GENETIC DIVERSITY OF SOUTHERN POPULATIONS OF *ABIES ALBA* MILL.

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ABSTRACT

Genetic structure and diversity of 25 populations of *Abies alba* from Italy, Bulgaria, Macedonia and Romania was analyzed in 18 isozyme gene loci, and compared with reference populations from Central Europe (Czech Republic, Slovakia and Slovenia). The total of 56 alleles was identified in the loci surveyed, resulting in an average of 3.05 alleles per locus. The highest total number of alleles (47) and mean number of alleles per locus (2.6) was revealed in Calabria. Proportion of polymorphic loci ranged from 100 % in Calabria to 62.5 % in Bulgaria. The highest genetic multiplicity (G_M) was detected in the Southern Carpathians, while the lowest one in Bulgaria. The polymorphisms at 10 loci including 23 alleles appeared to be area-specific. Southern populations of *A. alba* revealed mean observed heterozygosity (H_o) of 0.140–0.182, expected heterozygosity (H_e) 0.143–0.188, and effective number of alleles per locus (v) 1.167–1.235. Corresponding parameters were lower in the Central-European populations (H_o = 0.101–0.122, H_e = 0.102–0.124, v = 1.114–1.142). A slight deficiency of heterozygotes was observed in all populations but there was no difference between vital southern and reference Central-European populations affected by the long-term dieback. The among-population component of total gene diversity accounted for 8.7 %, of which 5 % was between the southern and Central-European part of natural range, 2.9 % among provenance regions, and 0.8 % among populations within provenance regions. Southern provenance regions revealed higher gene diversity. Nei's unbiased genetic distances (NEI 1978) between the southern provenance regions ranged from 0.003 to 0.019. Genetic distances between the southern and Central-European regions were much higher, ranging from 0.019 to 0.037. According to their genetic structures and diversity, the southern populations of *A. alba* can be divided into 3 to 4 groups: (i) Bulgaria and Macedonia, (iii) Romanian Carpathians, and (iv) Calabria. Several area-specific alleles seem to be different between geographically adjacent Macedonia and Bulgaria, however.

Key words: *Abies alba,* isozyme gene markers, genetic diversity, differentiation, Southern Europe

INTRODUCTION

The genetic research of European silver fir (*Abies alba* Mill.) has been relatively intensive over the last three decades. The hypothesis of LARSEN (1986) about primarily genetic reasons of the dieback of silver fir has been one of their outcomes and also an impetus for further research activities. According to this hypothesis, the long-term dieback of silver fir in Central Europe is basically caused by insufficient genetic variation resulting in its generally lower adaptability to stress than in other tree species. The reduced adaptability due to insufficient genetic variation is assumed thus the main predisposing factor exerting a permanent stress on silver fir and thereby making it sensitive to other factors (incidants) such as frost, drought, acidification and biotic pests. Reduced genetic variation of fir populations established in Central-Europe in the Postglacial period seems to be a consequence of directional selection and genetic drift triggered by climatic fluctuations during migration of the species to the northern part of its natural range.

The genetic and gene-ecological studies in silver fir focused on the geographic patterns of phenotypic (LANG 1994, WOLF 1992), genetic (BERGMANN & KOWNATZKI 1988, BERGMANN *et al.* (1990), eco-physiological and gene-ecological variation of the silver fir (LARSEN & FRIEDRICH 1988, LARSEN *et al.* 1988, LARSEN & MEKIĆ 1991). These studies and especially the international IUFRO 1982 experiment have found far more genetic diversity in the South-European and especially Calabrian provenances in comparison with the Central-European ones, and thus have supported Larsen's hypothesis about the primary genetic background of the long term dieback of the species. BERGMANN & GREGORIUS (1993) proved that the isozyme variation of silver fir may be influenced also by adaptation to climatic conditions, however.

