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Allozyme variation of European Black (*Pinus nigra* Arnold) and Scots pine (*Pinus sylvestris* L.) populations and implications on their evolution: A comparative study

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A comparative study of the type, magnitude, and pattern of variation among 13 Black pine (*Pinus nigra* Arn.) and 14 Scots pine (*Pinus sylvestris* L.) European populations was conducted by using allozyme markers. The evaluation of genetic diversity parameters, i.e. mean number of alleles per locus (A/L), effective number of alleles (Ae), percentage of polymorphic loci (P), expected heterozygosity per population (He), for the two species indicated that both Black and Scots pine are characterized by high levels of variation. The genetic differentiation coefficient (Gst) and genetic distances were higher for the Black pine populations. For Scots pine populations, excluding that of the Iberian Peninsula which seems to retain a Tertiary gene pool, genetic distances were low, even between populations of great geographical distance (Sweden-Balkan countries). The principal coordinate analysis and cluster analysis also confirmed the different genetic variation pattern of the two species. The results can be interpreted by the different evolutionary course of the two pine species, especially during the post-glacial period.

Key words: variation pattern, genetic distances, evolution, *Pinus nigra*, *Pinus sylvestris*.

INTRODUCTION

Pinus is considered one of the oldest genera of the plant kingdom. Today, approximately 100 species belong to the genus *Pinus*, the largest number of species than any other gymnosperm, mostly expanding to the northern hemisphere (Mirov, 1967; Vidakovic, 1991). In Europe, there are 11 pine species (Little & Critchfield, 1969). Black (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.) firstly originated during the Jurassic period (135-190 million years ago) (Klaus, 1989; Millar, 1993). From a taxonomic point of view, both Scots and Black pine belong to the subsection *Sylvestres* (Little & Critchfield, 1969; Vidakovic, 1991).

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European Black pine was defined as a species in 1957 when Rohrig (Rohrig, 1957) suggested the name *Pinus nigra*, previously given by Arnold in 1785. The species covers a discontinuous area of 2,300,000 ha in 13 countries, ranging from 6˚ W to 42˚ E and from 35˚ to 48˚ N (Lee, 1968). It is considered a widely expanding species and can be found from the altitude of 1800 m to the sea level. It occurs in southern Europe, Cyprus, Asia Minor, Crimea, and northwest Africa (Critchfield & Little, 1966). In Greece, Black pine grows mainly on the mountains of the continental country and in the islands of Thasos, Samos, Lesvos, and Evia.

Black pine is a relict of the Cenozoic and during that geological period had a greater expansion. It is considered that the species originated from south-

east Asia. In the Pliocene epoch, Black pine was found in the Balkan Peninsula at the very same sites where is currently located (Mirov, 1967). Also, populations of the Western Europe survived *in situ* during the last glacial according to a recent work based on molecular data (chloroplast DNA) (Rafii & Dodd, 2007). Even today, there is a controversy concerning the taxonomy of the species because of its wide and discontinuous expansion and long isolation of the populations (e.g. island populations, north Africa, Austria, Crimea). Another reason is the existence of intermediate forms between the subspecies, a fact that renders difficult their discrimination.

Scots pine is one of the conifers with the largest geographic expansion in northern Asia, central and northern Europe, situated within an area of 135 longitudinal (from 0° to 135° E) and 30 latitudinal (from 38°41′ to 70°20′ N) degrees. In Balkan Peninsula, the southernmost expansion point of the species is northern Greece (Vidakovic, 1991). According to many researchers, in central and eastern Europe the gene pools of *P. sylvestris* were formed in post-glacial times during the migration of the species from the glacial refugia and intensive gene exchange between populations. On the contrary, Scots pine from the Iberian Peninsula probably did not take part in the colonization of Europe after the last glaciations and represents original ancient Cenozoic gene pool (Mirov, 1967; Huntley & Birks, 1983; Bennett *et al*., 1991; Prus-Glowacki *et al*., 2003).

In Europe both species have been studied extensively, regarding the magnitude and type of their variation by means of morphological, anatomical, growth, and biochemical characteristics (Kinloch *et al.*, 1986; Prus-Glowacki & Stephan, 1994; Scaltsoyiannes *et al*., 1994; Gerber *et al*., 1995; Rafii *et al*., 1996; Aguinagalde *et al*., 1997).

Although Black and Scots pine belong taxonomically to the same subdivision, complete comparative studies at the level of population genetic analysis are limited. Past works were based firstly on morphological and anatomical characteristics, particularly of needles and cones, and secondly on phylogenetic relationships between the two species resulting from crossing experiments (Vidakovic, 1974; Moulalis & Mitsopoulos, 1975; Moulalis *et al*., 1976; Vidakovic, 1991).

A recent comparative study on Balkan populations indicated that Black and Scots pine had different evolutionary histories, especially during the postglacial period (Pasagiannis *et al*., 2000).

The aim of the present work was to perform a comparative analysis of allozyme variation in Black and Scots pine natural European populations, in order to reveal some trends of the post-glacial evolution of the two species in Europe.

MATERIALS AND METHODS

The plant material used for analyses consisted of endosperms of germinated seeds derived from 14 Scots pine and 13 Black pine natural populations from the entire expansion area of the two species in Europe (Table 1). The seeds were sampled from at least 30 trees per population and used as bulk samples after mixing equal amount of seeds from each tree and choosing randomly at least 80 seeds per population for the analysis.

The allozyme analysis was conducted on the haploid endosperms of germinated seeds (at least 80 endosperms per population) by using the technique of horizontal starch gel electrophoresis, following the protocols of Conkle *et al*. (1982) and Cheliak & Pitel (1984). After seed germination (root length 3-4 mm), their endosperms were homogenized by using the extraction buffer of Conkle *et al*. (1982). Both species were analyzed for the following nine enzyme systems: acid phosphatase (ACP; EC 3.1.3.2), glutamic dehydrogenase (GDH; EC 1.4.1.2), isocitrate dehydrogenase (IDH; EC 1.1.1.42), leucine aminopeptidase (LAP; EC 3.4.11.1), malate dehydrogenase (MDH; EC 1.1.1.37), menadione reductase (MNR; EC 1.6.5.2), phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), glucose-6-phosphate isomerase (PGI; EC 5.3.1.9), and phosphoglucomutase (PGM; EC 5.4.2.2).

By the method of Nei (1973, 1978) the following genetic parameters of variation were estimated: mean number of alleles per locus (A/L), effective number of alleles (Ae), percentage of polymorphic loci (P), expected heterozygosity per population (He), the differentiation coefficient (Gst), and the genetic distances (D). A UPGMA dendrogram was constructed and principal coordinate analysis (PCoA) was performed on the values of genetic distances. Data analysis was conducted by using the BIOSYS statistical program (Swofford & Selander, 1981).

