Allozyme Variation and Possible Phylogenetic Implications in Abies cephalonica Loudon and Some Related Eastern Mediterranean Firs

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Summary

A total of 22 loci were assayed in several populations of *Abies cephalonica, A. borisii regis, A. bornmuelleriana* and *A. alba* using horizontal starch gel electrophoresis. Within- and between-population diversity were analyzed as well as between-species diversity. Mean expected heterozygosity was high and ranged between 0.175 and 0.290. The percentage of loci polymorphic was also very high, from 52.9 to 87.5. All populations except the *A. borisii regis* provenance were either at equilibrium or significantly deficient in heterozygotes, which suggests a certain level of inbreeding. Some alleles were species-specific at the following loci: Pgi1, Pgi2, Lap, Got2 and Mnr1. Most of the genetic divergence between the studied species and provenances appeared recently, during and after the last glaciation.

Key words: Abies cephalonica, allozymes, genetic diversity, phylogeny, *Abies alba, Abies bornmuelleriana, Abies borisii regis.*

Introduction

Abies cephalonica is a fir species endemic to the mountains of central and southern Greece. From central Greece northwards, the species is progressively replaced by *A. borisii regis* which becomes the only fir species in northern Greece. These species are valuable for reforestation in Mediterranean France both from an ecological and a silvicultural perspective. *A. cephalonica* has a large and scattered geographic distribution (PANETSOS, 1975) under a wide range of ecological conditions contributing to isolation, hybridization and introgression phenomena.

Terpene analyses (KOEDAM, 1981; MITSOPOULOS and PANETSOS, 1987; FADY et al., 1992) tend to prove that *A. cephalonica* has a highly variable genetic structure and emerged from an ancestral species common to *A. alba* and *A. bornmuelleriana* during the Pleistocene (FADY et al., 1992). The only known electrophoretic study (SCALTSOYIANNES et al., 1991) also indicated that genetic variability in *A. cephalonica* is high, based on a limited number of enzyme systems.

The aim of this study was to quantify genetic diversity in *A. cephalonica* and to provide additional information on genetic variation patterns and probable phylogenetic history of *Abies* in the eastern Mediterranean.

Materials and Methods

Wind-pollinated seeds were collected from 5 provenances of *A. cephalonica and 1 of A. borisii regis* in Greece,

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Table 1. — Description of sampled material.

Provenances	coordinates mountain range	species
Pril	37°16 N, 22°18 E Taygetos (Greece)	*A. cephalonica*
Kolo	38°33 N, 22°29 E Parnassos (Greece)	*A. cephalonica*
Veti	37°33 N, 22°15 E Menalon (Greece)	*A. cephalonica*
Ceph	38°14 N, 20°32 E Cephallenia island (Greece)	*A. cephalonica*
Evia	38°40 N, 23°30 E Eubboea island (Greece)	*A. cephalonica*
Hybr	39°30 N, 21°30 E Pindos (Greece)	*A. borisii regis*
Kzlk	41°10 N, 33°50 E Kastamonu (Turkey)	*A. bornmuelleriana*
Bald	41°05 N, 33°50 E Kastamonu (Turkey)	*A. bornmuelleriana*
Alba	48°28 N, 6°58 E Vosges (France)	*A. alba*