KONNERT & BERGMANN (1995) attempted to assess

the postglacial phylogeny of European silver fir according to the geographic distribution of its area-specific isozyme alleles. The study was based on 54 provenance samples and 10 loci surveyed. The rare alleles detected allowed these authors to distinguish different provenance areas in the Alps, Carpathians, Massif Central, Apennines and Pyrenees, into which fir disseminated from different glacial refugia.

Due to uneven sampling, limited sample sizes and different loci analysed in individual studies, information about the genetic structures of silver fir is not always adequate. Information about genotypic structures appears to be particularly defficient. This applies to the populations from the southern, southeastern and eastern parts of the natural range, represented by no more than 15 population samples comprising either bulk seed or 25–60 trees in the studies of BERGMANN *et al.* (1990) and KONNERT & BERGMANN (1995). Many questions of Postglacial dissemination of European silver fir, where rare and area-specific variation patterns provide valuable information, are thus answered only partially.

The objective of the present study is to analyze genetic diversity and differentiation of natural populations from the southeasternmost parts of the natural range of European silver fir.

MATERIAL AND METHODS

The genetic structures and diversity of 25 populations of European silver fir from the southern and southeastern Europe were analysed (Figure 1). The Eastern Carpathians were represented by 8 populations, Southern Carpathians by 7 populations, Bulgaria by 3 populations, Macedonia & Serbia by 4, and Southern Italy (Calabria) by 3 populations. The reference Central-european provenance regions were represented by Southeastern Alps & Northern Dinaric Mts. (Slovenia), Bohemian Quadrangle (Czech Republic) and Western Carpathians (Slovakia) including 10, 11, and 20 populations, respectively.

The population samples originated either from natural forests or from autochthonous, naturally regenerated forest stands. Each population sample comprised approx. 50 dominant trees older than 80 years. In order to minimize a possibility of sampling of related individuals, the minimum spacing between sampled trees was 30–50 meters. The sampled area was always larger than 10 hectares. Branchlets with dormant buds were collected using a 10 m aluminum pole with scissors at the top. A shotgun was used in a limited number of stands with inaccessible crowns. Genetic structures and differentiation were studied in 18 enzyme loci (Table 1). The genetic control (codominant inheritance and stability of expression) of the isozymes in *Aap-A, -B, -C -D, Dia-A (= Mnr-A), Got-A, -B, -C, Idh-A, -B, 6Pgd-A, -B, Ndh-A (= Mnr-B), Pgi-A, -B, Pgm-A,-B* and *Skdh-A* was verified by HUSSENDÖRFER *et al.* (1995), and for *Aco, Mdh-A, Mnr-A* and *Mnr-B* by FADY and CONKLE (1992) in the closely related *Abies borisii-regis*. These authors revealed nearly no linkage between these loci. The interpretation of isozymes in *Skdh-B* was described by VICARIO *et al.* (1995), and in *Per-B and -C* by PARDUCCI *et al.* 1996 and SCALTSOYANNES *et al.* (1991). Due to insufficient reliability of scoring in zymograms, the allele denominated as $Mnr\text{-}B_1$ by FADY and CONKLE (1992), was pooled with $Mnr\text{-}A_2$, and the both are further referred as $Mnr(Nadh)\text{-}A_1$.

The laboratory procedures followed CONKLE *et al.* (1982), KONNERT (1992) and HUSSENDÖRFER *et al.* (1995). Horizontal electrophoresis was carried out using 11 % starch gels. For each tree, 5 to10 apices were dissected from dormant buds and homogenized in 50 ml of 0.2 M Tris-HCL extraction buffer pH 7.2, containing 1 % EDTA II, PVP 40, PVP 80, TWEEN, PEG, Na-ascorbate, 2-mercaptoethanol and 0.01 %

Table 1. Enzyme systems and gene loci surveyed.