RESULTS

Isozymes of nine enzyme systems were resolved with consistency and clarity and thus, 14 loci were recorded,

TABLE 1. Origin of 14 *Pinus sylvestris* and 13 *Pinus nigra* populations

	Population	Abbreviation	Provenance	Latitude	Longitude	Country
Scots pine	1	**XAN**	Xanthi (north Greece)	41°12′N	24°58′E	Greece
	2	**TRA**	Trachoniou Dipotamou (Drama, north Greece)	41°12′N	24°10′E	Greece
	3	**LEY**	Leukogeia (Drama, north Greece)	41°15′N	24°10′E	Greece
	4	**FLO**	Florina (north Greece)	40°47′N	21°30′E	Greece
	5	**ALM**	Almopia (north Greece)	40°48′N	22°03′E	Greece
	6	**ELA**	Elatia (Drama, north Greece)	41°12′N	24°10′E	Greece
	7	**LAI**	Lailias (Serres, north Greece)	40°58′N	23°34′E	Greece
	8	**PER**	Kato Neurokopi (Drama, north Greece)	41°21′N	23°52′E	Greece
	9	**JUN**	Jundola	42°01′N	23°49′E	Bulgaria
	10	**MAC**	Mala Krusa	42°17′N	20°38′E	FYROM
	11	**BER**	Berovo	41°42′N	22°51′E	FYROM
	12	**DEV**	Devin	41°43′N	24°23′E	Bulgaria
	13	**SWE**	North Sweden	66°00′N	18°39′E	Sweden
	14	**SPA**	Spain	40°10′N	02°28′W	Spain
Black pine	1	**FRA**	Gagnère Saint Anthème	45°32′N	03°53′E	France
	2	**SPA**	Algarbe	40°18′N	01°31′W	Spain
	3	**CAL**	Calabria (Verger de Bout)*	—	—	Italy
	4	**COR**	Pozzo di Najja-Casamacioli	42°19′N	09°00′E	Corsica
	5	**AUS**	Area south of Vienna	47°30′N	16°00′E	Austria
	6	**BUL**	Kustendil	42°16′N	22°46′E	Bulgaria
	7	**YUG**	Vhrovine	44°51′N	15°25′E	Croatia
	8	**ROD**	Petrota (north Greece)	41°09′N	25°26′E	Greece
	9	**THAS**	Thasos Island	40°40′N	24°40′E	Greece
	10	**MET**	Kalambaka (central Greece)	39°42′N	21°37′E	Greece
	11	**SAM**	Samos Island	37°45′N	29°50′E	Greece
	12	**MIT**	Mitilini Island	39°06′N	26°35′E	Greece
	13	**KAL**	Kalamata (south Greece)	37°03′N	22°10′E	Greece

* artificial plantation

coded by 53 alleles for *P. sylvestris* (Table 2) and 61 for *P. nigra* (Table 3).

All the loci tested were polymorphic for both species with the only exception of the IDH enzyme system, which was monomorphic for Scots pine. The increased number of alleles, observed in the present work, is attributed to the presence of rare alleles (alleles with frequencies less than 0.05). Unique alleles (appearing in only one population) were detected mainly in Black pine (Table 3) and particularly in the Balkan populations. Some rare alleles had frequencies higher than 0.1 (MNR-An in the Kalamata population with a frequency of 0.30, MDH-B4 in the same population with 0.19 frequency, MDH-A1 in the population of Kalambaka with a frequency of 0.16).The four parameters of population genetic diversity (A/L, Ae, P, He) for *P. sylvestris* and *P. nigra* are presented in Table 4.

Black and Scots pine showed on average high levels of heterozygosity, which compared with those of other conifers, justifies well why the two species belong to the most polymorphic conifers. Mean heterozygosity was 0.244 and 0.249 for Scots and Black pine, respectively. For Scots pine (Table 4), the Berovo population had the highest value (0.294), while that of Mala Krusa the lowest (0.206). For Black pine, the highest heterozygosity value (0.316) was observed in the Greek population of Kalambaka (central Greece) and the lowest one (0.191) in the population of Spain. The other parameters (e.g. Ae, P) followed similar trends for both species, reaching their highest and lowest values at the same populations. It is worth mentioning that the populations of Spain exhibited relatively low levels of heterozygosity (0.213 for Scots pine and 0.191 for Black pine). This is probably due to their isolation and consequently low gene flow. On the contrary, the populations of the Balkan Peninsula exhibited higher heterozygosity values.

TABLE 2. Allele frequencies at 14 gene loci in 14 populations of *Pinus sylvestris*

