GENETIC ANALYSIS OF SILVER-FIR POPULATIONS IN THE NORTH CARPATHIAN AND SUDETEN MOUNTAINS

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ABSTRACT

Seeds collected from individual trees in the 16 Carpathian and 2 Sudeten silver-fir (*Abies alba*) populations were studied with the starch gel electrophoresis in megagametophytes using 14 enzyme systems with 28 loci. The results show that the geographical distance between populations are in a small part reflected in genetic distances. There are two main groups of populations: Sudeten and Carpathian with a very big genetic distance between them. Other populations consist of a few small groups with low gene flow between them (N_m = 3.286). About 80% of genetic variation is located within populations (F_{ST} = 0.223). Average values for genetic multiplicity and diversity for Carpathian populations are as follows: number of alleles per locus: *Na* = 2.308, with effective number of alleles *Ne* = 1.552 and proportion of polymorphic loci 71.21%. The mean number of alleles per locus (*Na*) varied from 2.107 to 2.607 in population. The mean effective number of alleles per locus (*Ne*) ranged from 1.429 to 1.662. Average F_{is} for Carpathian populations was -0.021, which means that there is small excess of heterozygotes. The average observed heterozygosity amounted to *Ho* = 0.275 and expected heterozygosity was *He* = 0.269. The dendrogram structure and presence of rare alleles found in silver-fir of Czech, and Slovakian populations allow for a hypothesis that in postglaciation the silver-fir moved into the Polish Carpathians not westward from the east but from the south along river valleys from some Balkan refuges, getting North bypassing the High Tatra Range. This way, a highly diversified set of populations originated, differ in the presence of rare alleles. This differentiation is not prevented by a relatively small flow of genes between populations. The calculated gene flow N_m = 3.286 also indicates isolation between the populations. It means 3.3 immigrants per generation into the studied populations.

KEY WORDS: *Abies alba*, silver-fir isozymes, heterozygosity, genetic diversity, genetic distance, Carpathian Mts., Sudeten Mts.

INTRODUCTION

The northern and eastern limits of the occurrence of silver-fir (*Abies alba* Mill.) passes through Poland's territory with the Sudeten and Carpathian Mountains (Fig. 1). The Polish north Carpathian arc is formed by different groups of mountain chains called Western and Eastern Beskids. Many of these mountains are separated by deep valleys formed in the last glaciation. This type of orographic formation of the Beskids results in differentiations among forest populations including the silver-fir populations (Mejnartowicz 2003).

Till the end of the 17th century silver-fir was common in the forests of the Sudeten and Carpathians. Then, predatory exploitation, improper forest management, environment pollution, and some unclear natural factors led to the species decline, known as "silver-fir declining", on the entire area of European forests. Since about 1995 some recovery of the silver-fir has been noticed on the territory of Polish Carpathians.

In the western Sudeten Mts and in a major part of Saxony mountains, silver-fir became extinct and as a result appeared an anthropogenic disjunction in the population range of this species (Mejnartowicz 1983). It was believed that one of the reasons for the decline in the silver-fir stand was the species significant homozygosity (Kantor and Vincent 1970; Larsen 1986; Obmiński 1977), as silver-fir possesses one of the heaviest pollen grains. This should have caused high degree of self-pollination resulting in self-fertilization. This hypothesis was discredited in the early isozymes studies on the genetic structure of the silver-fir population (Mejnartowicz 1979). Populations of the species exhibited great genetic variation and diversity, with values comparable with those described in pine populations, and even higher than in spruce populations (Bergmann et al. 1990; Breitenbach-Dorfer et al. 1997; Konnert et al. Berg-