Enzyme system	E. C. code	Loci surveyed	Separation system
Aconitase	1.1.1.1	*Aco*	A
Glutamate dehydrogenase	1.4.1.3	*Gdh*	D
Glutamate-oxalacetate transaminase	2.6.1.1	*Got-A,-B,-C*	D
Isocitrate dehydrogenase	1.1.1.42	*Idh-B*	B
Leucine aminopeptidase	3.4.11.1	*Lap-A (Aap-A)*	A
Malate dehydrogenase	1.1.1.37	*Mdh-A*	B and C
Menadion reductase	1.6.99.2	*Mnr-A,-B (-B=Nadh-A)*	C
Peroxidase	1.11.1.7	*Per-B,-C*	A
Phosphogluco-isomerase	5.3.1.9	*Pgi-A,-B*	A
6-Phophogluconate dehydrogenase	1.1.1.44	*6Pgd-A,-B*	B and C
Shikimate dehydrogenase	1.1.1.25	*Skdh-A,-B*	B

dithiotreitol. Crude homogenates were absorbed onto paper wicks and inserted into a cathodally positioned slice across a gel. The separation systems were used:

A: *bridge*: Lithium-borate 0,2 M, pH 8.3, *gel*: Tris-citrate 0,12 M, pH 8.3, *run*: 5 hours at 55 mA/240 V,

B: *bridge*: Tris-citrate 0,135 M, pH 8.0, *gel*: Tris-citrate 0,025 M, pH 8.0, *run*: 6 hours at 75 mA/150 V,

C: *bridge*: Tris-citrate 0,135 M, pH 7.3, *gel*: Tris-citrate 0,025 M, pH 7.3, *run*: 6 hours at 75 mA/150 V,

D: *bridge*: Tris-citrate 0,1 M, pH 8.85, *gel*: Na-borate 0,3 M, pH 8.0, *run*: 4 hours at 35 mA/110 V.

The following genetic parameters were computed from single-tree genotypes:

- Allelic frequencies;
- Genetic multiplicity depicted in the total number of alleles (*M*), proportion of polymorphic loci (*PP*), mean number of alleles per locus (*A*) and maximum genotypic multiplicity (G_M). G_M results as the product for all loci of the number of genotypes that can arise for a specified number of alleles (BERGMANN *et al.* 1990);
- Mean expected (H_e) and observed (H_o) heterozygosities,
- Genetic diversity or effective number of alleles per locus (υ) and hypothetical gametic diversity (υ_{gam}), where (υ) equals the harmonic mean and υ_{gam} equals the product of single locus genetic diversities (υ), which measure the diversity that would result from given allelic frequencies under a linkage equilibrium at each locus (GREGORIUS 1978),
- Nei's genetic distances between all population pairs (NEI 1978),
- The Principal Coordinate Analysis based on Nei's genetic distances was carried out using the PC programme SYN-TAX III (PODANI 1988).

The allele frequencies, genotype frequencies and genetic distances were computed at the level of populations and provenance regions. The computer programmes BIOSYS-1 (SWOFFORD & SELANDER 1981) and GSED (GILLET 1994) were used for this purpose.

RESULTS

Genetic multiplicity

Each locus was polymorphic in at least one population. A total of 56 alleles was identified at 18 studied loci, resulting in an average of 3.05 alleles per locus. The mean number of alleles per locus ($p > 95\%$) varied from 2.1 to 2.6. The highest total number of alleles (47) and mean number of alleles (2.6) was revealed in Calabria.

Proportion of polymorphic loci ranged from 100 % in Calabria to 62.5 % in Bulgaria. Across all provenance regions, including Central Europe, the highest values of genetic multiplicity (G_M) were revealed in the Carpathians while the lowest ones in Bulgaria.

Area-specific polymorphisms

The polymorphisms or fixation at 10 loci, and the presence or absence of 23 alleles, appeared to be area-specific among the provenance regions. It is important to consider that the absence of an allele in a population sample does not prove its absence in the sampled population in absolute terms. In our study, however, the sample sizes allowed to conclude with 95 % reliability about the presence or absence of alleles with frequency 0.5–1.0 % in most provenance regions. Only in Calabria represented by 150, Macedonia by 205 and Bulgaria by 146 individuals, the allele frequency limits for reliable estimate were 2.2 %, 1.5 %, and 2.2 %, respectively.