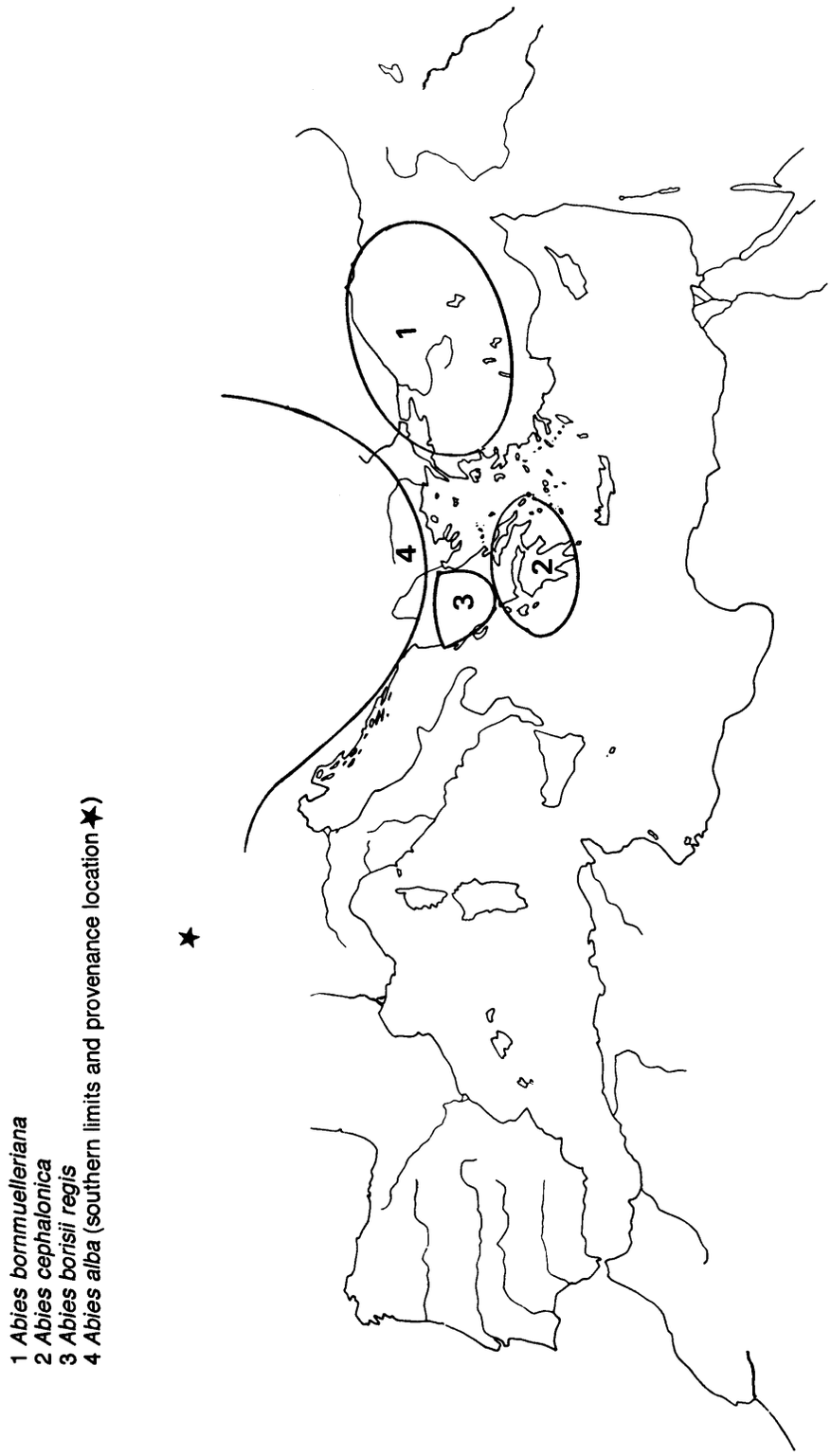

Figure 1. — Natural distribution of the studied Mediterranean firs.

2 provenances of *A. bornmuellerіana* in Turkey and 1 provenance of *A. alba* in France (*Table 1*). The species' natural distributions are shown in *figure 1*. Samples were made of bulked seeds using 30 trees per provenance for all populations except *A. borisii regis*. In *A. borisii regis*, parent identities were maintained: 403 seeds were assayed to calculate allele frequencies for 19 mother-trees. The *A. cephalonica* sample included provenances representative of the geographic variability found for other traits such as morphology, height growth, phenology and terpene composition (Fady et al., 1991). Both embryo and megagametophyte tissues were used for the isozyme analysis. Extraction and horizontal starch gel electrophoretic procedures were as described in Conkle et al. (1982) and Fady and Conkle (1992).

A total of 22 loci from 16 enzyme systems were scored: acid phosphatase (ACP, EC 3.1.3.2), aconitase (ACO, EC 4.2.1.3), catalase (CAT, EC 1.11.1.6), glutamate dehydrogenase (GDH, EC 1.4.1.3), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), glutathione reductase (GR, EC

Table 2. — Genetic diversity for 17 allozyme loci (14 loci for *A. borisii regis*) in 9 *Abies* provenances. Species means are given for 22 loci. Standard error in parentheses.

Provenance	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic(a)	Mean heterozygosity observed	Mean heterozygosity expected(b)	Mean F_{is}
Ceph	19.6 (3.3)	1.9 (0.2)	70.6	0.207 (0.056)	0.290 (0.062)	0.286
Evia	24.2 (3.2)	1.9 (0.2)	70.6	0.225 (0.063)	0.250 (0.064)	0.100
Kolo	28.5 (3.6)	1.7 (0.1)	64.7	0.140 (0.048)	0.242 (0.059)	0.421
Pril	23.2 (2.2)	1.9 (0.2)	70.6	0.200 (0.048)	0.223 (0.056)	0.103
Veti	18.1 (3.1)	1.7 (0.2)	52.9	0.147 (0.062)	0.192 (0.060)	0.234
Alba	37.4 (3.9)	1.6 (0.1)	58.8	0.174 (0.054)	0.209 (0.059)	0.167
Bald	29.6 (2.8)	1.7 (0.1)	64.7	0.179 (0.057)	0.227 (0.055)	0.211
Kzlk	11.9 (0.6)	1.6 (0.1)	52.9	0.163 (0.045)	0.175 (0.049)	0.069
Hybr	18.8 (0.1)	2.1 (0.2)	87.5	0.313 (0.068)	0.279 (0.057)	-0.122
A. cephal.(c)	96.0 (13.5)	2.0 (0.2)	72.7	0.161 (0.040)	0.221 (0.052)	0.271 (F_{it})
A. bornmu (c)	39.5 (2.9)	1.8 (0.2)	59.1	0.161 (0.044)	0.198 (0.047)	0.187 (F_{it})
A. alba	35.6 (3.4)	1.6 (0.1)	54.5	0.149 (0.044)	0. 182 (0.048)	0,181

(a) a locus is considered polymorphic if more than one allele was detected.
(b) unbiased estimate (NEI, 1978).
(c) estimate using pooled data without considering population subdivisions.

