GENETIC VARIATION OF *ABIES ALBA* MILL. IN POLISH PART OF SUDETY MTS.

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(Received: March 6, 2001. Accepted: June 18, 2001)

ABSTRACT

The genetic structure of silver fir (*Abies alba* Mill.) was investigated among 8 populations from Sudety Mts. and 2 additional populations from other parts of Poland. Nine enzyme systems controlled by 13 allozyme loci were analyzed using starch gel electrophoresis. The mean expected heterozygosity, ranging from 0.06 to 0.11 and was lower compared to that found in other conifers. The mean genetic distance among all silver fir populations was 0.005. The Sudeten group of populations appeared to be genetically different from the two populations from other parts of Poland, indicating that at least two different centers are responsible for the current distribution of allozyme polymorphism in the tested populations.

KEY WORDS: *Abies alba*, allozymes, genetic diversity, differentiation.

INTRODUCTION

Silver fir (*Abies alba* Mill.) in Poland attains the northern limit of its range. It is a mountain species, but it also disperse down to the lowlands. Silver fir is common in the Carpathians, Świętokrzyskie Mts. and in central and southern parts of Roztocze. In other regions of the country it occurs rarely, usually as an admixture in deciduous or mixed forests (Boratyński 1983). The fir-stands in the Sudety Mts. cover scarcely 0.2% of forest area and has a declining tendency. The health condition of the majority of these stands is unsatisfactory or simply bad (Boratyński and Filipiak 1997). Participation of Silver fir in Sudety Mts. was strongly reduced as a result of human activities (e.g. clear cuttings and reforestation with Norway spruce). During the last 50 years, the process of eliminating silver fir populations was enhanced by environmental pollution (Mejnartowicz et al. 1994). The reduction of forest areas resulted in a fragmentation of silver fir stands into many small sub-populations leading to the reduction of population sizes. In order to preserve the natural Sudeten populations of *A. alba*, a long-term conservation strategy is being elaborated (Boratyński, personal communication). However, designing efficient gene conservation strategies requires the information on the distribution of genetic diversity within and among populations. Allozyme markers have been useful in many aspects of forest genetics,

including applications in a wide range of genetic conservation situations (Millar and Westfall 1992). Nevertheless, the genetic structure of silver fir populations from Polish part of Sudety Mts. has been little studied (Mejnartowicz et al. 1994; Mejnartowicz 1996). Most of the previous works on this species have focused on populations from other parts of Europe (Kormutak et al. 1982; Schroeder 1989; Bergmann et al. 1990; Longauer 1994; Konnert and Bergmann 1995; Matusova 1995; Parducci et al. 1996; Breitenbach-Dorfer et al. 1997; Faddy et al. 1999; Hussendörfer 1999). The allozyme studies revealed a pronounced differentiation among European populations, with regional divergence remarkably higher than in many other conifer species.

The aim of the present work was to determine the amount and distribution of genetic variability in the *A. alba* populations from Polish part of Sudety Mts. by means of isozyme analysis.

MATERIALS AND METHODS

Laboratory analyses were performed using dormant buds collected in the winters of 1999. Samples from 8 populations: Rębiszów (1), Szrenica (2), Lubawka (3), Dobromyśl (4), Okrzeszyn (5), Łądek (6), Międzyzlesie (7), Prudnik (8) originated from different regions of Sudety

Fig.1. Location of the investigated populations, with distribution of *A. alba* in Poland.

Mts. (Fig. 1). For comparison, we also collected the material from two populations of two other regions. The South-Central region was represented by population Sucha Beskidzka (9) and South-Eastern region by Lesko (10). For each population approximately 30 trees were sampled (313 in total). The following 9 enzyme systems were assayed (Enzyme Commission number and abbreviations of scored locus/loci in parenthesis): fluorescent esterase (EC 3.1.1.1, Fle1), glutamate oxaloacetate transaminase (EC 2.6.1.1, Got1), isocitrate dehydrogenase (EC 1.1.1.42, Idh2), leucine aminopeptidase (EC 3.4.1.1.1, Lap1), menadione reductase (EC 1.6.99.2, Men1, Men2), 6-phosphogluconate dehydrogenase (6Pgdl, 6Pgdl2), phosphoglucose isomerase (EC 5.3.1.9, Pgi1, Pgi2), phosphoglucose mutase (EC 2.7.5.1, Pgm1, Pgm2), superoxide dismutase (EC 1.15.1.1, Sod1). All of the analyses were performed using 12% horizontal starch gel electrophoresis. Electrophoretic methods and staining procedures of Hussendörfer et al. (1995) and Cheliak and Pitel (1984) were followed. Alleles at each locus were numbered according to the electrophoretic mobility of allozymes. The most anodally migrating band was named 1, the next 2, and so on. Inheritance of the allozyme loci used in this study was determined earlier by Schroeder (1989) and Hassendörfer et al. (1995).