Provenance / **Gene loci & alleles**		**XAN**	**TRA**	**LEY**	**FLO**	**ALM**	**ELA**	**LAI**	**PER**	**JUN**	**MAC**	**BER**	**DEV**	**SWE**	**SPA**
PGI-B	PGI-B1	0.04	0.00	0.00	0.00	0.00	0.06	0.02	0.12	0.04	0.00	0.04	0.08	0.06	0.04
	PGI-B2	0.95	1.00	0.88	1.00	0.98	0.91	0.98	0.85	0.91	1.00	0.96	0.86	0.94	0.96
	PGI-B3	0.01	0.00	0.12	0.00	0.02	0.03	0.00	0.03	0.05	0.00	0.00	0.06	0.00	0.00
IDH-A	IDH-A1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-A	PGM-A1	0.02	0.05	0.06	0.05	0.02	0.06	0.00	0.07	0.10	0.00	0.10	0.01	0.14	0.00
	PGM-A2	0.90	0.91	0.91	0.91	0.93	0.89	1.00	0.90	0.86	0.91	0.85	0.88	0.86	1.00
	PGM-A3	0.08	0.02	0.00	0.02	0.05	0.05	0.00	0.00	0.04	0.09	0.05	0.10	0.00	0.00
	PGM-A4	0.00	0.02	0.03	0.02	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.00
MNR-A	MNR-A1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MNR-A2	0.53	0.81	0.65	0.81	0.65	0.64	0.57	0.65	0.60	0.71	0.68	0.45	0.70	0.82
	MNR-A3	0.04	0.00	0.00	0.00	0.02	0.01	0.03	0.05	0.04	0.03	0.07	0.04	0.05	0.10
	MNR-A4	0.18	0.00	0.10	0.00	0.18	0.04	0.14	0.00	0.10	0.00	0.06	0.08	0.05	0.00
	MNR-A5	0.24	0.19	0.25	0.19	0.15	0.31	0.26	0.30	0.26	0.26	0.19	0.41	0.20	0.04
	MNR-A6	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.04
ACP-A	ACP-A1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
	ACP-A2	0.89	0.75	0.85	0.75	0.83	0.89	0.93	0.83	0.81	0.96	0.71	0.87	0.88	0.92
	ACP-A3	0.03	0.25	0.03	0.25	0.00	0.03	0.00	0.02	0.05	0.00	0.11	0.08	0.02	0.04
	ACP-A4	0.08	0.00	0.12	0.00	0.17	0.08	0.07	0.15	0.14	0.04	0.18	0.05	0.10	0.00
LAP-A	LAP-A1	0.94	0.96	1.00	0.96	0.90	0.98	0.94	0.99	0.99	1.00	0.98	0.95	0.98	1.00
	LAP-A2	0.06	0.04	0.00	0.04	0.10	0.02	0.06	0.01	0.01	0.00	0.02	0.05	0.02	0.00
LAP-B	LAP-B1	0.05	0.03	0.00	0.04	0.00	0.02	0.07	0.00	0.01	0.00	0.05	0.00	0.00	0.00
	LAP-B2	0.95	0.94	1.00	0.95	0.95	0.98	0.92	1.00	0.99	0.95	0.91	0.99	0.99	1.00
	LAP-B3	0.00	0.03	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.05	0.01	0.01	0.01	0.00
	LAP-B4	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
GDH-A	GDH-A1	0.18	0.19	0.09	0.13	0.10	0.29	0.33	0.15	0.24	0.32	0.25	0.27	0.36	0.15
	GDH-A2	0.82	0.81	0.91	0.87	0.90	0.71	0.63	0.85	0.75	0.68	0.75	0.73	0.54	0.85
	GDH-A3	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.10	0.00
MDH-A	MDH-A1	0.07	0.01	0.00	0.07	0.05	0.07	0.01	0.02	0.01	0.02	0.02	0.02	0.06	0.02
	MDH-A2	0.93	0.99	1.00	0.93	0.84	0.93	0.94	0.98	0.99	0.98	0.98	0.98	0.94	0.98
	MDH-A3	0.00	0.00	0.00	0.00	0.11	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MDH-A4	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MDH-B	MDH-B1	0.00	0.03	0.02	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.02	0.03	0.00	0.00
	MDH-B2	1.00	0.89	0.96	1.00	1.00	1.00	0.98	0.98	0.96	1.00	0.94	0.93	1.00	0.96
	MDH-B3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.04
	MDH-B4	0.00	0.08	0.02	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.04	0.02	0.00	0.00
MDH-C	MDH-C1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	MDH-C2	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	MDH-C3	0.73	0.76	0.55	0.69	0.65	0.72	0.83	0.85	0.73	0.89	0.66	0.76	0.43	0.67
	MDH-C4	0.26	0.16	0.38	0.31	0.35	0.25	0.17	0.15	0.23	0.07	0.34	0.22	0.57	0.33
	MDH-C5	0.01	0.06	0.05	0.00	0.00	0.03	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00
MDH-D	MDH-D1	0.26	0.08	0.17	0.11	0.12	0.20	0.08	0.17	0.09	0.04	0.17	0.07	0.08	0.10
	MDH-D2	0.28	0.33	0.37	0.32	0.27	0.19	0.30	0.40	0.26	0.43	0.34	0.51	0.10	0.34
	MDH-D3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00
	MDH-D4	0.01	0.11	0.00	0.00	0.09	0.05	0.05	0.00	0.00	0.00	0.00	0.07	0.09	0.00
	MDH-D5	0.45	0.48	0.46	0.57	0.52	0.56	0.57	0.43	0.65	0.37	0.49	0.35	0.73	0.56
PGD-A	6PGD-A1	0.04	0.04	0.01	0.02	0.00	0.00	0.05	0.00	0.00	0.01	0.05	0.03	0.02	0.10
	6PGD-A2	0.49	0.48	0.70	0.60	0.46	0.74	0.59	0.55	0.62	0.50	0.50	0.64	0.34	0.30
	6PGD-A3	0.11	0.00	0.00	0.02	0.00	0.04	0.09	0.00	0.04	0.05	0.02	0.00	0.02	0.00
	6PGD-A4	0.36	0.48	0.29	0.31	0.54	0.22	0.27	0.45	0.34	0.44	0.40	0.31	0.62	0.66
	6PGD-A5	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.02
PGD-B	6PGD-B1	0.85	0.82	0.90	0.72	0.84	0.98	0.89	0.80	0.95	0.89	0.72	0.78	0.86	0.57
	6PGD-B2	0.15	0.18	0.10	0.28	0.16	0.02	0.09	0.20	0.05	0.11	0.26	0.22	0.14	0.33
	6PGD-B3	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00

