GENETIC ANALYSIS OF SILVER-FIR POPULATIONS IN THE BESKIDS[1]

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ABSTRACT

Twenty-eight isozymic loci were studied in the Beskid Mts., in four populations of common silver-fir (*Abies alba*): one in Beskid Makowski (BM) and three populations in Beskid Sądecki (BS). Their genetic variation and diversity were analyzed, and Nei's genetic distances between the populations were calculated. The results show that the geographical distance between the BM population and the three BS populations is reflected in genetic distances. The BM population is clearly distinct from the others. It has the lowest genetic diversity ($I = 0.42$), percentage of polymorphic loci ($\%PoL = 64.29$) and number of rare alleles ($NoRa = 5$). Besides, the BM population has the highest observed heterozygosity ($Ho = 0.291$), which exceeds the expected heterozygosity ($He = 0.254$), estimated on the basis of the Hardy-Weinberg Principle. On the contrary, BS populations are in the state of equilibrium, which is manifested, in similar values of $He = 0.262$ and $Ho = 0.264$.

KEY WORDS: isozymes, *Abies alba*, Beskids, populations, genetic structure, heterozygosity, genetic distances, genetic diversity, genetic variation.

INTRODUCTION

The six-hundred-kilometer chain of the Beskid Mountains forms the northern part of the Carpathians, stretching from the Czech territory across Poland and Ukraine. The western arm of the Carpathian arc is formed by the group of mountain chains called Western Beskids. They are separate, unconnected ranges, built from soft rocks of the Carpathian flysch, with gentle slopes and summits. The particular chains are separated by deep valleys formed during the last glaciation. This type of orographic formation of the Beskids causes differentiation in plant populations. The entire Beskids are an area of natural appearance of the silver-fir, which reaches out far to the north of the Beskids, entering the area of the Carpathian Uplands, Świętokrzyskie Mountains and southern Poland's uplands up to the Białowieża Primeval Forest (Mejnartowicz 1996). In the west, the silver-fir appears on the entire area of the Sudeten, entering into the area mountains of Saxony. There is, however, an anthropogenic disjunction in the silver-fir population range, resulting from the negligent forestry in the 19th and 20th centuries.

In the analysis of izoenzymatic genes, significant genetic differences were found between the silver-fir populations from the Sudeten and the Carpathians. On the dendrogram presenting genetic distances between populations the Sudeten and Carpathian populations form two separate groups (Mejnartowicz 2000). The first study on the silver-fir, in which the analysis of izoenzymes as genetic markers was used, revealed significantly greater differences between populations than those observed in the conducted then provenance tests (Mejnartowicz 1979). Later provenance tests showed great differences between silver-fir populations in their survival rate and quantitative traits. These differences may be conditioned both environmentally and genetically (Sabor et al. 1996; Skrzyszewska 1999). Below presented are the results of an izoenzymatic genetic analysis of the silver-fir population from the Makowski and Sądecki Beskids.

MATERIAL AND METHODS

Seed samples for the analysis came from four silver-fir populations from the Western Beskids. One population – Ukleina – came from the Makowski Beskid (BM), and the other three – Kamieniec, Przysietnica, Łomnica – from the Sądecki Beskid. In each population single-tree samples were collected from 20 trees. The trees in all populations were 80 to 140 years old. Geographic coordinates of the investigated silver-fir populations are shown in Table 1.

Isozyme analysis was carried out on megamaetophyte (endosperm) seed tissue on 20 trees per population. For

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TABLE 1. Geographic coordinates of the investigated silver-fir populations.

Population name	Geographic region	Lati-tude	Long.	Altitude [m]	Tree age
Ukleina (1)	Beskid Makowski	49° 49'	19° 58'	260-540	120
Kamieniec (2)	Beskid Sądecki	49° 34'	20° 36'	400-440	80
Przysietnica (3)	Beskid Sądecki	49° 30'	20° 36'	540-660	140
Łomnica (4)	Beskid Sądecki	49° 27'	20° 47'	50-730	140

each tree isozyme analysis was carried out on 7-megaga-metophyte. Enzyme was separated by starch gel (11.5%) electrophoresis in a discontinuous buffer system of Poulik (1957) and Siciliano and Shaw (1976). Isozyme analysis was performed following procedures of Bergmann and al. (1990), Konnert (1992), Mejnartowicz (1979), Mejnarto-wicz and Bergmann (1985), Pitel and Cheliak (1984) and Schröder (1989). Visualization of isozymes was mostly re-alized after Wendel and Weeden (1989).