TABLE 1. Frequencies of alleles in the investigated populations.

Locus	Populations No.*									
	1	2	3	4	5	6	7	8	9	10
Fle 1										
1	1	0.98	1	1	0.98	1	1	1	1	1
2	0	0.02	0	0	0.02	0	0	0	0	0
Got 1										
1	0	0	0	0.01	0	0	0	0	0	0
2	1	1	1	0.99	1	1	1	1	1	1
Idh 2										
1	0.16	0.19	0.25	0.28	0.17	0.23	0.23	0.32	0.50	0.45
2	0.84	0.81	0.75	0.72	0.83	0.77	0.77	0.68	0.50	0.55
Lap 1										
1	0	0	0.06	0	0	0	0	0	0.02	0.08
2	1	0.98	0.91	0.97	1	0.95	0.98	0.98	0.98	0.90
3	0	0.02	0.03	0.03	0	0.05	0.02	0.02	0	0.02
Men 1										
1	0	0	0	0	0	0	0	0	0	0.02
2	1	1	1	1	1	1	1	1	1	0.98
Men 2										
1	1	1	1	1	1	1	1	1	1	1
6Pgdl 1										
1	0.36	0.55	0.44	0.46	0.50	0.58	0.63	0.37	0.32	0.31
2	0.64	0.45	0.56	0.54	0.50	0.42	0.37	0.63	0.68	0.69
6Pgdl 2										
1	0	0	0	0	0	0	0	0.02	0	0
2	1	1	0.98	1	0.97	0.98	1	0.95	0.97	0.98
3	0	0	0.02	0	0.03	0.02	0	0.03	0.03	0.02
Pgi 1										
1	1	1	1	1	1	1	1	1	1	1
Pgi 2										
1	1	1	1	1	1	1	1	1	1	1
Pgm 1										
1	0.03	0	0.05	0.01	0.05	0.02	0	0	0.03	0
2	0.97	0.98	0.95	0.98	0.95	0.98	1	1	0.97	1
3	0	0.02	0	0.01	0	0	0	0	0	0
Pgm 2										
1	0	0	0.02	0.01	0	0.02	0.02	0.03	0.03	0.16
2	1	1	0.98	0.99	1	0.98	0.98	0.97	0.97	0.84
Sod 1										
1	1	1	1	1	1	1	1	1	1	1

* Population numbers are explained in Materials and Methods.

TABLE 2. Genetic variability at 13 loci in the investigated populations (standard deviations in parentheses).

Population	N_a	N_e	P	H_o	H_e
1. Rębiszów	1.23 (0.44)	1.10 (0.25)	0.23	0.06 (0.12)	0.06 (0.14)
2. Szrenica	1.38 (0.51)	1.12 (0.29)	0.38	0.07 (0.16)	0.07 (0.16)
3. Lubawka	1.54 (0.66)	1.15 (0.30)	0.46	0.08 (0.14)	0.09 (0.16)
4. Dobromyśl	1.54 (0.66)	1.14 (0.31)	0.46	0.09 (0.20)	0.08 (0.17)
5. Okrzeszyn	1.38 (0.51)	1.12 (0.29)	0.38	0.08 (0.16)	0.07 (0.15)
6. Łądek	1.46 (0.52)	1.13 (0.29)	0.46	0.09 (0.18)	0.08 (0.16)
7. Międzyzylesie	1.46 (0.66)	1.12 (0.27)	0.38	0.08 (0.16)	0.08 (0.15)
8. Prudnik	1.46 (0.66)	1.14 (0.30)	0.38	0.09 (0.19)	0.08 (0.16)
9. Sucha Beskidzka	1.38 (0.51)	1.15 (0.33)	0.38	0.09 (0.18)	0.09 (0.17)
10. Lesko	1.54 (0.66)	1.18 (0.32)	0.46	0.11 (0.19)	0.11 (0.18)

Genetic variability was described by the average (N_a) and effective (N_e) number of alleles per locus, the percentage of polymorphic loci (P – 99% criterion), expected (H_e) and observed (H_o) heterozygosities (Nei 1975). The amount of genetic differentiation at the gene pool level was estimated using the F_{ST} measure (Wright 1951). Genetic differences between populations were measured by the genetic distance index (D) of Nei (1972). The populations were clustered based on D values using the unweighted pair group method (UPGMA) (Sneath and Sokal 1973).