Table 3. — Fixation index (F_{is}) and probably of deviation from HARDY-WEINBERG equilibrium estimated using κ^2 test between expected and observed allele frequencies at polymorphic loci. Significant deviations ($p < 0.05$) indicate that F_{is} is significantly different from zero.

3a- Species estimates (pooled data without considering population subdivisions)

Locus	*A. cephalonica*		*A. alba*		*A. bornmuelleriana*		*A. borisii regis*	
	F_{is}	p	F_{is}	p	F_{is}	p	F_{is}	p
Aco	.605	.000	.392	.024	.384	.049	-.084	.743
Acp2	-.014	1.000	-.010	.893	.822	.003	-.056	.866
Got1	-.053	.639	-	-	-	-	-.027	1.000
Got2	.365	.028	.085	.563	-.012	1.000	-.161	.545
Got3	-.055	.812	.447	.016	-.072	.887	-.162	.833
Gr	.584	.000	.703	.000	.227	.166	.200	.331
Idh	-.099	.407	-.016	.970	-.137	.313	nd	nd
Lap	.441	.000	.109	.382	.619	.000	-.267	.282
Mdh2	-.013	.873	-.011	1.000	-	-	nd	nd
Mnr1	.259	.003	-.159	.339	.235	.077	-.080	.819
Mnr2	-	-	-	-	-	-	-.056	.866
Pep	.447	.023	-.333	.315	-.157	.104	nd	nd
6-Pgd	-.022	.794	.256	.088	-.119	.456	-.056	.866
Pgi1	-.065	.586	-	-	-.119	.441	-.512	.088
Pgi2	.067	.285	-	-	-.016	1.000	-.250	.543
Pgm	.660	.000	-	-	-	-	nd	nd
Skdh	-.051	.984	-	-	-.025	.910	nd	nd
Ugpp	-	-	1.000	.000	-	-	nd	nd

3b- *A. cephalonica* provenance estimates

Locus	Ceph		Evia		Kolo		Pril		Veti	
	F_{is}	p	F_{is}	p	F_{is}	p	F_{is}	p	F_{is}	p
Aco	.333	.346	.455	.346	1.00	.002	.200	.227	-	
Acp2	-	-	-	-	-	-	-.053	1.00	-	-
Got1	-.167	.595	-	-	-.061	.801	-.029	1.00	-.048	1.00
Gr	.784	.000	.649	.003	.357	.078	.267	.174	.792	.000
Idh	-.600	.343	-.190	.456	.308	.127	-.159	.487	-.538	.120
Lap	.451	.017	.220	.189	.793	.000	-.091	.703	-.050	.873
Mdh2	-.014	1.00	-.011	1.00	-.021	.918	-	-	-.014	1.00
Mnr1	.596	.004	-.117	.609	.165	.309	.184	.263	.286	.159
Pep	.491	.211	.083	.655	1.00	.011	.100	.633	1.00	.021
6-Pgd	-	-	-.014	1.00	-.043	.832	-.048	.825	-	-
Pgi1	.065	.635	-.200	.236	-.297	.058	-.067	.782	.093	.697
Pgi2	.042	.529	-.257	.162	.237	.027	-.078	.977	-.263	.562
Pgm	1.000	.000	-.032	1.00	-	-	-	-	-	-
Skdh	-.143	1.00	-.125	.796	-	-	-.069	.992	-	-

—: locus was monomorphic for the population.
nd: no data

1.6.4.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine amino peptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37) menadione reductase (MNR, EC 1.6.99.2), peptidase (PEP, EC 3.4.11.1), phosphoglucomutase (PGM, EC 2.7.5.1), 6-phosphogluconic dehydrogenase (6-PGD, EC 1.1.1.44), phosphoglucose isomerase (PGI, EC 5.3.1.9), shikimate dehydrogenase (SkDH, EC 1.1.1.25), UDP-glucose pyrophosphorylase (UGPP, EC 2.7.7.9).

Allele frequencies for all 22 loci are given only for species (pooled frequencies without considering population subdivisions). Comparisons between *A. cephalonica* provenances were made using 17 loci (see *Table 4*) and allele

Table 4. — Estimated allele frequencies for 17 allozyme loci and heterogeneity of allele frequencies for polmorphic loci (0.95 criterion) between *Abies cephalonica* provenances. (n) is the sample size per locus.