TABLE 3. Allele frequencies at 14 gene loci in 13 populations of *Pinus nigra*

Provenance / **Allele frequencies**		**FRA**	**SPA**	**CAL**	**COR**	**AUS**	**BUL**	**YUG**	**ROD**	**THAS**	**MET**	**SAM**	**MIT**	**KAL**
PGI-B	PGI-B1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
	PGI-B2	0.15	0.20	0.02	0.04	0.02	0.00	0.04	0.05	0.04	0.00	0.00	0.00	0.09
	PGI-B3	0.00	0.03	0.01	0.04	0.02	0.00	0.00	0.01	0.04	0.00	0.00	0.04	0.02
	PGI-B4	0.31	0.15	0.24	0.06	0.33	0.33	0.24	0.25	0.37	0.37	0.20	0.20	0.18
	PGI-B5	0.54	0.17	0.54	0.58	0.50	0.36	0.39	0.41	0.43	0.38	0.47	0.51	0.28
	PGI-B6	0.00	0.01	0.00	0.02	0.00	0.05	0.00	0.00	0.06	0.04	0.02	0.00	0.02
	PGI-B7	0.00	0.44	0.19	0.25	0.13	0.26	0.33	0.28	0.03	0.21	0.31	0.25	0.41
	PGI-B8	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IDH-A	IDH-A1	1.00	1.00	1.00	0.92	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00
	IDH-A2	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00
PGM-A	PGM-A1	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.08	0.00	0.00	0.03	0.00
	PGM-A2	1.00	1.00	1.00	1.00	1.00	0.92	0.96	0.97	0.92	1.00	1.00	0.97	1.00
	PGM-A3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
MNR-A	MNR-A1	0.00	0.00	0.16	0.00	0.55	0.42	0.47	0.53	0.36	0.66	0.45	0.23	0.34
	MNR-A2	0.95	1.00	0.84	1.00	0.41	0.53	0.48	0.47	0.54	0.30	0.49	0.77	0.36
	*MNR-An	0.05	0.00	0.00	0.00	0.04	0.05	0.05	0.00	0.10	0.04	0.06	0.00	0.30
ACP-A	ACP-A1	0.02	0.00	0.17	0.10	0.02	0.04	0.00	0.00	0.00	0.04	0.09	0.00	0.00
	ACP-A2	0.94	1.00	0.69	0.72	0.94	0.92	0.94	0.98	1.00	0.90	0.90	0.99	1.00
	ACP-A3	0.02	0.00	0.08	0.14	0.04	0.00	0.04	0.00	0.00	0.01	0.00	0.00	0.00
	*ACP-An	0.02	0.00	0.06	0.04	0.00	0.04	0.02	0.02	0.00	0.05	0.01	0.01	0.00
LAP-A	LAP-A1	0.94	0.90	1.00	0.99	0.96	0.99	0.99	0.98	1.00	0.97	1.00	0.93	0.98
	*LAP-An	0.06	0.10	0.00	0.01	0.04	0.01	0.01	0.02	0.00	0.03	0.00	0.07	0.02
LAP-B	LAP-B1	0.00	0.04	0.00	0.00	0.01	0.00	0.01	0.00	0.02	0.03	0.00	0.03	0.00
	LAP-B2	0.89	0.90	0.98	1.00	0.99	0.97	0.97	0.95	0.97	0.89	1.00	0.92	1.00
	LAP-B3	0.11	0.06	0.02	0.00	0.00	0.03	0.02	0.05	0.01	0.08	0.00	0.05	0.00
GDH-A	GDH-A1	0.00	0.00	0.02	0.03	0.00	0.00	0.03	0.00	0.04	0.00	0.00	0.00	0.00
	GDH-A2	1.00	1.00	0.98	0.97	1.00	0.99	0.96	1.00	0.96	0.91	1.00	0.99	1.00
	GDH-A3	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.09	0.00	0.01	0.00
MDH-A	MDH-A1	0.00	0.00	0.00	0.01	0.01	0.01	0.07	0.00	0.00	0.16	0.00	0.00	0.00
	MDH-A2	1.00	1.00	1.00	0.99	0.99	0.97	0.93	1.00	1.00	0.84	1.00	1.00	1.00
	MDH-A3	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MDH-B	MDH-B1	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
	MDH-B2	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	MDH-B3	0.15	0.08	0.31	0.13	0.48	0.49	0.35	0.45	0.34	0.43	0.29	0.36	0.06
	MDH-B4	0.00	0.01	0.00	0.01	0.02	0.01	0.00	0.01	0.00	0.02	0.00	0.01	0.19
	MDH-B5	0.85	0.91	0.67	0.81	0.48	0.46	0.65	0.45	0.65	0.54	0.59	0.51	0.75
	MDH-B6	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.04	0.00	0.01	0.12	0.08	0.00
	MDH-B7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
MDH-C	MDH-C1	0.04	0.01	0.00	0.01	0.02	0.01	0.06	0.00	0.00	0.00	0.00	0.00	0.00
	MDH-C2	0.01	0.03	0.05	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.60
	MDH-C3	0.64	0.56	0.68	0.83	0.46	0.38	0.35	0.49	0.26	0.31	0.29	0.34	0.44
	MDH-C4	0.00	0.00	0.04	0.00	0.27	0.01	0.00	0.00	0.00	0.00	0.05	0.04	0.00
	MDH-C5	0.00	0.23	0.02	0.00	0.00	0.02	0.00	0.01	0.01	0.02	0.00	0.01	0.19
	MDH-C6	0.31	0.17	0.20	0.16	0.23	0.54	0.59	0.49	0.73	0.67	0.66	0.60	0.31
	MDH-C7	0.00	0.00	0.01	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MDH-D	MDH-D1	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MDH-D2	1.00	1.00	1.00	0.99	0.94	1.00	1.00	1.00	1.00	0.83	0.84	0.93	0.92
	MDH-D3	0.00	1.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.17	0.16	0.07	0.08
	*MDH-Dn	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PGD-A	6PGD-A1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.03	0.20	0.05	0.07	0.04
	6PGD-A2	0.00	0.00	0.00	0.00	0.01	0.13	0.05	0.08	0.27	0.08	0.01	0.01	0.05
	6PGD-A3	0.60	0.74	0.60	0.48	0.81	0.69	0.74	0.69	0.65	0.57	0.60	0.81	0.72
	6PGD-A4	0.06	0.18	0.18	0.09	0.11	0.02	0.06	0.02	0.03	0.09	0.02	0.04	0.04
	6PGD-A5	0.34	0.08	0.22	0.43	0.06	0.16	0.15	0.06	0.02	0.06	0.32	0.07	0.15
PGD-B	6PGD-B1	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	6PGD-B2	0.66	0.73	0.64	0.58	0.78	0.52	0.61	0.65	0.42	0.54	0.28	0.46	0.79
	6PGD-B3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6PGD-B4	0.00	0.00	0.03	0.00	0.00	0.05	0.01	0.00	0.03	0.00	0.06	0.03	0.21
	6PGD-B5	0.19	0.27	0.27	0.42	0.16	0.41	0.35	0.34	0.55	0.46	0.55	0.43	0.00
	6PGD-B6	0.09	0.00	0.06	0.00	0.01	0.02	0.02	0.01	0.00	0.00	0.11	0.08	0.00
	6PGD-B7	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* null allele

TABLE 4. Estimated parameters of polymorphism for 14 populations of *Pinus sylvestris* and 13 populations of *Pinus nigra*

	Population	A/L	Ae*	P%**	He
Scots pine	XAN	2.64	1.35	85.71	0.263
	TRA	2.50	1.33	85.71	0.247
	LEY	2.29	1.29	71.43	0.228
	FLO	2.29	1.31	71.43	0.235
	ALM	2.29	1.34	85.71	0.256
	ELA	2.50	1.29	85.71	0.226
	LAI	2.64	1.31	85.71	0.237
	PER	2.21	1.3	85.71	0.235
	JUN	2.64	1.31	92.86	0.239
	MAC	2.14	1.25	71.43	0.206
	BER	2.79	1.41	92.86	0.294
	DEV	2.93	1.38	92.86	0.275
	SWE	2.79	1.33	92.86	0.260
	SPA	2.14	1.27	71.43	0.213
	Mean	**2.48**	**1.32**	**83.67**	**0.244**
Black pine	FRA	2.21	1.26	64.29	0.208
	SPA	2.21	1.23	50.00	0.191
	CAL	2.57	1.33	64.29	0.249
	COR	2.57	1.27	78.57	0.215
	AUS	2.93	1.32	78.57	0.244
	BUL	3.00	1.38	85.71	0.276
	YUG	2.64	1.35	78.57	0.260
	ROD	2.57	1.35	64.29	0.258
	THAS	2.71	1.32	85.71	0.245
	MET	2.71	1.46	85.71	0.316
	SAM	2.86	1.37	85.71	0.274
	MIT	2.36	1.34	57.14	0.255
	KAL	2.36	1.28	57.14	0.246
	Mean	**2.59**	**1.33**	**71.98**	**0.249**

* The monomorphic locus IDH in *Pinus sylvestris* is included.
** A locus was considered polymorphic if the frequency of the predominant allele was lower than 0.99.