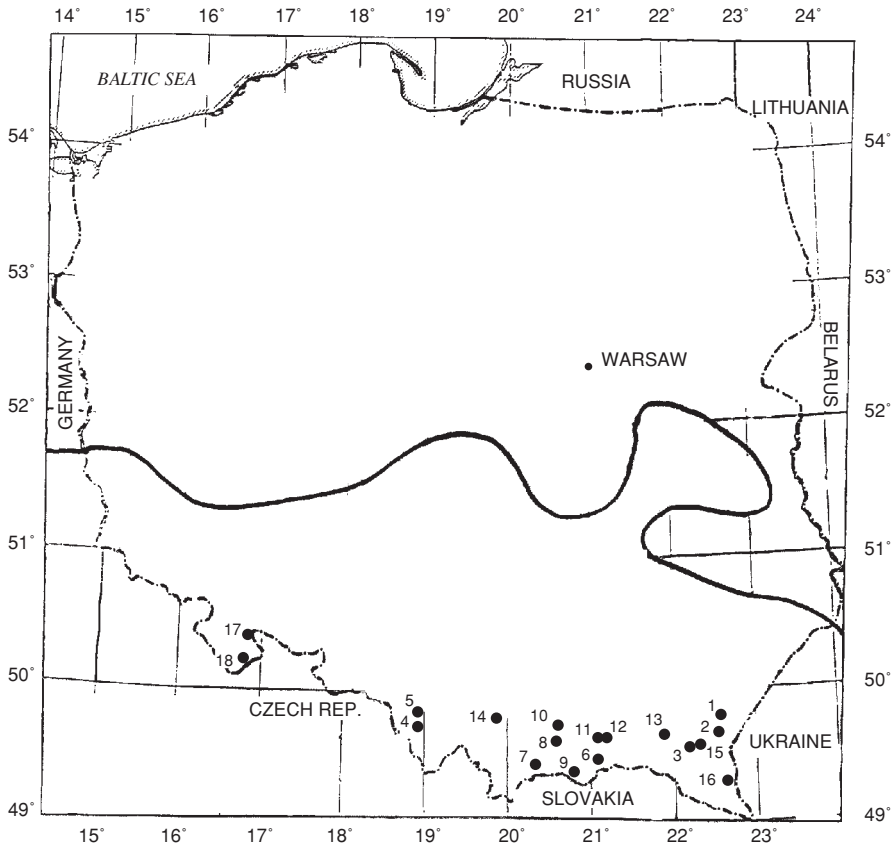

Fig. 1. North-eastern border of silver-fir distribution. Location of the investigated population 1-18.

mann 1995; Lewandowski et al. 2001; Liepelt et al. 2002; Mejnartowicz 1979a, 1996, 2000; Schroeder 1989).

MATERIAL AND METHODS

Seed samples for the analysis came from 16 silver-fir populations from the Eastern and Western Beskids. For the genetic distance comparison data published earlier were included on the two Sudeten populations (Mejnartowicz 2000). Single-tree samples were collected from a minimum 20 trees per population. In all populations the sampled trees were from 80 to 160 years old. Geographic location and coordinates of the studied populations as well as age of the trees are shown in Table 1 and Figure 1.

Each tree was genotyped using at least six megagametophytes. Isoenzyme was separated by horizontal starch gel (11.5%) electrophoresis in a discontinuous buffers system. For the method literature review, see Mejnartowicz (2000) and Mejnartowicz and Bergmann (2003). In the presented work, allele numbering corresponds to isoenzyme *Rf*: the fastest allozyme migrating toward anode was marked 1, next – 2, and so on. To estimate genetic variation the following parameters were used: mean number of alleles per lo-

TABLE 1. Geographic coordinate and the tree age of investigated silver-fir populations.

Population name	Geographic region	Latitude	Long.	Altiude [m]	Tree / Age
1. Krasiczyn–Cisowa	Pogórze Przemyskie	49°43’	23°38’	360	100
2. Bircza–Wojtkówka	Pogórze Przemyskie	49°34’	22°34’	425-500	100
3. Lesko–Malinki	Góry Słonne	49°30’	22°17’	320-340	110
4. Ustroń–Bukowa 2	Beskid Śląski	49°40’	18°51’	550	150
5. Ustroń–Bukowa 1	Beskid Śląski	49°40’	18°51’	670	150
6. Krynica–Tylicz	Beskid Sądecki	49°22’	20°57’	625	120
7. Szczawnica–Krościenko	Beskid Sądecki	49°25’	20°29’	610	100
8. Stary Sącz–Przysietnica	Beskid Sądecki	49°30’	20°37’	590	140
9. Piwniczna–Łomnica	Beskid Sądecki	49°27’	20°45’	580	140
10. Stary Sącz–Kamieniec	Beskid Sądecki	49°34’	20°38’	385	120
11. Nawojowa–Berest I	Beskid Niski	49°33’	20°57’	625-760	105
12. Nawojowa–Berest II	Beskid Niski	49°33’	20°57’	575-675	110
13. Rymanów–Szachty	Beskid Niski	49°26’	21°53’	500	120
14. Myślenice–Ukleina	Beskid Makowski	49°49’	19°58’	260-540	120
15. Lesko–Czarny Dział	Góry Słonne	49°30’	22°24’	500	100
16. Stuposiany–Czereszenka	Bieszczady	49°09’	22°41’	640-740	135
17. Międzylesie	Góry Bystrzyckie	50°14’	16°46’	750	100-140
18. Lądek Zdrój	Góry Złote	50°21’	16°52’	700	80-160

cus (*Na*), effective number of alleles at locus (*Ne*), number of rare alleles (*NoRa*) with the p < 5%, per cent of polymorphic loci (*%Pol*), Shannon's index of genetic diversity (*I*), observed (*Ho*) and expected (*He*) heterozygosity, Wright's fixation index (*F*), and gene flow (*Nm*). The statistical analysis of allele frequencies and cluster analysis were performed with the Yeh and Yang (1999) POGENE-32 program.

RESULTS AND DISCUSSION

The electrophoretic analysis of the 14 enzymes in megagametophytes of European silver-fir from the north Carpathians revealed 85 alleles coded in 28 isozymes loci (Table 2). Allele frequencies of the analyzed loci are given in the Table 3. Only in one locus of the twenty-eight studied – MEN2 – no variation was detected, neither in the north Carpathians, Sudeten, nor in the Thuringen populations (Hosius at al. 2000; Lewandowski at al. 2001; Mejnartowicz 2000). Variation in this locus was described by Longauer (1994) in many south and east Carpathians populations. Allele MEN-1 was found exclusively in the Krynica–Tylicz population. In the studied populations we found variation in loci PGI1, PGI2 and SOD1, which were previous considered to be monomorphic. From the twenty--eight studied loci, six were semi-monomorphic: GDH, GOT1, PGM1, SOD1, and PGI1 (Table 2). The last locus, PGI1, was without any variation in all populations from central and southern Germany (Konnert 1994), east Poland (Mejnartowicz 1996) as well as in Sudeten Mts (Lewandowski et al. 2001).