	Abies cephalonica provenances					heterogeneity		
Locus	Ceph	Evia	Kolo	Pril	Veti	χ^2	df	prob.
Aco (n)	3	3	8	6	1			
1	0.500	0.500	0.500	0.417	1.000			
2	0.000	0.333	0.500	0.417	0.000	6.533	8	0.5877
3	0.500	0.167	0.000	0.167	0.000			
Acp1 (n)	43	43	50	32	40			
	1.000	1.000	1.000	1.000	1.000	-	-	-
Acp2 (n)	6	4	10	10	7			
1	0.000	0.000	0.000	0.050	0.000	1.368	4	0.8497
2	1.000	1.000	1.000	0.950	1.000			
Got1 (n)	14	21	26	18	11			
1	0.143	0.000	0.058	0.028	0.045	3.871	4	0.4238
2	0.857	1.000	0.942	0.972	0.955			
Gr (n)	19	19	21	22	20			
1	0.579	0.342	0.333	0.545	0.600	5.54	4	0.2368
2	0.421	0.658	0.667	0.455	0.400			
Idh (n)	4	20	21	25	10			
1	0.375	0.300	0.405	0.340	0.350	0.519	4	0.9717
2	0.625	0.700	0.595	0.660	0.650			
3	0.000	0.000	0.000	0.000	0.000			
Lap (n)	28	33	31	24	21			
1	0.036	0.030	0.000	0.000	0.000			
2	0.750	0.894	0.806	0.917	0.952	8.18	8	0.4161
3	0.214	0.076	0.194	0.083	0.048			
Mdh1 (n)	27	32	47	34	24			
	1.000	1.000	1.000	1.000	1.000	-	-	-
Mdh2 (n)	36	44	49	34	37			
1	0.986	0.989	0.980	1.000	0.986	-	-	-
2	0.014	0.011	0.020		0.014			
Mnr1 (n)	21	27	31	31	20			
1	0.381	0.537	0.694	0.613	0.300	10.311	4	0.0355
2	0.619	0.463	0.306	0.387	0.700			
Pep (n)	7	11	5	9	4			
1	0.500	0.727	0.600	0.444	0.500	3.937	8	0.8628
2	0.429	0.273	0.400	0.556	0.500			
3	0.071	0.000	0.000	0.000	0.000			
6-Pgd (n)	27	36	36	33	29			
1	1.000	0.986	0.958	0.955	1.000	2.894	4	0.5758
2	0.000	0.014	0.042	0.045	0.000			
Pgi1 (n)	36	40	44	30	35			
1	0.389	0.500	0.432	0.283	0.243			
2	0.611	0.500	0.568	0.717	0.729	10.853	8	0.2099
3	0.000	0.000	0.000	0.000	0.029			
Pgi2 (n)	38	36	45	29	28			
1	0.355	0.375	0.522	0.914	0.357			
2	0.382	0.333	0.211	0.069	0.429	32.94	12	0.0100
3	0.263	0.292	0.267	0.017	0.196			
4	0.000	0.000	0.000	0.000	0.018			
Pgm (n)	16	16	16	19	12			
1	0.937	0.969	1.000	1.000	1.000	2.665	4	0.6153
2	0.063	0.031	0.000	0.000	0.000			
Skdh (n)	4	9	18	18	4			
1	0.875	0.889	1.000	0.917	1.000			
2	0.125	0.111	0.000	0.056	0.000	5.167	8	0.7396
3	0.000	0.000	0.000	0.028	0.000			
Ugpp (n)	5	18	27	20	4			
1	1.000	1.000	1.000	1.000	1.000	-	-	-
2	0.000	0.000	0.000	0.000	0.000			

frequencies fo *A. borisii regis* were calculated for 14 loci (see *Table 5*).

Genetic distances between species and provenances were calculated using 9 polymorphic loci: Lap, Pgi1, Pgi2, Gr, Aco, Got1, Mnr1, Acp2 and 6-Pgd. All followed Mendelian expectations when analyzed in *A. borisii regis* (FADY and CONKLE, 1992), except Aco and 6-Pgd where slight distortion was shown. However, both loci demonstrated Mendelian inheritance in other *Abies* (e.g. NEALE and ADAMS, 1981; SHEA, 1988) and the distortions observed in *A. borisii regis* could be due to sample size and/or the hybrid nature of this fir. The IDH and SKDH systems were not used as they could not be scored using the same buffer systems as the other polymorphic loci. Of the remaining systems: (1) 7 showed no allelic variants at the 0.90 criterion: ACP1, CAT, GDH, MDH1, MDH2, MNR2, PGM, UGPP and (2) 3 showed faint or blurred resolution in either *A. borisii regis* or some *A. cephalonica* populations (PEP, GOT2 and GOT3). Poor resolution was probably due to low quality seeds quite frequently found in these fir species.

Data analysis (i.e. estimation of allele frequencies, mean heterozygosity, fixation index, genetic diversity and genetic distance) was performed using BIOSYS-1 (SWOFFORD and SELANDER, 1981).

Results

Within-population diversity

Genetic diversity appeared to be similar over all populations studied: neither mean number of alleles per locus nor mean expected heterozygosity varied significantly among either *A. cephalonica* provenances or other *Abies* populations (see overlapping ranges in *Table 2*). Percentage of loci polymorphic was high and ranged from 52.9 (Veti) to 87.5 (Hybr). Hybr had a significantly higher ($\alpha = 0.05$) percentage of polymorphic loci than the other provenances.