The values of the differentiation coefficient Gst observed in Black pine were at least twice as high compared with those observed in Scots pine (8.8 and 3.8%, respectively) (Table 5). The above is considered an unexpected result, because Scots pine has a wider geographic range compared with that of Black pine and thus, a greater differentiation among its populations would be more plausible.

The genetic distances shown in Tables 6 and 7 for both species, also reveal the larger genetic differentiation between the populations of Black pine. Concerning the Scots pine populations, the largest genetic distances were observed between the Iberian and the other populations.

On the other hand, the Scots pine populations with the highest affinity (D = 0.003) were those of JUN (Bulgaria) and ELA (northern Greece), and BER (FYROM) and FLO (northern Greece). Another remarkable observation is the relatively low genetic distances between populations of distant geographical regions such as Sweden and Balkan countries. For Black pine populations, the lowest genetic distances (D = 0.004) were observed between the Bulgarian (BUL) and the Croatian (YUG) populations, as well as, between the ROD (northern Greece) and the Bulgarian (BUL) ones.

The principal coordinate analysis (PcoA) and the dendrograms (based on UPGMA clustering) of the two species are shown in figures 1, 2 and 3a, 3b, respectively. The figures clearly show the lower differentiation between the populations of Scots pine, with only the Iberian population differing substantially

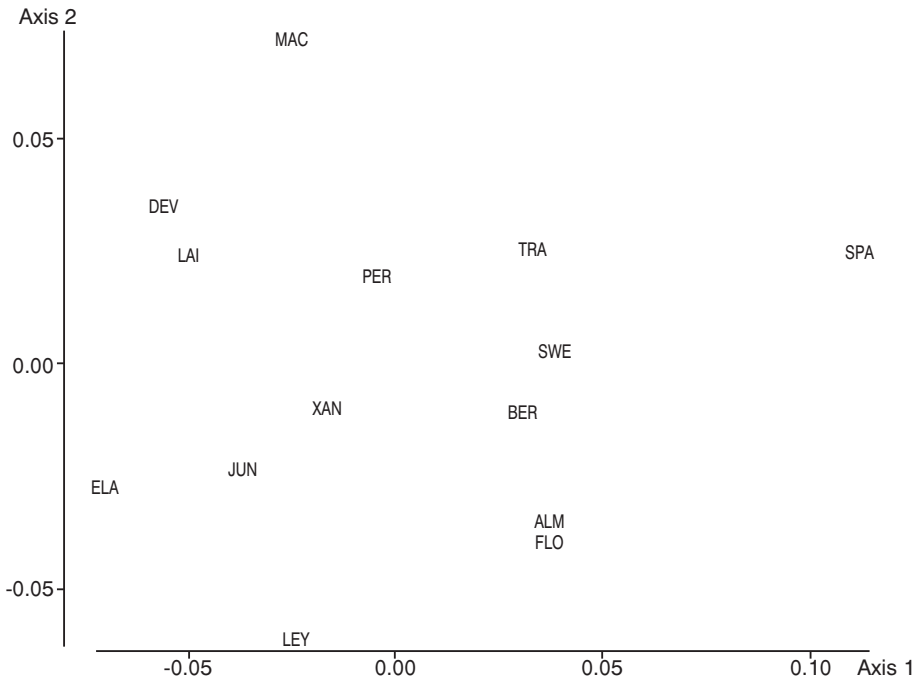

FIG. 1. Principal coordinate analysis (PcoA) of *Pinus sylvestris* populations based on the genetic distances.

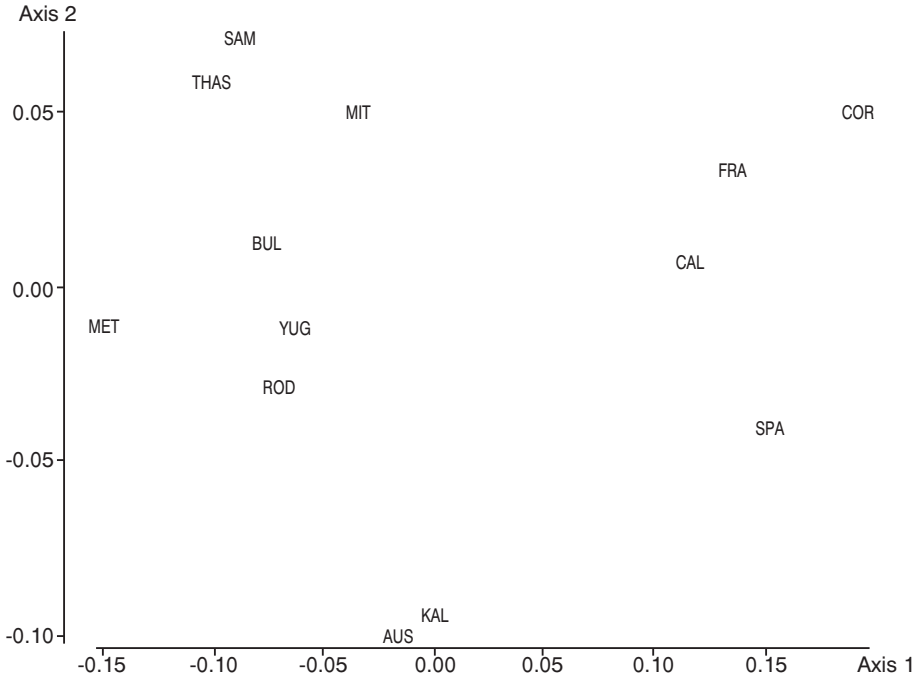

FIG. 2. Principal coordinate analysis (PcoA) of *Pinus nigra* populations based on the genetic distances.