Southern populations revealed much more area-specific polymorphisms when compared to the Central-European ones (Slovenia, Czech Republic and Slovakia). The loci of *Skdh-A* and *-B* were polymorphic only in Calabria, *Pgi-A* and *-B* in Calabria and in the Balkan Peninsula. *Gdh*, *Got-B* and *Nadh-A* with rare polymorphisms in most provenance regions, revealed fixation to one allele in Macedonia and Bulgaria. The same applies to *Got-A* in Bulgaria and *Dia-A* (*Mnr-A*) in Macedonia. *Per-C* showed an opposite trend with two common alleles in Southern Europe whereas nearly fixation to the slower-migrating allele in Central Europe. The loci of *Idh-B*, *6Pgd-A*, *Got-C*, *Aco* and *Ap-A* were polymorphic in all provenance regions (Table 2).

Calabria has a quite distinct position among the provenance regions, due to the presence of 18 area-specific alleles, 5 of which were not found outside this region. In the Balkan Peninsula, 14 area specific alleles were detected. Two of these alleles are private to this region while another 2 alleles were found also in Calabria.

Of 16 area-specific alleles detected in southern populations, 12 alleles are different while only 4 shared between Bulgaria and Romanian Carpathians. There are thus more area-specific alleles between Romania and Bulgaria than, for example, between Slovenian and Romanian populations certainly originating in 2 different postglacial migration routes.

According to the area-specific alleles, the southern populations of silver fir can be divided into 4 groups: (i) Bulgaria and (ii) Macedonia, (iii) Romanian Carpa-

Table 2. Estimated allele frequencies for 18 allozyme loci and the eight provenance regions of *Abies alba*.

Locus		Calabria	Macedonia & Serbia	Bulgaria	Southern Carpathians	Eastern Carpathians	Slovakia	Slovenia	Czech Republic
Sample size		152	171	146	365	421	1036	511	566
Per-A	1	–	.067	–	–	–	–	–	–
	2	.993	.889	.966	.992	.987	.994	.995	.981
	3	–	.032	.007	.008	.013	.006	.005	.019
	4	.007	.012	.027	–	–	–	–	–
Per-B	1	.214	.278	.386	.490	.373	.092	.025	.008
	2	.786	.722	.614	.510	.627	.908	.975	.992
Aco-A	1	.037	.149	.165	.096	.058	.033	.092	.073
	2	.110	.118	.119	.299	.253	.092	.057	.074
	3	.853	.727	.709	.596	.689	.874	.846	.846
	4	–	.006	.004	.008	–	.001	.005	.007
	5	–	–	.004	–	–	–	–	–
Lap-A	1	.070	.257	.182	.176	.147	.032	.051	.020
	2	.907	.743	.800	.822	.847	.939	.885	.917
	3	.013	–	.018	.001	.006	.029	.064	.063
	4	.010	–	–	–	–	–	–	–
6Pgdh-A	1	.116	.257	.268	.347	.253	.403	.566	.530
	2	.884	.743	.732	.653	.747	.597	.434	.470
6-Pgdh-B	1	.928	.883	.914	.944	.968	.970	.961	.989
	2	.063	.117	.062	.049	.025	.026	.035	.007
	3	.010	–	.024	.007	.007	.004	.004	.004
Idh-A	1	.737	.529	.676	.696	.696	.463	.316	.222
	2	.263	.471	.324	.304	.304	.537	.684	.778
Got-A	1	.010	.020	–	.014	.012	.007	.003	.003
	2	.990	.980	1.000	.981	.988	.992	.997	.997
	3	–	–	–	.005	–	.001	–	–
Got-B	1	.023	–	–	–	.001	–	–	.001
	2	.957	.985	1.000	.999	.998	.990	.966	.970
	3	.020	.015	–	.001	.001	.010	.034	.030
Got-C	1	.123	.149	.144	.143	.209	.121	.130	.107
	2	.806	.836	.796	.840	.772	.832	.774	.802
	3	.071	.015	.060	.017	.019	.047	.096	.091
Mdh-A	1	.007	.006	–	–	.001	.011	.056	.015
	2	.888	.918	.911	.963	.967	.970	.913	.975
	3	.105	.076	.089	.037	.032	.019	.031	.010
Gdh-A	1	.057	–	–	.007	.004	.010	–	.004
	2	.937	1.000	1.000	.993	.996	.990	1.000	.996
	3	.007	–	–	–	–	–	–	–
Mnr-A	1	–	.006	–	–	–	–	–	–
	2	.007	.035	.024	.005	.004	.002	.004	.003
	3	.993	.959	.976	.995	.996	.998	.996	.997