Mean expected heterozygosity (H_e, *Table 2*) ranged between 0.175 and 0.290. *Table 2* shows that all provenances except Hybr were deficient in heterozygotes. The highest mean deficiency was found for *A. cephalonica* provenances. High mean F_{is} values seem to be the result of a limited number of loci within each provenance, as shown in *table 3*. Deviations were found for 1 or more loci for all but 2 provenances (Pril and Hybr). The loci that deviated from HARDY-WEINBERG equilibrium most frequently were Aco, Gr and Lap. It should be noted that there can be considerable variation in the resolution and segregation patterns of some enzyme systems depending on the buffer systems used (e.g. for all dehydrogenases where so-called "nothing-dehydrogenase" bands can appear). The use of different buffer systems might be advisable in future studies to test the origin of these deviations. Other deviations could be due to sampling error: Aco for Kolo (8 samples only) and *A. alba* (15 samples) and Acp2 for *A. bornmuelleriana* (12 samples).

Between-population diversity in A. cephalonica

Over all loci, allele frequencies were not very different when only *A. cephalonica* provenances were considered (*Table 4*). Frequencies were significantly heterogeneous ($p < 0.05$) at only 2 loci: the "fast" Mnr1 allele was under represented in Veti and Ceph and the "fast" Pgi2 allele was over represented in Pril.

The proportion of genetic variation attributable to differences among populations (F_{st}) of *A. cephalonica* can be estimated in comparison with the proportion of genetic variation attributable to differences within populatons (F_{is}, fixation index) using:

$$F_{it} = F_{is} + (1 - F_{is})\, F_{st},$$

where F_{it} is the level of deviation from HARDY-WEINBERG proportions in the total population (WRIGHT, 1965).

Pooling all *A. cephalonica* frequencies without considering subdivisions into populations yielded F_{it} (0.271, *Table 2*). Mean weighted F_{is} for all 5 *A. cephalonica* provenances was 0.234. Thus, $F_{st} = 0.048$. When only the 9 polymorphic loci used in the phylogenetic studied are used, the figures become: mean $F_{is} = 0.234$, $F_{it} = 0.297$, and $F_{st} = 0.086$.

Between-species diversity

Frequencies were significantly heterozygous ($p < 0.05$) at all polymorphic loci (0.90 criterion) except for Got1, Gr, Pep and Skdh where data were pooled by species (*Table 5*).

Some alleles at the following loci were species specific: Pgi1, Pgi2, Mnr1, Lap and Got2. *A. alba* was homozygous for alleles Pgi1-1 and Pgi2-1 and *A. bornmuelleriana* was almost totally homozygous for allele Pgi2-1, while *A. cephalonica* and *A. borisii regis* had 2 alleles at intermediate frequencies and a third rare Pgi1 allele which was lacking in *A. alba* and *A. bornmuelleriana*. The Mnr1-2 allele was not detected in *A. alba*. The "fastest" Lap allele was lacking in *A. borisii regis* and almost totally lacking in *A. cephalonica*, but was in comparatively high frequency (0.198 and 0.250) in *A. alba* and *A. bornmuelleriana*. The "slowest" Lap allele was missing in *A. alba* and *A. bornmuelleriana*, but was in comparatively high frequency (0.211 and 0.127) in *A. borisii regis* and *A. cephalonica*. The "fastest" Got2 allele was not detected in *A. alba* and was almost lacking (0.012) in *A. bornmuelleriana* and the "slowest" Got2 allele was detected only in *A. alba*.

Other alleles are in very different frequencies depending on the species. One Acp2 allele was almost entirely missing in *A. cephalonica* and *A. borisii regis*, but very common (0.550 and 0.375) in *A. alba* and *A. bornmuelleriana*. The least common of 2 6-Pgd alleles was in much higher frequency (0.145 and 0.298) in *A. alba* and *A. bornmuelleriana* than in the other 2 taxa. *A. cephalonica* and *A. alba* were almost homozygous (0.948 and 0.905) for the "fastest" Got3 allele although the Got3-2,3 alleles had non negligeable frequencies in *A. borisii regis* and *A. bornmuelleriana*.

Genetic distance and phylogeny

Quantitative differences were analyzed using a multivariate analysis. A phylogenetic tree was constructed with CAVALLI-SFORZA and EDWARD's (1967) arc distance (WAGNER procedure) using combined data. It separated *A. cephalonica* and *A. borisii regis* from *A. alba* and *A. bornmuelleriana* (*Figure 2*). Although *A. alba* is geographically as distant from *A. cephalonica* as from *A. bornmuelleriana*, it appeared genetically closer to *A. bornmuelleriana*. *A. borisii regis*, which is considered as a hybrid between *A. alba* and *A. cephalonica*, was included in the *A. cephalonica* cluster. As *A. cephalonica* provenances did not appear clearly grouped according to geographic distance. NEI's unbiased minimum distance (1978) was used to provide a basis for comparison with geographic distance (estimated in kilometers using a straight line between provenances) within the *A. cephalonica* cluster. The correlation between genetic and geographic distances proved to be non significant.