TABLE 5. Overall genetic differentiation coefficient Gst per gene locus for *Pinus sylvestris* and *Pinus nigra* populations

	Pinus sylvestris	*Pinus nigra*
Gene loci	**Gst**	**Gst**
PGI-B	0.043	0.057
IDH-A	0.000	0.048
PGM-A	0.029	0.050
MNR-A	0.044	0.220
ACP-A	0.050	0.089
LAP-A	0.038	0.036
LAP-B	0.027	0.035
GDH-A	0.043	0.054
MDH-A	0.036	0.095
MDH-B	0.033	0.094
MDH-C	0.037	0.134
MDH-D	0.034	0.098
PGD-A	0.049	0.069
PGD-B	0.062	0.148
Mean	**0.038**	**0.088**

TABLE 6. Genetic distances (Nei, 1978) between the 14 studied *Pinus sylvestris* populations

Population	**XAN**	**TRA**	**LEY**	**FLO**	**ALM**	**ELA**	**LAI**	**PER**	**JUN**	**MAC**	**BER**	**DEV**	**SWE**	**SPA**
XAN	*	.013	.008	.012	.006	.008	.007	.006	.006	.011	.007	.010	.013	.020
TRA		*	.016	.010	.013	.018	.016	.009	.012	.010	.007	.018	.011	.014
LEY			*	.009	.011	.009	.017	.010	.007	.021	.010	.013	.020	.028
FLO				*	.009	.016	.017	.009	.011	.019	.003	.021	.008	.016
ALM					*	.018	.016	.012	.011	.019	.009	.022	.013	.013
ELA						*	.006	.013	.003	.014	.014	.014	.015	.039
LAI							*	.012	.005	.007	.013	.011	.012	.030
PER								*	.008	.007	.007	.007	.012	.018
JUN									*	.012	.008	.012	.008	.028
MAC										*	.014	.010	.016	.024
BER											*	.013	.005	.012
DEV												*	.022	.033
SWE													*	.012
SPA														*

TABLE 7. Genetic distances (Nei, 1978) between the 13 studied *Pinus nigra* populations

Population	**FRA**	**SPA**	**CAL**	**COR**	**AUS**	**BUL**	**YUG**	**ROD**	**THAS**	**MET**	**SAM**	**MIT**	**KAL**
FRA	*	.025	.013	.017	.053	.054	.050	.057	.063	.091	.068	.041	.051
SPA		*	.029	.034	.067	.069	.057	.067	.087	.110	.088	.052	.041
CAL			*	.012	.033	.041	.040	.041	.064	.076	.057	.037	.047
COR				*	.077	.080	.075	.080	.103	.127	.085	.066	.072
AUS					*	.026	.024	.018	.051	.040	.053	.037	.035
BUL						*	.004	.004	.010	.015	.014	.010	.033
YUG							*	.006	.014	.014	.014	.012	.021
ROD								*	.020	.014	.023	.015	.028
THAS									*	.020	.019	.016	.047
MET										*	.020	.031	.045
SAM											*	.016	.043
MIT												*	.040
KAL													*

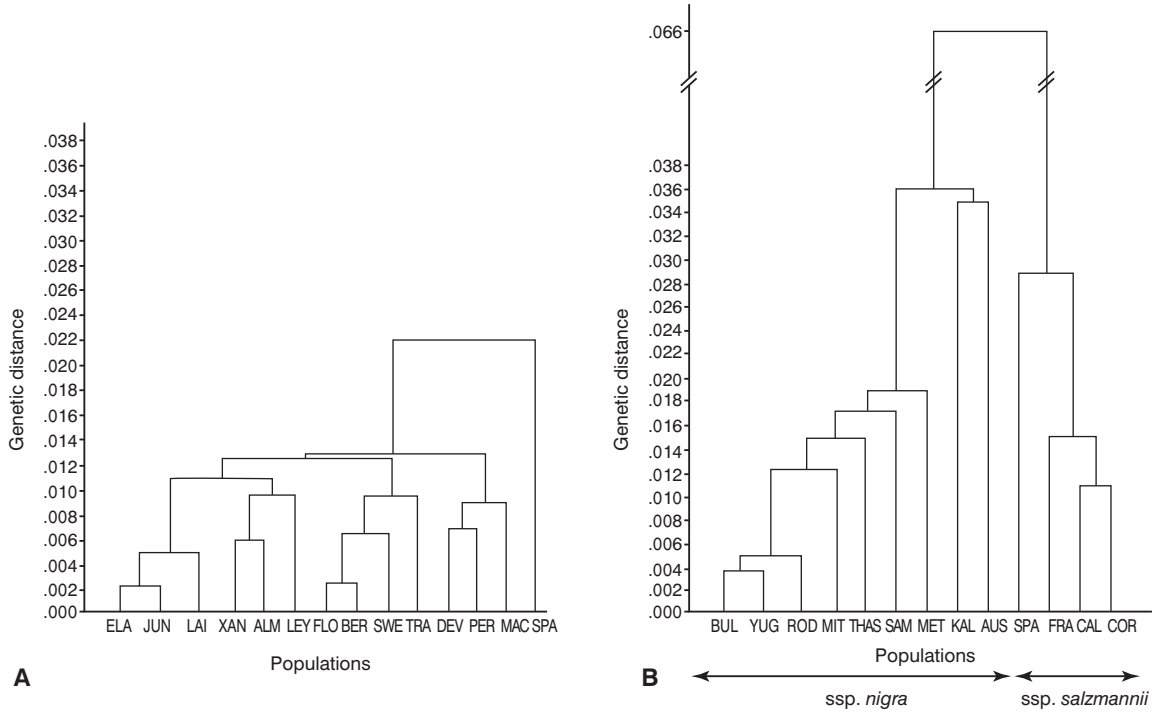

FIG. 3. UPGMA dendrograms of A) 14 *Pinus sylvestris* and B) 13 *Pinus nigra* populations.

from the others, while the populations of Black pine form two distinct groups, corresponding to the subspecies *nigra* and *salzmannii*.

As indicated, the populations of Scots pine possess a tendency to form smaller groups while the opposite occurs in the case of Black pine populations. This is attributed to the closer phylogenetic relation that exists between the Scots pine populations and to the larger differentiation observed between the populations of Black pine.

DISCUSSION AND CONCLUSIONS

Biochemical markers and specifically, isozymes were used successfully in the last decades both in phylogenetic and evolutionary studies of conifers (Hamrick *et al*., 1979, 1981; Mitton, 1983; Gullberg *et al*., 1985; Hamrick & Godt, 1990; Bergmann, 1991; Scaltsoyiannes *et al*., 1997, 1999; Scaltsoyiannes, 1999). Pines are, on average, among the most genetically diverse plants, both among and within populations, as measured by quantitative traits (Cornelius, 1994), and diversity at isozyme loci (Hamrick *et al*., 1979; Hamrick & Godt, 1990; Ledig, 1998).