Table 2. (continued).

Locus		Calabria	Macedonia & Serbia	Bulgaria	Southern Carpathians	Eastern Carpathians	Slovakia	Slovenia	Czech Republic
Sample size		152	171	146	365	421	1036	511	566
Mnr-B	1	.990	.968	1.000	1.000	.995	.992	.964	.985
	2	.010	–	–	–	.005	.008	.034	.015
	3	–	–	–	–	–	–	.002	–
	4	–	.032	–	–	–	–	–	–
Pgi-A	1	.167	.058	.167	.019	.013	–	–	–
	2	.833	.942	.833	.981	.987	1.000	1.000	1.000
Pgi-B	1	.010	.044	.017	–	–	–	–	–
	2	.013	.009	–	–	–	–	–	–
	3	.954	.942	.983	1.000	1.000	1.000	1.000	1.000
	4	.023	.006	–	–	–	–	–	–
Skdh-A	1	.760	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	.237	–	–	–	–	–	–	–
	3	.003	–	–	–	–	–	–	–
Skdh-B	1	.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	.003	–	–	–	–	–	–	–

Table 3. Genetic multiplicity characterized by the total number of alleles (*M*), mean number of alleles per locus (*A*), percentage of polymorphic loci (*PP*), and maximum genotypic multiplicity (G_M). For given sample sizes, α is the frequency limit over which all alleles have been detected with $p > 95\%$.

	Calabria	Macedonia & Serbia	Bulgaria	Southern Carpathians	Eastern Carpathians	Slovakia	Slovenia	Czech Republic
M	47	42	38	38	39	39	38	39
A	2.6	2.4	2.1	2.1	2.2	2.2	2.1	2.2
PP	100.0	83.3	66.7	77.8	83.3	77.8	77.8	72.2
G_M	143.10^{10}	312.10^6	42.10^6	765.10^6	306.10^6	510.10^6	85.10^6	155.10^6

thians, and (iv) Calabria. Some of the area-specific alleles appeared to be different also between geographically adjacent Macedonia and Bulgaria, however.

Allele frequencies

The allele frequencies in analyzed populations are provided in Table 2. Contrary to the area-specific alleles, the southern populations revealed a relatively big affinity in allele frequencies at loci where major polymorphisms are present. This phenomenon can be associated with the geographic clines, which follow climatic gradients from the northwest to south of the species' natural range. Such clines were reported in *Idh-B*, *6Pgd-A* (BERGMANN & KOWNATZKI 1988, LONGAUER 1995 1996), *Per-C*, *Ap-A* and *Aco* (LONGAUER 1995 1996).

Levels of genetic diversity

Genetic diversity varied between 1.167 and 1.214 and observed heterozygosity between 0.140 and 0.182 in the southern populations of silver fir. These values are incomparably higher than those revealed in Slovenia, Bohemian Quadrangle and Slovakia (v = 1.114–1.142, H_o = 0.099– 0.110). The heterozygosity where the biggest difference was revealed between the Southern and Central-European populations, is by 80 % higher in Macedonia or Bulgaria than in the Bohemian Quadrangle! It is of worth to mention in relation to it that observed heterozygosity measures the number of alleles present in individual genomes, i.e. the genetic diversity at the level of trees.