RESULTS AND DISCUSSION

Genetic variation at 13 loci were studied in all investigated populations. Allele frequencies of analyzed loci are presented in Table 1. Four out of 13 loci (Men2, Pgi1, Pgi2 and Sod1) appeared to be monomorphic. These loci have been found invariant (or with very low variation) in populations of silver fir from other parts of its natural range (Longauer 1994; Konnert and Bergmann 1995; Mejnartowicz 1996; Breitenbach-Dorfer et al. 1997; Fady et al. 1999). The highest level of heterozygosity we found at Idh2 and 6Pgd1. Allozyme frequencies at these loci varied among populations (Table 1). Frequencies of allele 2 at Idh2 and allele 1 at 6Pgd1 were higher in Sudeten populations than in population from Sucha Beskidzka and Lesko. Longauer (1994) found the clinal distribution of alleles in these loci among populations of silver fir in Eastern part of its natural range. The mean frequencies of allele 2 at Idh2 locus in Sudety (0.77) and South-Eastern region of Poland (0.53) revealed in our study were very consistent to those reported by Longauer (1994) for the same regions. The similar pattern may be observed for 6Pgd1 locus.

The genetic variability measures of the investigated *A. alba* populations are given in Table 2. Generally, a low and similar level of allozyme variation was observed across populations. On average, 40% of loci were polymorphic. The mean number of alleles per locus (N_a) ranged from 1.23 in Rębiszów to 1.54 in Lubawka, Dobromyśl and Lesko, while the effective number of alleles (N_e) ranged from 1.10 in Rębiszów to 1.18 in Lesko. The observed heterozygosity, averaged over populations for all 13 loci was estimated to be 0.08. Individual population heterozygosities ranged from 0.06 in Rębiszów to 0.11 in Lesko. The mean estimates of heterozygosity, mean and effective numbers of alleles per locus, and the percentage of polymorphic loci were lower than those observed for the same species by other authors (Kormutak et al. 1982; Schroeder 1989; Bergmann et al. 1990; Longauer 1994; Matusova 1995; Parducci et al. 1996; Breitenbach-Dorfer et al. 1997; Faddy et al. 1999; Hussendörfer 1999). Comparison between our results and those presented in the literature are hampered by differences in the number and sets of loci studied. However, the low level of allozyme variation in Sudeten populations may result from a limited gene pool of populations located at the species range boarder. Longauer (1994) found, that the lowest level of heterozygosity existed in the populations from the northern parts of the species distribution.

Nine of the investigated populations showed negative fixation indices, similarly to other conifers, which usually exhibit a slight excess of heterozygotes at the adult stage. Only Rębiszów population had positive fixation index indicating a slight (nonsignificant) deficiency of heterozygotes.

The F_{ST} coefficient, estimated for polymorphic loci, ranged from 0.013 (Fle1) to 0.074 (Pgm2). The mean F_{ST} for all the 13 loci was equal to 0.049. This indicates that

TABLE 3. Matrix of Nei's genetic distance coefficients between the studied populations.

Population No.*	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	-----									
2.	0.0031	-----								
3.	0.0017	0.0019	-----							
4.	0.0021	0.0014	0.0005	-----						
5.	0.0017	0.0005	0.0014	0.0014	-----					
6.	0.0024	0.0043	0.0015	0.0011	0.0036	-----				
7.	0.0049	0.0004	0.0022	0.0016	0.0013	0.0047	-----			
8.	0.0065	0.0009	0.0036	0.0029	0.0018	0.0069	0.0005	-----		
9.	0.0102	0.0126	0.0071	0.0060	0.0122	0.0031	0.0122	0.0150	-----	
10.	0.0102	0.0133	0.0070	0.0068	0.0128	0.0038	0.0127	0.0158	0.0020	-----

* Population numbers are explained in Materials and Methods.

about 95% of the total genetic diversity of *A. alba* may be attributed to variation within populations and only about 5% is due to interpopulation diversity. The amount of genetic differentiation among investigated silver fir populations seems to be typical for conifer species. However, taking into account that we investigated only a minor part of the distribution range of silver fir, the amount of subpopulation differentiation may be considered high, compared to other European conifers (Lagercrantz and Ryman 1990; Lewandowski and Mejnartowicz 1991; Prus-Głowacki and Stephan 1994).