Enzyme systems, presented in Table 2, were the same as in the studies of silver-fir populations from the Eastern Be-skids and the Sudeten (Mejnartowicz 2000), but allele numbering is different – it corresponds to enzyme *Rf* and not to the enzyme order of appearance in the analysis (Mej-nartowicz 2000): the fastest migrating allele is no.1, the slower one no. 2, etc. The statistical analysis was perfor-med with the Yeh and Yang (1999) Popgene program.

RESULTS AND DISCUSSION

Beskid Makowski Population: Ukleina (1)

In 28 investigated izoenzymatic loci of 4 silver-fir popu-lations from the Western Beskids, 4 loci were monomor-phic: MEN-A, MEN-B, PGI-A, and SOD-A. These loci had two or more alleles in the Eastern Beskids silver-fir populations (Mejnartowicz 2000). The smallest percentage of polymorphic loci (64.29%) and the smallest number of rare alleles (5) were found in the Ukleina population from the Makowski Beskid. In this population the silver-fir is also characterized by the lowest rate of genetic variability ($I =$

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

Enzyme system	Abbreviations	E.C. number	Investigated loci	Allele number	Number of frequent Allele
Alcohol dehydrogenase	ADH	E.C. 1.1.1.1	ADH-A	A-4	3
			ADH-B	B-3	2
Esterase	EST	E.C. 3.1.1.1	EST-B	B-5	3
			EST-C	C-6	5
Fluorescence esterase	FLE	E.C. 3.1.1.2	FLE-A	A-3	2
			FLE-B	B-4	2
Glutamate dehydrogenase	GDH	E.C. 1.4.1.2	GDH-A	A-2	2
Glutamate-oxalacetate-transaminase	GOT (AAT)	E.C. 2.6.1.1	GOT-A	A-2	2
			GOT-B	B-2	1
			GOT-C	C-5	3
			GOT-D	D-4	4
Isocitrate dehydrogenase	IDH	E.C. 1.1.1.42	IDH-A	A-4	3
			IDH-B	B-2	3
Leucine aminopeptidase	LAP	E.C. 3.4.11.1	LAP-A	A-3	2
			LAP-B	B-5	4; 5
Malate dehydrogenase	MDH	E.C. 1.1.1.37	MDH-A	A-5	2
			MDH-B	B-4	1; 3
			MDH-C	C-2	1
Menadione reductase	MEN (DIA)	E.C. 1.6.4.3	MEN-A	A-1	1
			MEN-B	B-1	1
Phosphoglucomutase	PGM	E.C. 5.4.2.2	PGM-A	A-1	1
			PGM-B	B-2	2
Phosphoglucoisomerase	PGI	E.C. 5.3.1.9	PGI-A	A-1	1
			PGI-B	B-4	2; 5
6-Phosphogluconic dehydrogenase	6-PGDH	E.C. 1.1.1.44	6PGD-A	A-3	1; 2; 3
			6PGD-B	B-5	1; 4
Shikimate dehydrogenase	SHDH	E.C. 1.1.1.25	SHDH-A	A-3	1
Superoxide dismutase	SOD	E.C. 1.15.1.1	SOD-A	A-1	1

TABLE 3. Results of genetic analysis of four *Abies alba* populations in the Western Beskids.

Population	Na*	Ne	I	Ho	He	Fis	% PoL	No Ra
1. Ukleina (BM)	2.25	1.51	0.42	0.291	0.254	-0.061	64.29	5
2. Kamieniec (BS)	2.21	1.55	0.43	0.250	0.259	0.038	67.96	6
3. Przysietnica (BS)	2.25	1.54	0.44	0.277	0.269	-0.020	71.43	10
4. Łomnica (BS)	2.46	1.51	0.44	0.266	0.259	-0.023	67.86	14
Average for BS	2.31	1.53	0.44	0.264	0.262	-0.002		10
West Beskids	2.29	1.53	0.43	0.271	0.260	-0.017		4.56
East Beskids*	2.26	1.60	0.45	0.300	0.277	-0.064		

Na – Mean number of observed allele; *Ne* – Mean number of effective allele; *I* – Shanon's index of genetic diversity; *Ho* – observed heterozygosity; *Fis* – Wright' fixation index; *%PoL* – percent of polimorphic loci; *NoRa* – number of rare alleles. Coefficients for East Beskid Mts. are from Mejnartowicz (2001).