The results of the analysis of hierarchical apportion-

Table 4. Geographic distribution of area-specific alleles: ▌- allele frequency >0.5%, ▒ - frequency <0.5%).

Provenance region	α (p≥95 %)	*Aco-A*$_4$	*Aco-A*$_5$	*Ap-A*$_4$	*6-pgd-B*$_1$	*Got-A*$_1$	*Got-A*$_3$	*Got-B*$_1$	*Got-B*$_3$	*Mdh-A*$_1$	*Gdh-A*$_1$	*Gdh-A*$_3$	*Dia-A*$_1$	*Dia-A*$_2$	*Nadh-A*$_2$	*Per-B*$_1$	*Per-C*$_2$	*Pgi-A*$_1$	*Pgi-B*$_1$	*Pgi-B*$_2$	*Pgi-B*$_4$	*Skdh-A*$_2$	*Skdh-A*$_3$	*Skdh-B*$_2$
Calabria	2.1			▌	▌	▌			▌	▌	▌	▌	▌	▌	▌		▒	▌	▌	▌	▌	▌	▒	▒
Macedonia & Serbia	1.6						▌		▌	▌			▒	▒		▌	▌	▌	▌	▌	▌			
Bulgaria	2.3	▒	▒		▌								▒	▒		▒	▌	▒	▌					
Southern Carpathians	1.0	▌			▌	▌	▌		▒		▒							▒						
Eastern Carpathians	1.0				▌	▌		▒	▒	▌	▒		▒		▒			▒						
Slovakia	0.5	▒			▒	▌	▒	▒	▌	▌	▌		▒		▌									
Slovenia	0.5	▌			▒	▒			▌	▌	▌		▒		▌									
Czech Republic	0.5	▌			▒					▌	▒		▒											

Table 5. Mean observed (H_o) and expected (H_e) heterozygosity, effective number of alleles per locus (υ) and hypothetic gametic diversity (υ_{gam}) according to the provenance regions. Standard errors to the estimates of mean heterozygosity are in parentheses.

	Calabria	Macedonia & Serbia	Bulgaria	Southern Carpathians	Eastern Carpathians	Slovakia	Slovenia	Czech Republic
H_o	.162 ± .030	.182 ± .039	.176 ± .044	.150 ± .046	.140 ± .043	.105 ± .037	.122 ± .037	.101 ± .038
H_e	.168 ± .032	.188 ± .040	.177 ± .044	.155 ± .047	.143 ± .044	.110 ± .038	.124 ± .038	.102 ± .035
υ	1.203	1.235	1.214	1.184	1.167	1.124	1.142	1.114
υ_{gam}	34.768	57.857	2.939	37.235	25.759	11.651	15.533	9.365

ment of gene diversity (NEI 1987) and F-statistics (WRIGHT 1987) are presented in Table 6. The among-population component of total gene diversity accounted for 8.7 %, of which 5 % was between the southern and northern parts of natural range, 2.9 % among provenance regions, and 0.8 % among populations within the provenance regions. Separate computations of F-statistics for the northern and southern part of natural range revealed higher gene diversity among southern provenance regions of silver fir.

Genetic differentiation

Nei's unbiased genetic distances (NEI 1978) between the southern provenance regions range from 0.003 to 0.019 (Table 7). Genetic distances between the Southern and Central-European provenance regions were much higher, ranging from 0.019 to 0.037, however. The area-specific genes and North-South clinal variation of allele frequencies (LONGAUER 1994 1996) contributed apparently to the observed patterns genetic differentiation.