The average genetic distance (D) for all pair-wise comparisons between the ten analyzed populations was 0.0051, with the D values ranging from 0.0004 to 0.0158 (Table 3). The average distance within Sudeten populations was smaller ($\bar{D} = 0.0024$), ranging from 0.0004 to 0.0069. The greatest distances were found when Sudeten populations (1-8) were compared to Beskides Mts. population from Sucha Beskidzka (9) and to Bieszczady Mts. population from Lesko (10). The mean distance between Sudeten and these two populations was 0.0093. A linear relationship among geographic and genetic distance did not exist within Sudeten group of populations. However, if all populations were considered, a distinct relationship appeared ($r = 0.753$). Genetic distances among Polish silver fir populations are comparable to those reported for Italian (Vicario et al. 1995; Parducci et al. 1996), French (Fady et al. 1999) or German and Austrian (Breitenbach-Dorfer et al. 1997) *Abies alba* populations. The UPGMA dendrogram based on Nei's genetic distances revealed a weak, but distinct differentiation among the investigated populations (Fig. 2). The Sudeten group of populations formed a separate, heterogeneous cluster. The second cluster comprised the two populations from Sucha Beskidzka and Lesko. Similarly, results of provenance tests and monoterpene investigations differentiated genetically the trees of *Abies alba* of provenances from the Sudety Mts. and western regions of the Carpathians, from the Central and Eastern Carpathians (Sabor et al. 1996; Skrzyszewska 1999).

It seems, that the genetic differentiation between Sudeten group and the remaining populations can be mainly explained by re-immigration history events. During the last ice age Silver fir survived in refuges in southern Europe: the Pyrenees, Central Italy, Calabria, and the Balkan

Peninsula (Kral 1980). Historical data indicate that the Silver fir entered the present territory of Poland about 5000 years ago, probably from two centers located south of the Carpathians and Sudety Mts. (Huntley and Birks 1983; Środoń 1983). Similarly to our results, Longauer (1994) postulated that the difference he found, between populations from Moravia (eastern part of Czech Republic) and Slovakia might have a polygenetic background related to different Postglacial migration routes. The occurrence of the Balkan-specific allele 3 at the locus 6Pg2 (Schroeder 1989; Konnert and Bergmann 1995) in Sudeten populations suggests that the populations from Balkans refuge participated in the processes of establishing of those populations.

CONCLUSIONS

Silver fir populations are usually characterized by lower levels of intra-population genetic variation, and more distinct differentiation among populations, as compared to other European conifers. This trend can be confirmed by the results of this study.

ACKNOWLEDGEMENTS

We thank J. Kozłowska, M. Ratajczak and J. Samoćko for technical assistance. This work was supported by grant 6PO4G04915 from KBN.

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Fig. 2. UPGMA dendrogram based on genetic distance (Nei 1972) for the investigated populations of *Abies alba*.

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ZMIENNOŚĆ GENETYCZNA JODŁY POSPOLITEJ *ABIES ALBA* MILL. W POLSKIEJ CZĘŚCI SUDETÓW

STRESZCZENIE

Badano strukturę genetyczną 8 populacji jodły pospolitej z Sudetów oraz dwóch populacji z rejonu południowo-wschodniego Polski. Stosując rozdziały elektroforetyczne ekstraktów białkowych, z paków spoczynkowych, na żelu skrobiowym analizowano zmienność dziesięciu systemów enzymatycznych, kodowanych w 13 loci. Obliczona średnia wartość heterozygotyczności oczekiwanej była niższa, w porównaniu do innych gatunków drzew iglastych i zawierała się w granicach od 0,06 do 0,11. Średnia wartość dystansu genetycznego wg Nei'a, wyliczona pomiędzy wszystkimi populacjami, wynosiła 0,005. Stwierdzono znaczną odrębność genetyczną populacji jodeł rosnących w Sudetach od populacji z Suchej Beskidzkiej i Leska, co sugeruje istnienie w przeszłości co najmniej dwóch odrębnych centrów, z których jodła rozprzestrzeniła się na obszar dzisiejszej Polski.

SŁOWA KLUCZOWE: *Abies alba*, allozomy, zmienność genetyczna, genetyczne zróżnicowanie.