Discussion

High heterozygosity is commonly found in fir species as demonstrated by SCALTSOYIANNES et al. (1991) for *A. cephalonica* (mean of 0.304, 8 loci) and *A. alba* (0.233, 8 loci), by KORMUTAK et al. (1982) for *A. alba* (range 0.446 to 0.554, 9 loci), by DIEBEL and FERET (1991) for *A. fraseri* (0.258, 4 loci), by NEALE and ADAMS (1985) for *A. balsamea* (range 0.261 to 0.292, 8 loci). However, comparison with these figures would not be accurate as they were calculated using only polymorphic loci for a limited number of enzyme systems, which overestimates genetic variability. Compared to the heterozygosity found for *A. lasiocarpa* (H_e = 0.081, 18 loci, SHEA, 1990) and to the values generally found for conifers (LEDIG, 1986), mean heterozygosity is high in *A. cephalonica*, *A. alba*, *A. borisii regis* and *A. bornmuelleriana*, indicating high levels of genetic diversity. Percentage of loci polymorphic appears higher than that observed for *Abies* populations in the eastern U.S. (range: 25 to 60, JACOBS and WERTH, 1984), another indication of high levels of genetic variability.

Less than 10% of the total observed variation appeared among populations of *A. cephalonica* as shown by F_{st}, which seems typical for outcrossed wind-pollinated conifers (HAMRICK, 1989; HAMRICK and GODT, 1989). Most of the genetic diversity lies within populations.

Deviations from HARDY-WEINBERG equilibrium were noticeable and mean F_{is} coefficients showed a significant heterozygote deficiency for some populations. Heterozygote deficiency is commonly found in outbreeding species (BROWN, 1979). It can be attributed to several factors:

Table 5. — Estimated allele frequencies for 22 allozyme loci and heterogeneity of allele frequencies for polymorphic loci (0.95 criterion) between *Abies* species. (n) is the sample size per locus.

	Abies species				heterogeneity		
Locus	*A. ceph*	*A. alba*	*A. bornm*	*A. boris*	χ^2	df	prob.
Aco (n)	21	15	23	18			
1	0.500	0.267	0.304	0.611	16. 836	6	0.0099
2	0.357	0.400	0.696	0.333			
3	0.143	0.333	0.000	0.056			
Acp1 (n)	208	55	49	19			
	1.000	1.000	1.000	1.000	-	-	-
Acp2 (n)	37	10	12	19			
1	0.014	0.550	0.375	0.053	24.474	3	0.0001
2	0.986	0.450	0.625	0.947			
Cat (n)	30	36	37	nd			
	1.000	1.000	1.000	nd	-	-	-
Gdh (n)	67	51	45	19			
	1.000	1.000	1.000	1.000	-	-	-
Got1 (n)	90	39	45	19			
1	0.050	0.000	0.000	0.026	4.344	3	0.2304
2	0.950	1.000	1.000	0.974			
Got2 (n)	33	30	42	18			
1	0.394	0.000	0.012	0.139	31.912	6	0.0001
2	0.606	0.850	0.988	0.861			
3	0.000	0.150	0.000	0.000			
Got3 (n)	29	21	29	19			
1	0.948	0.905	0.552	0.711	19.232	6	0.0038
2	0.052	0.095	0.379	0.158			
3	0.000	0.000	0.069	0.132			
Gr (n)	101	23	32	18			
1	0.480	0.326	0.281	0.417	4.868	3	0.1817
2	0.520	0.674	0.719	0.583			
Idh (n)	80	48	52	nd			
1	0.350	0.437	0.135	nd	24.175	4	0.0001
2	0.650	0.562	0.750	nd			
3	0.000	0.000	0.115	nd			
Lap (n)	137	53	56	19			
1	0.015	0.198	0.250	0.000	46.641	6	0.0001
2	0.858	0.802	0.750	0.789			
3	0.127	0.000	0.000	0.211			
Mdh1 (n)	164	59	54	nd			
	1.000	1.000	1.000	nd	-	-	-
Mdh2 (n)	200	48	51	nd			
1	0.988	0.990	1.000	nd	-	-	-
2	0.012	0.010	0.000	nd			
Mnr1 (n)	130	40	52	19			
1	0.531	0.863	0.654	0.579	52.158	6	0.0001
2	0.469	0.000	0.346	0.421			
3	0.000	0.137	0.000	0.000			
Mnr2 (n)	21	10	11	19			
	1.000	1.000	1.000	0.947	-	-	-
	0.000	0.000	0.000	0.053			

Pep (n)	36	12	16	nd			
1	0.569	0.500	0.281	nd			
2	0.417	0.500	0.656	nd	3.226	4	0.5208
3	0.014	0.000	0.063	nd			
6-Pgd (n)	161	38	47	19			
1	0.978	0.855	0.702	0.947	50.971	3	0.0001
2	0.022	0.145	0.298	0.053			
Pgi1 (n)	185	53	47	19			
1	0.378	1.000	0.106	0.526	93.837	6	0.0001
2	0.616	0.000	0.894	0.447			
3	0.005	0.000	0.000	0.026			
Pgi2 (n)	176	55	49	19			
1	0.494	1.000	0.980	0.737	75.022	9	0.0001
2	0.284	0.000	0.010	0.105			
3	0.219	0.000	0.010	0.158			
4	0.003	0.000	0.000	0.000			
Pgm (n)	79	22	37	nd			
1	0.981	1.000	1.000	nd	-	-	-
2	0.019	0.000	0.000	nd			
Skdh (n)	53	29	41	nd			
1	0.943	1.000	0.976	nd	2.228	4	0.6939
2	0.047	0.000	0.024	nd			
3	0.009	0.000	0.000	nd			
Ugpp (n)	74	36	43	nd			
1	1.000	0.028	1.000	nd	-	-	-
2	0.000	0.972	0.000	nd			

ud: no data for *A. borisii regis*.