TABLE 4. Unbiased Measures of Nei's (1978) Genetic Identity (above diagonal) and genetic distance (below diagonal) between investigated populations.

Population	1	2	3	4
1. Ukleina (BM)	***	0.9760	0.9631	0.9707
2. Kamieniec (BS)	0.0243	***	0.9902	0.9897
3. Przysietnica (BS)	0.0376	0.0099	***	0.9870
4. Łomnica (BS)	0.0298	0.0103	0.0131	***

0.42), yet it shows high values in its observed ($H_o = 0.291$) and expected ($H_e = 0.254$) heterozygocity.

Ukleina is the most heterozygotic silver-fir population in the West Beskid populations examined so far. In this population, value $H_o = 0.260$ far exceeds the average heterozygocity of the Carpathian silver-fir trees in Poland (Mejnartowicz 2000). The negative value $F_{is} = -6.1\%$ (Table 3) indicates the outcrossing of trees within the Ukleina population. On the dendrogram, drawn on the basis of interpopulational genetic distances (Table 4) according to Nei (1978), the Ukleina population from the Makowski Beskid is separated from the Sądecki populations (Fig. 1).
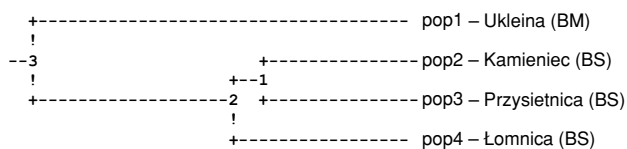
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+----------------------------------- pop1 – Ukleina (BM)
!
--3                    +--------------- pop2 – Kamieniec (BS)
!                   +--1
+-------------------2 +--------------- pop3 – Przysietnica (BS)
               !
               +----------------- pop4 – Łomnica (BS)
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Fig. 1. Dendrogram Based Nei's (1972) Genetic Distance: Method = UPGMA Modified from NEIGHBOR procedure of PHYLIP Version 3.5.

Beskid Sądecki Populations: Kamieniec (2), Przysietnica (3) and Łomnica (4)

The set of silver-fir populations from the Sądecki Beskid is separated by the Wyspowy Beskid range from the Ukleina population in the Makowski Beskid and there is a great genetic distance between them (Table 4). The Sądecki Beskid populations form one consistent subgroup, separated from the Ukleina population (Fig. 1). Two silver-fir populations – Kamieniec and Przysietnica from the area of Stary

Sącz – have similar values in the number of alleles ($N_a = 2.21$ and 2.25) and in the number of effective alleles ($N_e = 1.55$ and 1.54). The two populations, however, differ significantly in the percentage of polymorphic loci (67.96% and 71.43% in Kamieniec and Przysietnica respectively). Similarly great interpopulational differences occur in the number of rare alleles (6 in Kamieniec and 10 in Przysietnica). The Kamieniec population is the only one possessing a positive value of coefficient $F_{is} = 3.8\%$, which indicates that about 4% of the seeds originate from consanguine mating (Table 3). This mating system is also one of the causes of lower observed heterozygocity ($H_o = 0.250$) than was expected from the H-W principle ($H_e = 0.259$). It must be noted though, that the Kamieniec population is much younger – 80 years old – than the other populations – about 140 years old (Table 1); quite frequently, in the gymnospermous trees heterozygocity increases with the populations' age.

The Łomnica (3) population has the highest mean number of alleles in locus $N_a = 2.46$ and, at the same time, a smaller than the average number of effective alleles $N_e = 1.51$. It is due to the fact that as many as 14 rare alleles occur in this population, which is almost three times more than in the Ukleina population from the Makowski Beskid.

The great number of alleles can have some influence, assuming random selection, on the observed heterozygocity $H_o = 0.266$, bigger than the expected heterozygocity $H_e = 0.259$. As has been said, the surplus of heterozygotes is quite frequent in old populations of gymnospermous trees, particularly in mountain populations, where selection against homozygotes can occur.