The UPGMA dendrogram based on the unbiased genetic distances (NEI 1978) illustrates the patterns of differentiation between the investigated regional populations (Figure 1). In the southern group, genetic relationships correspond with the geographical position of provenance regions. There are two less differentiated subclusters comprised of the populations from Romanian Carpathians and Balkan Peninsula, while Calabria has a relatively specific position. In the less heterogeneous cluster of northern provenance regions, the Bohemian populations of *A. alba* are genetically closer to the Slovenian ones than those from geographically less distant Moravia and Western Carpathians.

DISCUSSION AND CONCLUSIONS

Even without the Calabrian populations with specific genetic structures, a high level of genetic differentiation was observed between the southern and Central-Eastern European populations of silver fir. It is higher than in common beech within the Eastern part of its natural range

Table 6. Hierarchical analysis of population differentation based on apportionment of genetic diversity within and among populations according to NEI (1987) and respective F-statistics according to WRIGHT (1978), combined across loci.

Hierachical level	D_{st}	F_{st}
1. Diversity among populations	0.087	0.087
2. Diversity among southern and northern areas	0.050	0.050
3. Diversity among provenance regions	0.029	
*Southern provenance regions**	*0.025*	0.029
*Northern provenance regions**	*0.021*	
4. Diversity among populations within provenance regions	0.08	
*Southern populations**	*0.08*	0.008
*Northern populations**	*0.09*	
5. Total diversity within populations	0.913	

* Computed separately for northern and southern part of the natural range

Table 7. Matrix of Nei's unbiased estimates of genetic distances between southern provenance regions (NEI 1978).

Provenance region		1	2	3	4	5	6	7	8
1	Calabria	***							
2	Macedonia & Serbia	.013	***						
3	Bulgaria	.010	.003	***					
4	Southern Carpathians	.019	.008	.004	***				
5	Eastern Carpathians	.011	.005	.003	.002	***			
6	Slovakia	.017	.009	.014	.019	.013	***		
7	Slovenia	.033	.018	.026	.032	.027	.004	***	
8	Czech Republic	.037	.020	.031	.037	.031	.005	.001	***

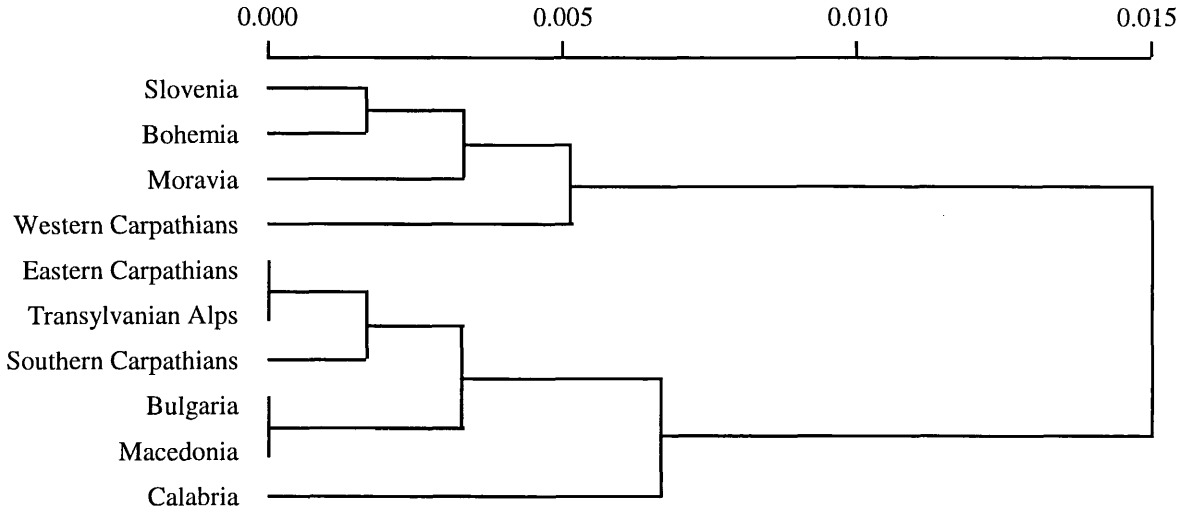

Figure 1. UPGMA dendrogram based on unbiassed genetic distances (NEI 1978) for the sets of southern and northern populations of *A. alba.*