sampling error, WAHLUND effect, selection and non-random mating within populations. Terpene analysis has indicated a relatively high consanguinity in *A. cephalonica* populations (FADY et al., 1992). Spatial clustering of related trees can be expected as heavy seeds tend to fall close to the seed-bearing tree. As firs are predominantly outcrossed (male and female flowers at different levels on the tree, 96% outcrossing using isozymes in *A. borisii regis*, FADY, unpublished), inbreeding due to crosses between related trees rather than selfing is thus the most likely explanation for high F_{is}.

The negative F_{is} coefficient of *A. borisii regis* were calculated using inferred allele frequencies of mature trees which are known to be less heterozygote deficient than seeds (e.g. PLESSAS and STRAUSS, 1986). It is assumed that inbreeding occurs followed by natural selection for outcrossed offsprings (LEDIG, 1986). Another reason for heterozygote excess as well as high percentage of polymorphic loci in *A. borisii regis* could be the presence of a mixed gene pool, because this species originates from hybridization between *A. cephalonica* and *A. alba*.

It should be noted that not all loci contributed to significant heterozygote deficiency. For example, 6-Pgd never contributed to deviation from HARDY-WEINBERG equilibrium, as also demonstrated by SCHROEDER (1989) for *A. alba*. This emphasizes the need for multi-locus studies when characterizing population genetic structures. In addition, further studies are needed to understand why some loci tend to be systematically implicated in heterozygote deficiency, especially as some loci seem to be directly sensitive to environmental factors (e.g. MÜLLER-STARCK, 1985).

Some levels of genetic diversity have been detected both between species and between *A. cephalonica* provenances as shown by heterogeneity tests. Reasons for this diversity are probably manyfold. The first reason could be geographic isolation: collapse of the Aegean sea-floor during the Pliocene and the Pleistocene followed by cycles of marine transgressions and regressions (PAPAPETROU-

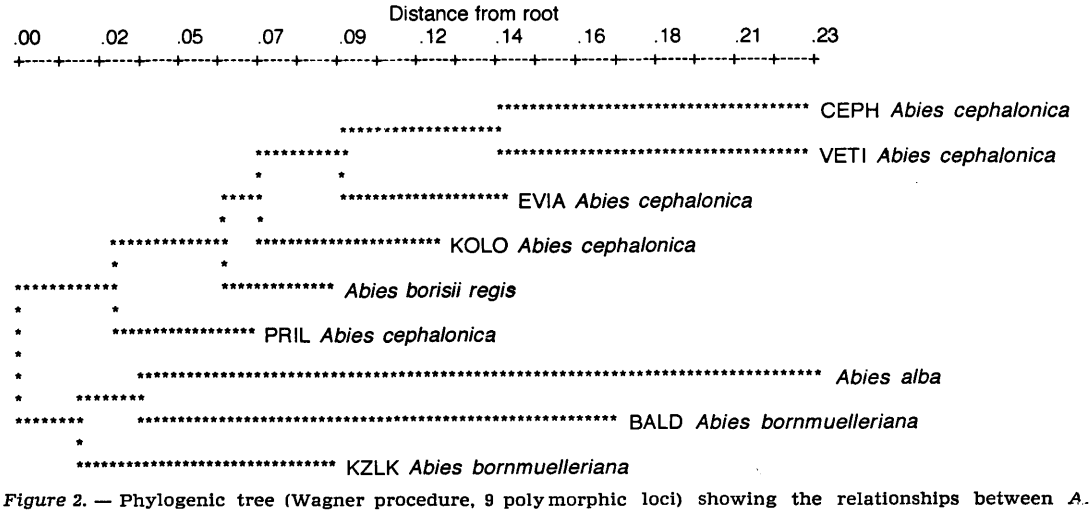

Figure 2. — Phylogenic tree (Wagner procedure, 9 polymorphic loci) showing the relationships between *A. cephalonica*, *A. borisii regis*, *A. alba* and *A. bornmuelleriana* provenances.

Table 6. — Estimated time in years (and range) since phylogenetic divergence using NEI's average genetic distance (1975) on 17 loci and the equation, $t = D/2\alpha$, where D is the genetic distance and α is the mutation rate approximated as 10^{-6}. Species represented by more than one population can be used to estimate time since divergence between sub-populations within the species.