In the present study, 14 Scots and 13 Black pine populations covering most of the distribution range of the two species in Europe were analyzed for allozyme variation in 14 loci. Our data revealed that, all the tested loci were polymorphic for both species, except for the IDH enzyme system, which was monomorphic for Scots pine. A previous comparative study of the two species (Pasagiannis *et al*., 2000) on the Balkan Peninsula populations, reported the same number of gene loci for the particular nine enzyme systems, coded by 49 and 56 alleles for Scots and Black pine, respectively. It appears that both species in the Balkan Peninsula contain a highly diverse gene pool. The above is in accordance with previous studies conducted on the two species in question (Scaltsoyiannes *et al*., 1994; Moulalis *et al*., 1996). Of the two species, unique alleles were detected mainly in the Balkan Black pine populations.

The high average heterozygosities estimated credit the two species as being among the most polymorphic coniferous species. Similar diversity rates were also found in pines by other researchers (Goncharenko *et al*., 1994; Silin & Goncharenko, 1996; Ledig, 1998).

The isolation and consequently, the low gene flow of Black and Scots pine Spanish populations are considered responsible for the relatively low diversity observed. To the contrary, the populations of the Balkan Peninsula had higher values for both species. High values in the diversity parameters for the Bal-

kan populations of both species were also recorded in previous works by Tsaktsira *et al*. (1997) and Pasagiannis *et al*. (2000), while low values for the populations of the Iberian Peninsula were reported by Prus-Glowacki & Stephan (1994) and Prus-Glowacki *et al*. (2003) for Scots pine and by Aguinagalde *et al*. (1997), Tsaktsira & Scaltsoyiannes (1998) for Black pine. The above, support the hypothesis of many researchers that the populations of the Iberian Peninsula were geographically isolated for considerable periods of time and were not affected drastically by glaciations (Mirov, 1967; Vidakovic, 1991; Prus-Glowacki & Stephan, 1994; Salvador *et al*., 2000). According to the previous authors and others (Huntley & Birks, 1983; Bennett *et al*., 1991), Cenozoic gene pools have survived in Iberian Peninsula more or less unchanged through the glaciation period.

Gst was at least twice higher for Black pine compared with Scots pine, a paradox result considering the fact that Scots pine is more expanded than Black pine and thus, a greater differentiation among its populations would be expected. This is well explained by the different evolutionary course of the two species, especially during the post-glacial period (Mirov, 1967; Gullberg *et al*., 1985; Vidakovic, 1991; Prus-Glowacki & Stephan, 1994; Moulalis *et al*., 1996). Similar Gst values were also found by Goncharenko *et al*. (1994), Prus-Glowacki & Stephan (1994) for Scots pine and by Tsaktsira (1992), Scaltsoyiannes *et al*. (1994) and Silin & Goncharenko (1996) for Black pine.

The genetic distances of both species also confirm the larger genetic differentiation between the populations of Black pine. The majority of Black pine populations exhibited the largest genetic distances from the Spanish and Corsican ones. Various morphological and biochemical data have also demonstrated the distinctive differences of the above-mentioned populations from other European ones (Barbero *et al*., 1998).

The distinction of the Iberian Scots pine population from the rest indicates that the Iberian Peninsula represents original ancient Tertiary gene pools. The discontinuity and high altitude of the Pyrenees contributes to this isolation. The data reported by Sinclair *et al*. (1999) based on mitochondrial DNA further support the above statements.

By comparing the respective data of PcoA and the dendrograms for Black and Scots pine, it is evident that each species follows almost the same genetic variation pattern in both analyses. Black pine populations follow an east-west geographic distribution model corresponding to two subspecies (*P. nigra* subsp. *nigra* and *P. nigra* subsp. *salzmannii*), while the differentiation model of the Scots pine populations does not express a clear trend. Pasagiannis *et al*. (2000), working on Balkan populations of the two species, resulted in the same conclusions. These findings confirm previous statements on the different post-glacial evolution of these particular species in Europe. According to geobotanical and other studies (Mirov, 1967; Gullberg *et al*., 1985; Moulalis *et al*., 1996), European populations of Scots pine, apart from those of the Iberian Peninsula, drew back southwards in refugia, as these became subject to great pressure by glaciations, which occupied a significant area in northern and central Europe during the Cenozoic era. All the currently existing European populations of Scots pine except those of the Iberian Peninsula are considered an end-product of the post-glacial expansion of the species, and therefore, they are relatively uniform genetically. The above hypothesis seems to be in accordance with the theory formulated by Gullberg *et al*. (1985), that natural species, as Scots pine, strongly affected by the glaciations, now present little differentiation among their populations. On the contrary, other pine species, such as Black pine, that were not affected significantly by the glaciations, may preserve, even today, a genetic diversity that already existed during the Cenozoic era and therefore, are characterized by higher interpopulation differentiation and higher number of rare alleles.

REFERENCES

Aguinagalde L, Llorente F, Benito C, 1997. Relationships among five populations of European Black pine (*Pinus nigra* Arn.) using morphometric and isozyme markers. *Silvae genetica*, 46: 1-5.

Barbero M, Loisel R, Quezel P, Richardson DM, Romane F, 1998. Pines of the Mediterranean basin. In: Richardson DM, ed. *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge: 153-170.

Bennett KD, Tzedakis PC, Willis KJ, 1991. Quarternary refugia of north European trees. *Journal of biogeography*, 18: 103-115.

Bergmann F, 1991. Isozyme gene markers. In: Mueller-Starck G, Ziehe M, eds. *Genetic variation in European populations of forest trees*. Sauerlaender's Verlag, Frankfurt am Main: 67-78.

Cheliak WM, Pitel JA, 1984. *Techniques for starch gel electrophoresis of enzymes from forest tree species*. Information report PI-X-42, Petawawa Natural Forest Institute.

Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC, 1982. *Starch gel electrophoresis of conifer seeds: a laboratory manual*. General technical report PSW-64, U.S. Forest Service, Washington DC.

Cornelius J, 1994. Heritabilities and additive genetic coefficients of variation in forest trees. *Canadian journal of forest research*, 24: 372-379.

Critchfield WB, Little EL, 1966. *Geographic distribution of the pines of the world*. Miscellaneous publication 991, USDA, Washington DC.

Gerber S, Baradat P, Marpeau A, Arbez M, 1995. Geographic variation in terpene composition of *Pinus nigra* Arn. *Forest genetics*, 2: 1-10.

Goncharenko GG, Silin AE, Padutov VE, 1994. Allozyme variation in natural populations of Eurasian pines. *Silvae genetica*, 43: 119-132.

Gullberg U, Yazdani R, Rudin D, Ryman N, 1985. Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. *Silvae genetica*, 34: 193-201.

Hamrick JI, Godt MJW, 1990. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sinauer Associates, Sunderland MA: 43-63.