In the provenance experiment on the silver-fir, carried out in the testing area in Stary Sącz, where the examined populations originate, the Sądecki Beskid populations ranked low in their survival rate at the age of 15: Przysietnica ranked 48, Kamieniec – 69, and Łomnica – 74 in the total of 99 populations tested in the experiment. The Ukleina population from the Makowski Beskid ranked in the 14th, much higher position. Interestingly, the same populations exhibited a survival rate significantly above the average, on the experimental area established at the lowlands, beyond the range of the silver-fir, in the Masurian region and in the Białowieża Primeval Forest (Skrzyszewska 2000). These were populations with both a small number, like Ukleina, and a great number, like Łomnica, of rare alleles. In these studies also the Beskid Sądecki populations were included in the class of high breeding value (Skrzyszewska 1999). The considerable participation of rare alleles can have a significantly positive influence on the population's adaptation to vegetative conditions, or at least they do not exert a harmful influence on the adaptive processes.

The comparison of genetic parameters of the Western Beskid silver-fir population with those of the Eastern Beskid silver-fir populations shows that the latter has a slightly greater heterozygocity (H_o and H_e), a greater number of effective alleles (N_e), and a greater value of I – index of genetic diversity. It can be assumed that the decrease in genetic variability and diversity occurred during the silver fir migration from the Balkan refuges into the post-glacial.

It should be mentioned, here that heterozygosity calculated from silver-fir buds is much lower; Ho and He amounted about 0.130, than received in this studies of megagametophyte: Ho = 0.270 and He = 0.260 (Longauer 1994; Mejnartowicz 1996), so it is very important to know on what

analytical material the data for genetic interpretation were received.

A relatively great spatial isolation between the populations, particularly in the mountainous terrain, can influence significantly the genetic variability and diversity of silver-fir populations. The calculated gene flow $N_m = 4.565$ also indicates the populations' isolation. It means 4.7 immigrants per generation into the studied populations. Considering the large size of the forest tree populations, these values are of course very small, but it should be noted that the silver-fir tree stands on the studied area are separated by large Norway spruce and beech populations, which undoubtedly greatly hinders the gene flow between populations.

CONCLUSIONS

There is a significant genetic diversity between the Sądecki and Makowski Beskid populations, expressed in the formation of two separate groups on the dendrite. The result is similar to the dendrite formed on the basis of the mean value of breeding index.

The Sądecki Beskid populations have a slightly smaller genetic variability and diversity in comparison to the Eastern Beskid populations. Eastern Beskid populations have a greater heterozygocity (H_o and H_e), a greater number of effective alleles (N_e), and a greater value of I – index of genetic diversity. Decrease in genetic variability and diversity could have occurred during the silver fir migration from the Balkan refuges into the postglacial period.

Heterozygosity calculated from silver-fir buds is much lower; Ho and He amounted to about 0.130, than that received in the studies of megagametophyte: Ho = 0.270 and He = 0.260

There is a limited gene flow of $N_m = 4.565$ immigrants per one generation into the population.

Genetic distances between populations correspond to geographical distances.

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GENETYCZNA ANALIZA
POPULACJI JODŁY POSPOLITEJ Z BESKIDÓW

STRESZCZENIE

Zbadano 28 loci izozymowych w czterech populacjach jodły pospolitej z Beskidów: z Beskidu Makowskiego (BM) jedną populację i trzy populacje z Beskidu Sądeckiego (BS). Poddano analizie ich zmienność i różnorodność genetyczną, a także obliczono międzypopulacyjne dystanse genetyczne według Neiego. Otrzymane wyniki wskazują, że dystans geograficzny między populacją BM, a grupą populacji BS znajduje swój wyraz w dystansie genetycznym. Populacja BM różni się zdecydowanie od pozostałych populacji. Ma ona najniższą różnorodność genetyczną ($I = 0,42$), najmniejszy procent polimorficznych loci ($\%PoL = 64,29$) i najmniejsza liczbę rzadkich alleli ($NoRa = 5$). Populacja BM ma jednakże najwyższą heterozygotyczność obserwowaną ($Ho = 0,291$), znacznie przewyższającą heterozygotyczność oczekiwaną z prawa Hardego-Weinberga. Przeciwnie populacje BS – są one w stanie równowagi H-W, co wyraża się podobieństwem wartości $He = 0,262$ i $Ho = 0,264$.

SŁOWA KLUCZOWE: izozymy, *Abies alba*, Beskidy, populacje, struktura genetyczna, heterozygotyczność, dystans genetyczny, różnorodność genetyczna, zmienność genetyczna.