(D_{max} = 0.036, 12 loci, PAULE *et al.* 1995) or Norway spruce (D_{max}= 0,045, 22 loci, LAGERCRANTZ & RYMAN 1990) throughout its incomparably larger natural range. High differentiation of fir populations is due to. Genetic diversity and differentiation of studied populations are influenced by the both area specific and clinal patterns of isozyme variation, which can be explained by:

- Phylogenetic divergence between Glacial refugia and populations established along different Postglacial migration routes,
- Former genetic contacts with neighboring fir species – *A.nebrodensis* in Calabria, *A. borisii-regis A. cephalonica* and *A. bornmuelleriana* in the Balkan Peninsula (FADY 1995).
- Long-term adaptation to prevalent site conditions, *e.g.* while the natural range of silver fir is influenced by the Mediterranean climate in Calabria (MENGUZZATO 1988), there is a mountain climate in the Balkan Peninsula (Macedonia, Bulgaria, GAGOV 1985) and even a continental mountain climate in the Southern Carpathians (LONGAUER 1996).

As to the clinal variation, distinction between the effects of genetic drift during postglacial migration, and non-neutral variability (adaptation to different site conditions), would require a more detailed analysis of genetic structures along phylogenetically independent climatic gradients. The adaptive significance of isozyme variation in *Idh-B* was proved, as mentioned in the above, by BERGMANN & GREGORIUS (1993). Clinal distribution of allele frequencies in 7 out of 18 surveyed loci may indicate thus, at least in some of the loci, genetic adaptation to prevalent site conditions. Such adaptation may contribute to a vulnerability (lower adaptability) to a more dramatic change of climatic conditions. Higher genetic diversity may buffer this potential threat in the southern populations of silver fir, however. Several alleles which are rare in Central Europe and also area-specific alleles, occur with frequencies high enough (>10 %) to consider them a part of operating genetic potential there.

Differences in the area-specific alleles and genetic diversity suggest phylogenetic divergence of Carpathian and Bulgarian populations of silver fir. This observation corresponds well with analyses of pollen and macroscopic remnants proving a very early presence of silver fir (8000-6000 years B.C.) in the Northeastern Carpathians (OPRAVIL 1976, ŚRODOŃ 1983, KRIPPPEL 1986) but its late dissemination in Southern Carpathians (ENESCU 1995 and references therein). A refugial area of silver fir eastwards to the Carpathian Mts. would explain the limited number area-specific alleles shared between Bulgarian and Romanian populations. The shared area-specific alleles would be a consequence of introgression (ancient genetic contacts) rather than common Glacial and Postglacial phylogeny of these populations.

In the Dinaric region, only 4 out of 10 area-specific genes detected in Macedonia were present also in Slovenia. The sample sizes in Slovenia are big enough for reliable (95 %) conclusions about a presence or absence of alleles even if their expected frequencies are less than 0.5 %. This allows thus to conclude about a limited gene flow from southern part of the Dinaric Mts. further northwards. Genetic structure of populations from the central part Dinarids (Bosnia and Monte Negro) has not been analyzed yet, however.

Apparently higher genetic diversity revealed in the vigorous southern populations, confirms LARSEN's hypothesis about the primarily genetic background of the dieback of European silver fir. Clinal variation patterns, high heterozygosity and relatively big genetic differentiation of southern populations of silver fir, correspond well also with the knowledge obtained from the provenance experiments and analyses of monoterpene, morphological and eco-physiological variability. Similar trends in the isozyme and phenotypic variability confirm applicability of isozyme gene markers in the delineation of provenance regions, seed zones, and designs of gene conservation strategies of the species.

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