COMPARISON EASTERN AND WESTERN CARPATHIANS POPULATIONS

From the east Carpathians 5 populations were examined: Krasiczyn, Bircza, Lesko–Malinki, Lesko–Czarny Dział, and Stuposiany. Only in Stuposiany and Krasiczyn populations variations were found in locus Pgm1 (Table 3). In all studied population, silver-fir locus SOD1 was monomorphic, but in the population Bircza–Wojtkówka two alleles exist: common Sod1-2 and private Sod1-1. Allele Sod1-1 distinguished Bircza population from the other populations. In the east Carpathian populations there was smaller allelic multiplicity at the considered loci (*Na* = 2.286) than in the western Carpathian populations (*Na* = 2.318) but genetic diversity was greater (*I* = 0.454), and higher than expected heterozygosity (*Ho* = 0.29 vs. *Ho* = 0.27) and percentage of polymorphic loci (72.14%) than was observed in the western Carpathian populations (70.78) (Table 4). Among the 11 examined western Carpathian silver-fir populations the smallest genetic variation and diversity was found in the population from Szczawnica–Krościenko in the Sądecki Beskid. It had the smallest number of alleles: *Na* = 2.107 and *Ne* = 1.429, and extremely small number of rare alleles: *NoRa* = 2 (p < 5%), and the lowest expected heterozygosity *He* = 0.238. These are the lowest values both for the eastern and western Carpathian populations (Table 4). Significant differences in the number of alleles can be seen in the Beskid Sądecki populations – from the lowest values in Szczawnica population to the highest in Krynica–Tylicz populations (*Na* = 2.536). Populations from Beskid Niski and Beskid Makowski are characterized by the number of alleles per locus (*Na* = 2.25 and *Na* = 2.179 respectively) smaller than average for the western

TABLE 2. Enzyme systems assayed in macrogametophytes of individual silver-fir trees.

Enzyme system	Abbre.	E.C. number	Invest. Loci	Allele no.	No. of frequent allele
Alcohol dehydrogenase	ADH	E.C. 1.1.1.1	ADH1	5	3
			ADH2	3	2
Esterase	EST	E.C. 3.1.1.1	EST2	6	3
			EST3	8	5
Fluorescence esterase	FLE	E.C. 3.1.1.2	FLE1	4	2
			FLE2	5	2
Glutamate dehydrogen.	GDH	E.C. 1.4.1.2	GDH1	2	2
Glutamate-oxaloacetate-transaminase	GOT (AAT)	E.C. 2.6.1.1	GOT1	3	2
			GOT2	3	1
			GOT3	5	3
			GOT4	5	4
Isocitrate dehydrogenase	IDH	E.C. 1.1.1.42	IDH1	4	3
			IDH2	3	1; 3
Leucine aminopeptidase	LAP	E.C. 3.4.11.1	LAP1	4	2
			LAP2	8	4; 5
Malate dehydrogenase	MDH	E.C. 1.1.1.37	MDH1	5	2
			MDH2	5	1; 3
			MDH3	3	1
Menadione reductase	MEN (DIA)	E.C. 1.6.4.3	MEN1	3	1
			MEN2	1	1
Phosphoglucomutase	PGM	E.C. 5.4.2.2	PGM1	3	2
			PGM2	2	2
Phosphoglucose isomerase	PGI	E.C. 5.3.1.9	PGI1	2	2
			PGI2	4	2; 3
6-Phosphogluconic dehydrogenase	6-PGD	E.C. 1.1.1.44	6PGD1	4	2; 3; 4
			6PGD2	6	2; 4
Shikimate dehydrogen.	SHDH	E.C. 1.1.1.25	SHDH1	4	1
Superoxide dismutase	SOD	E.C. 1.15.1.1	SOD1	2	2

TABLE 3. Allele frequencies in the macrogametophytes of 18 *Abies alba* populations. For population names see Table 1.