Species and populations	*A. cephalonica*	*A. alba*	*A. bornmuelleriana*
A. cephalonica	6 500 (0 - 14 000)		
A. alba	64 500 (54 500 - 87 000)	- -	
A. bornm	27 500 (4 500 - 46 500)	59 500 (56 500 - 63 000)	16 000 (-)
A borisii regis (14 loci)	5 000 (-)	27 500 (-)	20 000 (-)

ZAMANI and PSARIANOS, 1977; PHILIPPSON, 1898) and global warming after 13 000 years Before Present (BP). Populations thus migrated to higher elevations on isolated mountain ranges. The second reason could be recolonization after the last glaciation from several refugia: each refugia was made of individuals different from one refugia to the next which can account for between provenances and species diversity. However, among-individuals within-refugia diversity must have been high to account for current individual genetic diversity. The last more recent reason is human disturbance which was probably the most critical for among-populations within-species diversity: clear cutting, over-grazing and burning resulted in forest fragmentation and reduction of population size. It promoted drift and inbreeding within populations and increased diversity among populations.

The phylogenetic tree suggests that divergence from an ancestral *Abies* species led to the emergence of 2 groups, one including *A. cephalonica* and *A. borisii regis* and the other a loose cluster formed of *A. alba* and *A. bornmuelleriana*. *A. alba* and *A. bornmuelleriana* are more closely related than either is with *A. cephalonica*, in agreement with data from terpene analysis (FADY et al., 1992).

Average genetic distance between taxa can be used as a measure of time since divergence between taxa under a neutral gene model (NEI, 1975). In the equation:

$t = D/2\alpha$

"D" is the genetic distance and "α" is the mutation rate which can be assumed to be between 10^{-5} and 10^{-7} (mean = 10^{-6}). The use of this equation is only approximate because migration, due to tectonic activity and glaciations, followed by hybridization between species, probably occurred after their first divergence. Nevertheless, a rough sequence in the emergence of the different taxa can be constructed using this procedure *(Table 6)*.

Divergence between *A. cephalonica*, *A. alba* and *A. bornmuelleriana* started at the beginning of the most recent glaciation (Würm in Alpine chronology, 90 000 to 12 000 years BP, DE LUMLEY, 1976). Proof of evolution prior to that date as supposed by FADY et al. (1992) using terpenes could not be found in this study. As *A. alba* fossils have been found in Europe and dated to the Peistocene (e.g. LIU, 1971), it is probable that some degree of divergense had occured before. *A. alba* probably colonized middle Europe during the Pleistocene and the Holocene, and benefited from gene flow from southern-European refugia (BERGMANN and KOWNATZKI, 1987), yielding the rather distinct genetic composition found in the population analyzed. It is also probable that variations of sea levels in the Aegean Basin during the Pleistocene are responsible for some degree of divergence between *A. cephalonica* and *A. bornmuelleriana* prior to the last glaciation. Isozyme data thus reflect the fact that the Würm glaciation was extremely severe (PONS, 1984) and that most of the within-species genetic diversity found today was probably shaped at that time.

All present-day species were reconstructed from a few small populations migrating from refugia which is also demonstrated by low F_{st} values. Contact between *A. bornmuelleriana* and *A. cephalonica* obviously continued for some time during the Holocene, probably through the island of Euboea (north-east of Athens). Divergence between *A. cephalonica* populations clearly occurred during the Holocene. *A. borisii reglis* is generally recognized as a hybrid between *A. cephalonica* and *A. alba* (MITSOPOULOS and PANETSOS, 1987), but divergence from *A. alba* proved to be older than from *A. cephalonica*. Even though eastern European populations of *A. alba* were not included in this study, *A. borisii regis* is clearly within the divergence range of all *A. cephalonica* provenances. As hybridization naturally occurs between *A. cephalonica* and *A. borisii regis* (FADY et al., 1991), it is thus unclear why they should be recognized as separate species rather than two subgroups of the same taxonomic unit.

Some phylogenetic points remain unclear and could probably be answered using a wider population sample, especially from *A. borisii regis* and eastern-European populations of *A. alba*. Other populations of *A. bornmuelleriana*, which were found to be possible "parents" for *A. borisii regis* using terpene analysis (FADY et al., 1992), might also be useful to detect recent hybridization. *A. cilicica* populations from Turkey and Lebanon should also be sampled as MITSOPOULOS and PANETSOS (1987) noted possible ancient gene flow between this species and fir populations in north-eastern Greece. In addition, some electrophoretic and stain procedures should be modified to increase the number of scorable loci and reduce the occurrence of possible nothing-dehydrogenase effects.

Evidence of high genetic diversity within populations and low genetic variability between populations was also found for quantitative traits in *A. cephalonica*. It is an

indication that breeding programs should concentrate on individual and family sampling within well-adapted provenances for optimum genetic gain on desirable traits.

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An Advanced-Generation Tree Improvement Plan for Slash Pine in the Southeastern United States

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Abstract

The Cooperative Forest Genetics Research Program has adopted an advanced-generation breeding strategy designed to maximize genetic gain in the short term and provide for continued improvement in the long term, while maintaining a broad genetic base for gene conservation. The breeding population consists of 933 members with an effective population size of 625. The population is divided into 4 strata based on genetic quality, with the top stratum functioning as an elite population. Clones in higher strata of the breeding population will receive more emphasis for both breeding and progeny testing, while those in the bottom stratum will serve to maintain a broad genetic base and infuse potentially valuable genes into upper stratum. Superimposed on the quality stratification is a division into 2 superlines, with each superline composed of 12 breeding groups (for a total of 24). These sub-divisions of the population will allow long-term breeding, while