Hamrick JL, Linhart YB, Mitton JB, 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual review of ecology and systematics*, 10: 173-200.

Hamrick JL, Mitton JB, Linhart YB, 1981. Levels of genetic variation in trees: influence of life history characteristics. In: Conkle MT, ed. *Proceedings of a symposium on isozymes of North American forest trees and forest insect*. Forest service general technical report PSW-48, USDA: 35-41.

Huntley B, Birks HJB, 1983. *An atlas of past and present pollen maps for Europe: 0-13000 years ago*. Cambridge University Press, Cambridge.

Kinloch Jr BB, Westfall RD, Forrest GI, 1986. Caledonian Scots pine: origins and genetic structure. *New phytologist*, 104: 703-729.

Klaus W, 1989. Mediterranean pines and their history. *Plant systematics and evolution*, 162: 133-163.

Ledig FT, 1998. Genetic variation in *Pinus*. In: Richardson DM, ed. *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge: 251-280.

Lee CH, 1968. Geographic variation in European Black pine. *Silvae genetica*, 17: 165-172.

Little EL, Critchfied WB, 1969. *Subdivisions of the genus Pinus (Pines)*. Miscellaneous publication 1144, Forest service, USDA.

Millar CI, 1993. Impact of the Eocene on the evolution of *Pinus* L. *Annals of the Missouri botanical garden*, 80: 471-498.

Mirov NT, 1967. *The genus Pinus*. The Ronald Press Company, New York.

Mitton JB, 1983. Conifers. In: Tanksley SD, Orton TJ, eds. *Isozymes and plant genetics and breeding, Part B*. Elsevier Science Publishers B.V., Amsterdam: 443-472.

Moulalis D, Mitsopoulos D, 1975. Artificial hybrids between some pine species in Greece. *Scientific annals of the forestry faculty*, IH: 57-96.

Moulalis D, Bassiotis C, Mitsopoulos D, 1976. Controlled pollinations among pine species in Greece. *Silvae genetica*, 25: 95-107.

Moulalis D, Scaltsoyiannes A, Tsaktsira M, 1996. Isozyme analysis of Scots pine (*Pinus sylvestris* L.) of the most southern part of its distribution in south-east Europe. *Proceedings of the 6th conference of the Greek scientific society of genetics and breeding of plants*, Florina: 206-216.

Nei M, 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the national academy of sciences USA*, 70: 3321-3323.

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.

Pasagiannis G, Tsaktsira M, Scaltsoyiannes A, 2000. Contribution of isozyme analysis in the interpretation of postglacial evolution of Scots pine (*Pinus sylvestris* L.) and Black pine (*Pinus nigra* Arnold) in the Balkan Peninsula. *Proceedings of the 9th conference of the Greek forestry society*: 701-711.

Prus-Glowacki W, Stephan BR, 1994. Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. *Silvae genetica*, 43: 7-14.

Prus-Glowacki W, Stephan BR, Bujas E, Alia R, Marciniak A, 2003. Genetic differentiation of autochthonous populations of *Pinus sylvestris* (Pinaceae) from the Iberian Peninsula. *Plant systematics and evolution*, 239: 55-66.

Rafii ZA, Dodd RS, 2007. Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of Black pine (*Pinus nigra*) in western Europe. *Molecular ecology*, 16: 723-736.

Rafii ZA, Dodd RS, Zavarins E, 1996. Genetic diversity in foliar terpenoids among natural populations of European Black pine. *Biochemical systematics and ecology*, 24: 325-339.

Rohrig E, 1957. Uber die Schwarzkiefer (*Pinus nigra* Arn.) und ihre Formen. I. Teil: Die naturlichen Standorte. *Silvae genetica*, 6: 39-53.

Salvador L, Alia R, Agundez D, Gil L, 2000. Genetic variation and migration pathways of maritime pine (*Pinus pinaster* Ait.) in the Iberian Peninsula. *Theoretical and applied genetics*, 100: 89-95.

Scaltsoyiannes A, 1999. Allozyme differentiation and phylogeny of Cedar species. *Silvae genetica*, 48: 61-68.

Scaltsoyiannes A, Rohr R, Panetsos KP, Tsaktsira M, 1994. Allozyme frequency distributions in five European

populations of Black pine (*Pinus nigra* Arnold). I. Estimation of genetic variation within and among populations. II. Contribution of isozyme analysis to the taxonomic status of the species. *Silvae genetica*, 43: 20-30.

Scaltsoyiannes A, Panetsos KP, Drouzas A, Tsaktsira M, 1997. Adaptive polymorphism in Mediterranean fir species. In: *8th IUFRO symposium of European silver fir on "Ecology and silviculture of European silver fir"*, Jundola (Bulgaria): 168-184.

Scaltsoyiannes A, Tsaktsira M, Drouzas A, 1999. Allozyme differentiation in the Mediterranean firs (*Abies*, Pinaceae). A first comparative study with phylogenetic implications. *Plant systematics and evolution*, 216: 289-307.

Silin AE, Goncharenko GG, 1996. Allozyme variation in natural populations of Eurasian pines. IV. Population structure and genetic variation in geographically related and isolated populations of *Pinus nigra* ARNOLD on the Crimean Peninsula. *Silvae genetica*, 45: 67-75.

Sinclair WT, Morman JD, Ennos RA, 1999. The postglacial history of Scots pine (*Pinus sylvestis* L.) in western Europe: evidence from mitochondrial DNA variation. *Molecular ecology*, 8: 83-88.

Swofford DL, Selander RB, 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *The journal of heredity*, 72 : 281-283.

Tsaktsira M, 1992. *Variation isoenzymatique inter-, intra-populations de Pinus nigra ARNOLD et contribution des isoenzymes à l'étude taxonomique de cette espèce*. D.E.S., Université Claude-Bernard Lyon 1.

Tsaktsira M, Scaltsoyiannes A, 1998. Isozyme polymorphism of 18 European Black pine populations and its contribution to the taxonomic status of the species. *Proceedings of the 7th conference of the Greek scientific society of genetics and breeding of plants*, Heraklion: 245-254.

Tsaktsira M, Scaltsoyiannes A, Athanassiadis N, 1997. Allozyme variation in nine Balkan pine (*Pinus nigra* Arnold) populations. In: Moustakas M, Tsekos I, eds. *Proceedings of the 1st Balkan botanical congress*. Kluwer Academic Publishers, Thessaloniki: 573-576.

Vidakovic M, 1974. Genetics of European Black pine (*Pinus nigra* Arn.). *Annales forestales*, 6/3: 57-86.

Vidakovic M, 1991. *Conifers: Morphology and variation*. Grafički Zavod Hrvatske, Zagreb.