		Populations																	
Locus	Allele	KC 1	BW 2	LM 3	UB2 4	UB1 5	KT 6	Sz 7	Prz 81	Pi 9	SK 10	NB-1 11	NB-2 12	RS 13	MU 14	LC 15	ST 16	LZ 17	Mg 18
ADH1	**1**	0	0	0	0.075	0	0.050	0	0.125	0.050	0.125	0.050	0	0	0	0.075	0	-	-
	2	0.025	0	0	0.025	0.075	0.075	0.111	0.025	0.025	0	0	0.050	0.050	0	0.025	0	-	-
	3	0.975	1	1	0.850	0.925	0.875	0.806	0.850	0.900	0.850	0.925	0.900	0.950	1	0.875	0.975	-	-
	4	0	0	0	0.025	0	0	0	0	0	0	0	0.025	0	0	0	0.025	-	-
	5	0	0	0	0.025	0	0	0.083	0	0.025	0.025	0.025	0.025	0	0	0.025	0	-	-
ADH2	**1**	0	0	0	0.025	0.150	0.075	0	0.025	0	0	0.025	0	0.050	0.150	0.025	0.175	-	-
	2	1	1	0.975	0.950	0.800	0.900	0.972	0.950	1	1	0.950	0.950	0.950	0.850	0.975	0.825	-	-
	3	0	0	0.025	0.025	0.050	0.025	0.028	0.025	0	0	0.025	0.050	0	0	0	0	-	-
EST2	**1**	0.225	0.100	0.425	0.225	0.075	0.375	0.056	0.375	0.150	0.225	0.175	0.200	0.250	0.050	0.325	0.175	-	-
	2	0	0	0	0	0.025	0	0	0	0	0	0.050	0	0	0	0	0	-	-
	3	0.750	0.800	0.550	0.700	0.800	0.550	0.888	0.500	0.675	0.775	0.725	0.725	0	0.900	0.650	0.775	-	-
	4	0.025	0.100	0.025	0.075	0.100	0.075	0	0.125	0.125	0	0.050	0.050	0.100	0.050	0.025	0.050	-	-
	5	0	0	0	0	0	0	0	0	0	0	0	0	0.650	0	0	0	-	-
	6	0	0	0	0	0	0	0.056	0	0.050	0	0	0.025	0	0	0	0	-	-
EST3	**1**	0	0	0	0.025	0.050	0	0	0	0	0	0	0	0	0.075	0	0	0	0
	2	0.025	0.050	0	0	0.025	0	0	0	0	0.025	0.025	0	0.050	0	0.025	0.050	0.029	0
	3	0.475	0.325	0.500	0.300	0.150	0.350	0.278	0.425	0.125	0.325	0.600	0	0	0.200	0.375	0.175	0.059	0.075
	4	0	0	0	0	0.025	0	0	0	0	0	0	0.475	0.525	0	0	0	0	0
	5	0.475	0.500	0.250	0.625	0.575	0.500	0.611	0.500	0.775	0.500	0.325	0.300	0.250	0.650	0.475	0.425	0.883	0.925
	6	0.025	0.025	0.187	0.050	0.150	0.125	0.111	0.075	0.075	0.100	0.050	0.125	0.125	0.025	0.125	0.325	0.029	0
	7	0	0.100	0.063	0	0.025	0.025	0	0	0.025	0.050	0	0.100	0.025	0.050	0	0.025	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0	0.025	0	0	0	0	0
FLE1	**1**	0	0	0.050	0	0	0.050	0	0	0	0	0.125	0	0	0	0.075	0	0	0
	2	1	1	0.850	0.950	0.975	0.925	1	0.975	0.850	1	0.875	0.975	1	1	0.925	1	1	1
	3	0	0	0.025	0.050	0	0	0	0	0.100	0	0	0	0	0	0	0	0	0
	4	0	0	0.075	0	0.025	0.025	0	0.025	0.050	0	0	0.025	0	0	0	0	0	0
FLE2	**1**	0.175	0.100	0.300	0.275	0.075	0.075	0.056	0.300	0.150	0.125	0.150	0.150	0.175	0.050	0.175	0.175	-	-
	2	0.800	0.875	0.700	0.700	0.875	0.825	0.944	0.700	0.800	0.850	0.800	0.850	0.750	0.950	0.800	0.825	-	-
	3	0.025	0.025	0	0.025	0.050	0.100	0	0	0	0.025	0	0	0.075	0	0.025	0	-	-
	4	0	0	0	0	0	0	0	0	0	0	0.050	0	0	0	0	0	-	-
	5	0	0	0	0	0	0	0	0	0.050	0	0	0	0	0	0	0	-	-
GDH	**1**	0	0.025	0	0	0	0.075	0	0	0.025	0	0	0	0	0.025	0	0.025	0	0
	2	1	0.975	1	1	1	0.925	1	1	0.975	1	1	1	1	0.975	1	0.975	1	1
