.005	.006	–	.054
MAL	.024	.011	.007	.002	.004	.006	.005	–

morphological and physiological traits, and on biochemical and molecular markers, in order to integrate results obtained from various approaches and verify the taxonomic status of Scots pine vegetating in the Ligurian-Emilian Apennine.

The notable differentiation of the Apennine population suggests a fairly distinct evolutionary history, in relation to the history of the post-glacial recolonization of the Alps, which could have started from shelter areas along the borders or within the mountain range, namely from Illyrian refugia in the far east of the Alpine range, as well as from refugia in the Ligurian-Emilian Apennine and in the Tyrrhenian coastal plane near Viareggio (Versilia, Tuscany) in the far west (GIACOMINI 1958; JEDLOWSKI & MINERBI 1967; AGOSTINI 1972; WATSON 1996). Indeed, during glacial expansions, southern Mediterranean Europe was the area most densely covered with forests in the continent. This region was characterized by the mountain and subalpine boreal

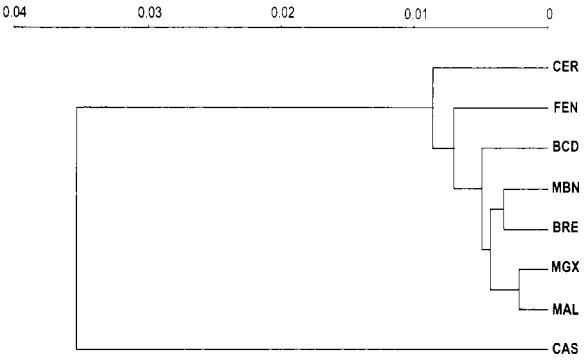

Figure 4. Dendrogram constructed on the basis of Nei's genetic distance values with the UPGMA method.

forest ecosystems, whose most important species were Scots pine, Swiss mountain pine (*Pinus mugo* Turra), silver fir (*Abies alba* Mill.), Norway spruce and others, which descended to the sea level along Italian coastal planes resembling those of Swedish coasts (CHIARUGI 1950).

If the recolonization started from the Illyrian refugia extending westward, according to the most commonly accepted theory for Norway spruce (GIANNINI *et al.* 1991; MORGANTE & VENDRAMIN 1991), the differentiation of the Scots pine Appennine population (CAS) could be due to a different origin from the Alpine ones – besides its geographic isolation – assuming that Scots pine followed the same migration route, since data relating to Scots pine recolonization are not available. For Norway spruce, GIANNINI *et al.* (1991) and MORGANTE & VENDRAMIN (1991) hypothesized a recolonization of the western Alpine range starting from Northern Apennine and Versilia refugia, while silver fir is assumed to have recolonized all the Alpine range starting from Central Italy refugia (BERGMANN 1991). Nevertheless, contrary to what GIANNINI *et al.* (1991) and MORGANTE & VENDRAMIN (1991) observed in Norway spruce populations, our data do not show any obvious similarity between the Apennine population and the western Alpine populations of Scots pine, in support of the hypothesis that the high differentiation of the former could be due to a different origin; as a matter of fact, the Apennine population of Norway spruce studied by the above-mentioned authors is not as differentiated as *P. sylvestris* population CAS from the Alpine ones.

SINCLAIR *et al.* (1999) studied mitochondrial DNA variation among several European populations of Scots pine, including two Italian ones: the Apennine population Casina (the same we surveyed) and the Alpine one Naz-Sciaves (close to our population BRE). In both populations they found the same mitotype, observed also in some of the surveyed Spanish, Scottish and Scandinavian populations, which is different from the mitotype observed in the other studied populations from central Europe (France, Germany and Poland). Nevertheless – as the authors themselves acknowledge – this identity does not demonstrate the genetic identity of the whole mtDNA, since the mitotype diversity they observed does not represent its total variation; consequently, the two Italian populations were not necessarily derived from the same refugium.

Because of its peculiarity, the investigated relict population, whose intrapopulation genetic variation is almost as high as in the Alpine populations, is an important genetic resource that requires protection. It is a small stand, completely surrounded by cultivated fields. Since it is an official seed collection area (MORANDINI & MAGINI 1975), it is protected by existing laws. However, due to its distinct genetic value, more specific measures of protection and habitat preservation should be introduced, to include other Apennine relict populations as well through the establishment of nature reserves.

Dynamic *in situ* conservation is the most suitable strategy for populations of forest tree species, because of their biological features. This form of conservation can preserve genetic adaptability under conditions of global environmental change; as a matter of fact, it allows populations to evolve and therefore to maintain their genetic variation by means of a continuous adaptation to spatial and temporal heterogeneity of environmental conditions (GREGORIUS 1989, 1991; ZIEHE *et al.* 1989).

In this context, it is important to apply suitable silvicultural treatments, either when natural regeneration is scanty or in order to prevent the settlement of different species – mostly broad-leaves – under the adult trees (AGOSTINI 1972), as it is much in evidence for instance in population CER (Liguria).

Nevertheless, when this kind of conservation strategy is applied to minor and isolated relict populations – as in the case of the Apennine population CAS – it should be matched with some appropriate forms of dynamic *ex situ* conservation (establishment of experimental plots or planting programs for range expansion) and, in some particular cases, of static conservation (seed or clonal banks). Accordingly, it is possible to ensure preservation of threatened gene resources even if the original populations do not survive to environmental adversities (ZIEHE *et al.* 1989), such as extended and recurrent fires, which are so common in Mediterranean regions.

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