GOT1	**1**	0	0	0	0	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0
	2	1	1	1	1	1	1	1	0.975	1	1	1	1	1	1	1	1	0.941	1
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.059	0
GOT2	**1**	0.800	0.975	0.850	0.975	0.975	1	0.944	0.975	1	0.950	0.925	0.975	0.950	1	1	0.950	0.971	0.850
	2	0.150	0	0	0	0.025	0	0	0.025	0	0.050	0.025	0	0.050	0	0	0.025	0	0.100
	3	0.050	0.025	0.150	0.025	0	0	0.056	0	0		0.050	0.025	0	0	0	0.025	0.029	0.050
GOT3	**1**	0.300	0.400	0.225	0.200	0.375	0.250	0.083	0.075	0.275	0.225	0.300	0.275	0.375	0.475	0.125	0.225	-	-
	2	0	0.025	0	0	0	0	0	0	0.025	0	0	0	0	0	0	0.025	-	-
	3	0.425	0.350	0.550	0.675	0.375	0.400	0.834	0.725	0.500	0.425	0.375	0.400	0.400	0.375	0.525	0.325	-	-
	4	0.025	0.125	0.075	0	0.075	0.075	0	0.125	0.025	0.150	0.150	0.225	0.075	0.025	0.275	0.100	-	-
	5	0.250	0.100	0.150	0.125	0.175	0.275	0.083	0.075	0.175	0.200	0.175	0.100	0.150	0.125	0.075	0.325	-	-
GOT4	**1**	0.150	0.175	0.200	0.075	0.100	0.175	0.278	0.300	0.175	0.125	0.150	0.125	0.225	0.250	0.100	0.125	-	-
	2	0.050	0.025	0.150	0.050	0	0	0	0	0.050	0	0.050	0	0.025	0	0.150	0.050	-	-
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
	4	0.800	0.800	0.650	0.875	0.850	0.825	0.722	0.700	0.750	0.875	0.800	0.875	0.750	0.750	0.750	0.800	-	-
	5	0	0	0	0	0.050	0	0	0	0.025	0	0	0	0	0	0	0.025	-	-
IDH1	**1**	0	0	0	0	0.050	0	0	0	0	0	0	0	0	0.025	0	0	0.029	0
	2	0.325	0.150	0.300	0.275	0.175	0.125	0.139	0.225	0.125	0.250	0.275	0.275	0.250	0.375	0.350	0.225	0.029	0.200
	3	0.500	0.700	0.450	0.450	0.425	0.625	0.722	0.500	0.450	0.450	0.475	0.350	0.375	0.525	0.400	0.550	0.677	0.525
	4	0.175	0.150	0.250	0.275	0.350	0.250	0.139	0.275	0.425	0.300	0.250	0.375	0.375	0.075	0.250	0.225	0.265	0.275
IDH2	**1**	0.525	0.575	0.375	0.400	0.400	0.525	0.472	0.425	0.425	0.475	0.425	0.475	0.200	0.500	0.450	0.575	0.088	0.275
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.025
	3	0.475	0.425	0.625	0.600	0.600	0.475	0.528	0.575	0.575	0.525	0.575	0.525	0.800	0.500	0.550	0.425	0.912	0.700
LAP1	**1**	0.050	0	0	0	0.025	0	0.056	0	0.025	0	0	0	0	0	0.025	0.075	0.088	0
	2	0.925	0.975	0.975	0.875	0.975	0.950	0.944	0.975	0.975	0.950	1	1	0.950	0.975	0.875	0.900	0.883	0.950
	3	0.025	0.025	0.025	0.025	0	0.050	0	0.025	0	0.050	0	0	0.050	0.025	0.100	0.025	0.029	0.050
	4	0	0	0	0.100	0	0	0	0	0	0	0	0	0	0	0	0	0	
LAP2	**1**	0	0	0	0	0	0	0.083	0	0	0	0.025	0	0	0.025	0	0.050	-	-
	2	0	0	0.150	0	0	0.025	00	0	0	0	0	0.025	0	0	0	0	-	-
	3	0	0	0	0.025	0.075	0.150	0	0	0	0	0		0	0	0	0.050	-	-
	4	0.700	0.450	0.325	0.400	0.550	0.375	0.139	0.475	0.525	0.450	0.475	0.525	0.475	0.525	0425	0.375	-	-
	5	0.300	0.500	0.400	0.550	0.350	0.400	0.639	0.500	0.425	0.500	0.500	0.450	0.500	0.375	0.475	0.500	-	-

TABLE 3. Cont.

Locus	Allele	Populations																	
		KC 1	BW 2	LM 3	UB2 4	UB1 5	KT 6	Sz 7	Prz 81	Pi 9	SK 10	NB-1 11	NB-2 12	RS 13	MU 14	LC 15	ST 16	LZ 17	Mg 18
	6	0	0.050	0.100	0.025	0	0.025	0.083	0	0.025	0.050	0	0	0	0.025	0.100	0.025	-	-
	7	0	0	0	0	0.025	0.025	0.056	0	0	0	0	0	0	0	0	0	-	-
	8	0	0	0.025	0	0	0	0	0.025	0.025	0	0	0	0.025	0.050	0	0	-	-
MDH1	**1**	0.350	0.350	0.250	0.100	0.300	0.150	0.055	0.125	0.100	0.075	0.175	0.400	0.300	0.200	0.375	0.275	0.059	-
	2	0.650	0.600	0.700	0.825	0.650	0.850	0.806	0.825	0.850	0.875	0.800	0.600	0.700	0.750	0.625	0.675	0.941	-
	3	0	0.025	0	0	0	0	0	0.025	0.025	0	0.025	0	0	0	0	0.050	0	-
	4	0	0.025	0.025	0.075	0.025	0	0	0.025	0.025	0	0	0	0	0.050	0	0	0	-
	5	0	0	0.025	0	0.025	0	0.139	0	0	0.050	0	0	0	0	0	0	0	-
	1	0.350	0.400	0.350	0.375	0.625	0.350	0.444	0.350	0.500	0.375	0.600	0.325	0.575	0.400	0.525	0.675	1	1
MDH2	**2**	0	0.025	0.075	0	0	0	0	0.050	0.050	0	0	0	0	0	0	0	0	0
	3	0.650	0.575	0.575	0.600	0.350	0.650	0.556	0.600	0.450	0.550	0.375	0.675	0.425	0.600	0.475	0.325	0	0
	4	0	0	0	0	0.025	0	0	0	0	0.075	0.025	0	0	0	0	0	0	0
	5	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MDH3	**1**	0	0	0	0	0	0	0	0	0	0	0.075	0	0	0	0	0	0	0
	2	0.975	1	1	1	1	0.875	1	1	1	0.950	0.925	0.975	1	1	1	1	1	1
	3	0.025	0	0	0	0	0.125	0	0	0	0.050	0	0.025	0	0	0	0	0	0
MEN1	**1**	0	0	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0	0
	2	0.975	1	1	1	1	0.975	1	1	1	1	1	0.975	1	1	0.975	1	1	1
	3	0.025	0	0	0	0	0	0	0	0	0	0	0.025	0	0	0.025	0	0	0
MEN2	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6-PGD-1	**1**	0	0	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0.025	0	0
	2	0.300	0.625	0.350	0.350	0.400	0.250	0.111	0.350	0.375	0.475	0.425	0.475	0.350	0.475	0.425	0.625	0.412	0.525
	3	0.400	0.025	0.375	0.250	0.500	0.275	0.611	0.375	0.550	0.250	0.100	0.400	0.500	0.025	0.400	0.325	0.441	0.475
	4	0.300	0.350	0.275	0.400	0.100	0.450	0.278	0.275	0.075	0.275	0.475	0.125	0.150	0.500	0.175	0.025	0.147	0
6-PGD2	**1**	0	0	0	0.125	0.075	0	0.139	0.075	0	0.025	0.025	0	0	0.225	0.100	0	0	0.025
	2	0.375	0.575	0.275	0.400	0.150	0.425	0.278	0.200	0.350	0.475	0.350	0.475	0.300	0.400	0.175	0.700	0	0
	3	0	0	0	0	0	0	0	0	0	0.025	0	0.025	0	0	0.025	0	0	0
	4	0.625	0.425	0.725	0.475	0.725	0.525	0.555	0.675	0.550	0.450	0.600	0.475	0.650	0.375	0.625	0.300	1	0.950
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.075	0	0	0
	6	0	0	0	0	0.050	0.050	0.028	0.050	0.100	0.025	0.025	0.025	0.050	0	0	0	0	0.025
PGI1	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.050
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.950
PGI2	**1**	0	0	0	0	0	0	0	0	0.025	0	0	0.425	0	0.175	0.025	0.050	0	0
	2	0.850	0.575	0.550	0.350	0.650	0.425	0.389	0.300	0.425	0.250	0.625	0.475	0.525	0.650	0.475	0.575	0	0
	3	0.150	0.425	0.450	0.650	0.350	0.475	0.611	0.675	0.550	0.750	0.375	0.100	0.475	0.175	0.500	0.375	1	1
	4	0	0	0	0	0	0.100	0	0.025	0	0	0	0	0	0	0	0	0	0
PGM1	**1**	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.025	0	0
	2	0.975	1	1	1	0.950	0.975	1	1	1	1	1	1	1	1	0.975	0.975	1	1
	3	0	0	0	0	0.050	0.025	0	0	0	0	0	0	0	0	0.025	0	0	0
PGM2	**1**	0.050	0.350	0.275	0.025	0.125	0.050	0.194	0.175	0.075	0.150	0.100	0.150	0.100	0.250	0.175	0.125	0.029	0.025
	2	0.950	0.650	0.725	0.975	0.875	0.950	0.806	0.825	0.925	0.850	0.900	0.850	0.900	0.750	0.825	0.875	0.971	0.975
ShDh	**1**	0	0	0.025	0	0.075	0.050	0	0	0	0	0	0	0	0	0.025	0	0	0
	2	0.975	0.825	0.950	0.775	0.825	0.900	0.944	1	0.975	0.975	0.925	0.975	1	0.975	0.975	0.825	0.676	0.700
	3	0	0	0	0.175	0.075	0.025	0.056	0	0.025	0	0.050	0	0	0	0	0	0.324	0.300
	4	0.025	0.175	0.025	0.050	0.025	0.025	0	0	0	0.025	0.025	0.025		0.025	0	0.175	0	0
SOD1	**1**	0	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	0.975	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

* Population numbers are explained in Table 1.

Carpathians. On average in the Carpathian silver-fir populations we find *Na* = 2.308 and *Ne* = 1.552, which is similar to the data for Germany as presented by Hussendörfer and Konnert (2000).

The Sudeten silver-fir populations have significantly smaller than Carpathian numbers of observed (*Na* = 1.731) and effective alleles (1.227) (Table 4). Heterozygosity is also higher in the Carpathian populations (*Ho* 0.275, *He* = 269) than in the Sudeten populations where *Ho* = 130 and *He* =0.129 (Table 4). Müller-Starck (1993) and Ziehe and Hattemer (1999) presented results that selection under field conditions is characterized by a viability advantage of those individuals with a large degree of heterozygosity. It was concluded that the value of estimated (*Ho*) and expected (*He*) heterozygosity in a population depends on the age, space, and species composition of the stand (Bergmann 1995; Hussendörfer and Konnert 2000). Evaluation of heterozygosity depends however on the type of analyzed material. Similar heterozygosity values are described for Polish, German, and Italian populations (Bergmann 1995, 1996; Mejnartowicz 1996) when megagametophytes are analyzed. Lower heterozygosity values appear in publications analyzing bud material (Konnert 1994; Lewandowski et al. 2001; Scaltsoyiannes et al. 1999). In the provenance tests the offspring of Szczawnica population is characterized by high survival rate while Lesko–Czarny Dział popu-

TABLE 4. Summary of Genetic Variation and Heterozygosity for the Polish Carpathians and Sudetes Mts populations of silver-fir.

Population		*Na**	*Ne*	*I*	*Ho*	*He*	F_{is}	*%PoL*	*NoRa*
1. Krasiczyn–Cisowa		2.143	1.500	0.407	0.248	0.253	-0.023	75.00	12
2. Bircza–Wojtkówka		2.214	1.524	0.423	0.248	0.262	0.013	67.86	11
3. Lesko–Malinki		2.286	1.662	0.491	0.329	0.300	-0.071	67.86	10
15. Lesko–Czarny Dział		2.357	1.605	0.479	0.366	0.291	-0.163	75.00	18
16. Stuposiany–Czereszenka		2.429	1.583	0.472	0.250	0.281	0.049	75.00	18
Mean for East Carpathians		**2.286**	**1.575**	**0.454**	**0.29**	**0.277**	**-0.039**	**72.14**	**13.8**
	SD	0.113	0.065	0.037	0.056	0.020	0.082	3.911	3.9
4. Ustron–Bukowa 2		2.392	1.537	0.455	0.259	0.273	0.023	71.43	16
5. Ustron–Bukowa 1		2.607	1.562	0.484	0.234	0.276	0.080	75.00	16
Mean for Beskid Śląski		2.499	1.55	0.47	0.25	0.275	0.051	73.22	16
6. Krynica–Tylicz		2.536	1.625	0.501	0.295	0.295	-0.018	82.14	16
7. Szczawnica–Krościenko		2.107	1.429	0.397	0.298	0.238	-0.153	67.86	2
8. Stary Sącz–Przysietnica		2.250	1.538	0.440	0.277	0.269	-0.020	71.43	12
10. Stary Sącz–Kamieniec		2.214	1.555	0.432	0.250	0.259	0.035	67.86	13
9. Piwniczna–Łomnica		2.464	1.517	0.443	0.266	0.259	-0.034	67.86	13
Mean for Beskid Sądecki		2.314	1.533	0.443	0.28	0.264	-0.038	71.43	11.2
11. Nawojowa–Berest 1		2.393	1.548	0.456	0.279	0.273	0.035	71.43	18
12. Nawojowa–Berest 2		2.250	1.588	0.443	0.261	0.270	-0.005	75.00	14
13. Rymanów–Szachty		2.107	1.551	0.428	0.257	0.262	-0.019	64.29	10
Mean for Beskid Niski		2.25	1.562	0.442	0.27	0.268	0.0037	70.24	14
14. Myslenice–Ukleina (Beskid Makowski)		2.179	1.504	0.409	0.289	0.249	-0.070	64.29	15
Mean for West Carpathians		**2.318**	**1.541**	**0.444**	**0.270**	**0.266**	**-0.013**	**70.781**	**13.2**
	SD	0.171	0.050	0.030	0.020	0.015	0.062	5.251	4.3
Mean for the Carpathians		**2.308**	**1.552**	**0.448**	**0.275**	**0.269**	**-0.021**	**71.21**	**13.5**
	SD	0.144	0.050	0.029	0.033	0.015	0.065	4.63	3.5
17. Lądek Zdrój		1.762	1.208	0.208	0.132	0.120	-0.091	35.71	7
18. Międzygórze		1.700	1.246	0.228	0.127	0.138	0.010	35.71	4
Mean for the Sudetes		**1.731**	**1.227**	**0.218**	**0.130**	**0.129**	**-0.041**	**35.71**	**5.5**

Na – mean number of observed allele; *Ne* – mean number of effective allele; *I* – shanon's index of genetic diversity; *Ho* – observed heterozygosity; *He* – expected heterozygosity; F_{is} – wright' fixation index; *%PoL* – percent of polymorphic loci; *NoRa* – number of rare allele.

TABLE 5. Matrix of Nei's genetic distances between the studied *Abies alba* populations.

pop	ID*	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	***																
2	0.026	***															
3	0.022	0.031	***														
4	0.030	0.029	0.024	***													
5	0.021	0.031	0.030	0.030	***												
6	0.022	0.024	0.021	0.015	0.027	***											
7	0.043	0.041	0.033	0.022	0.034	0.027	***										
8	0.032	0.036	0.014	0.013	0.032	0.017	0.022	***									
9	0.028	0.033	0.027	0.016	0.014	0.019	0.023	0.017	***								
10	0.030	0.020	0.024	0.010	0.028	0.013	0.025	0.014	0.015	***							
11	0.017	0.020	0.018	0.020	0.022	0.017	0.037	0.024	0.026	0.017	***						
12	0.030	0.034	0.037	0.040	0.030	0.036	0.053	0.043	0.032	0.034	0.037	***					
13	0.058	0.069	0.045	0.055	0.045	0.051	0.071	0.047	0.044	0.055	0.054	0.044	***				
14	0.023	0.015	0.038	0.030	0.028	0.027	0.042	0.042	0.034	0.028	0.022	0.032	0.073	***			
15	0.021	0.027	0.013	0.018	0.017	0.021	0.027	0.013	0.018	0.016	0.016	0.028	0.044	0.032	***		
16	0.032	0.020	0.037	0.032	0.024	0.029	0.044	0.043	0.026	0.024	0.026	0.031	0.060	0.030	0.027	***	
17	0.246	0.240	0.205	0.182	0.176	0.194	0.193	0.173	0.168	0.192	0.202	0.252	0.184	0.242	0.187	0.212	***
18	0.238	0.236	0.202	0.179	0.173	0.192	0.191	0.169	0.161	0.184	0.199	0.245	0.185	0.237	0.181	0.203	0.01

* Population numbers are explained in Table 1.

lation has high death rate (Skrzyszewska 2003). It seems that low genetic load of Szczawnica population contributes to its high survival rate, while high genetic load results in high death rate in Lesko–Czarny Dział population. The latter population not only has a great number of rare alleles (*NoRa* = 18) but also high heterozygosity (*Ho* = 0.366). It is maintained that rare genes are lethal or sublethal in character and remain in populations mainly in the heterozygotic form. Indeed, all rare alleles detected in northern Carpathian and Sudeten silver-fir populations appeared only in the form of heterozygotes. According to Althukov et al. (1987), Scots pine heterozygotes with sublethal genes produce less of the vital seeds than trees with heterozygosity lower than population average. On the other hand though, presence of rare alleles is relevant for the future of a population because they may be playing an adaptative role in the changing environment. The proportion of polymorphic loci (*%Pol*), calculated as a measure of genetic variation,

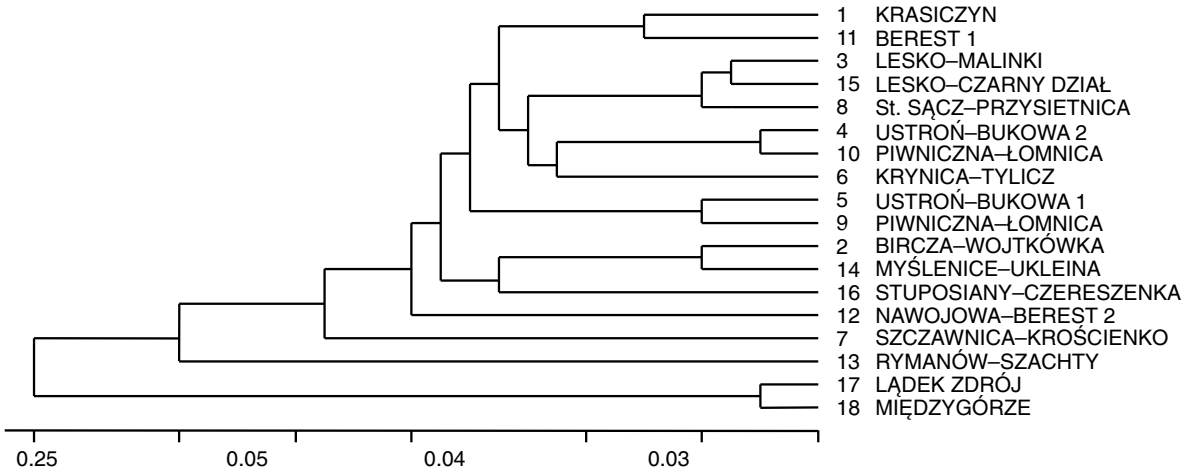

Fig. 2. UPGMA cluster analysis based on genetic distance (Nei 1972) modified from NEIGHBOR procedure of PHYLIP Version 3.5.

for Carpathian populations amounted to 71.21%, which is very similar to the results obtained by Scaltsoyiannes et al. (1999) for Mediterranean firs. The minimum *%Pol* = 64.29 was found in Rymanów and Myślenice populations, the maximum 82.14% – in Krynica–Tylicz population. For the Sudeten populations *%Pol* amounted 35.71.

The F_{ST} coefficient for 27 polymorphic loci ranged from 0.021 at MEN1 to 0.865 at MDH3 with the average F_{ST} = 0.203, which means that 20% of the total genetic variation is due to interpopulation diversity and 80% is located within populations. The inbreeding in the studied silver-fir populations amounted to 2% only. The heterogeneity of fixation indices suggests no strong influence of inbreeding on the genotypic diversity of all studied silver-fir populations (Table 4).

In the dendrogram drawn according to Nei (1978) on the basis of interpopulation genetic distances (Table 5) two main groups were observed. The first one includes Carpathian silver-fir populations, the other one – Sudeten populations. In the Carpathian group, highly differentiated, the biggest genetic distance in relation to other populations is seen between Rymanów, Szczawnica and Berest-2 populations. The remaining populations form several smaller groups (Fig. 2). The dendrogram structure and presence of rare alleles found in silver-fir of Czech, Moravian and Slovakian populations allow for a hypothesis that in postglaciation the silver-fir moved into the Polish Carpathians not westward from the east but from the south along river valleys from some Balkan refuges, getting north bypassing the High Tatra Range. This way, a highly diversified set of populations originated, differing in the presence of rare alleles. This differentiation is not prevented by a relatively small flow of genes between populations. The calculated gene flow N_m = 3.286 also indicates isolation between the populations. It means 3.3 immigrants per generation into the studied populations.

CONCLUSIONS

There is a significant diversity within Carpathian silver-fir populations. Still greater genetic distance separates Sudeten and Carpathian populations. Restricted gene flow between populations, their isolation, great differentiation in the silver-fir population environments, and different postglacial immigration routes from the Balkan Peninsula to the Carpathian area result in great interpopulation differences (F_{ST} = 0.203) shown on the dendrogram. Sudeten populations have much lower genetic diversity and great genetic distance compared with Carpathian populations. The results of isoenzyme analyses suggest that Sudeten silver-fir populations derive from populations that in glaciation found refuge on the Apennine Peninsula.

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