Silva Gabreta	vol. 3	p. 173–182	Vimperk, 1999

Genetic structure of three natural Norway spruce populations in the Šumava mountains determined by isoenzyme analysis

Genetická struktura tří přirozených šumavských populací smrku ztepilého sledovaná isoenzymovou analýzou

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Abstract
The genetic structure of three populations (Trojmezná, Plesná and Radvanovický hřbet – all of them are considered to be autochthonous) of Norway spruce (*Picea abies* (L.) Karst.) in the Šumava Mts. was investigated by starch gel electrophoresis. These populations were analyzed through eleven gene-enzyme systems encoding 17 loci. On average, more than 80% of the loci per population were polymorphic, the number of alleles per locus was 2.12 and the mean expected and observed heterozygosites were 0.115 and 0.113 respectively. These results are more or less corresponding with another studies from Central Europe. According to the occurrence of some rare alleles, it is possible to recognize one population from another. Some genetic differences between the Radvanovický hřbet population (the mixed forest comprised of Norway spruce, common beech, silver fir, maple) and two others (climatic spruce forests) were found. Compared to the other populations, in Radvanovický hřbet a little bit lower amount of mean number of alleles per locus and lower number of polymorphic loci were found. In this population was the highest observed heterozygosity. Some of the genetic differences might be caused by different natural conditions pertinent to altitude and to forest vegetation degree.

Key words: Picea abies, genetic structure, isoenzymes, polymorphism, electrophoresis, differentiation, diversity, heterozygosity

Introduction

Isoenzymes are gene markers that are electroforetically detectable variants of an enzyme system in the same species which have different structures but identical or nearly identical functions (MÜLLER-STARCK & ZIEHE 1991). In higher plants, isoenzymes were first used as gene markers in the late sixties by Allard (BROWN & ALLARD 1969, MARSHALL & ALLARD 1969). Since 1970, isoenzyme polymorphism were also used in genetic studies with forest tree species. More than twenty years ago, one of the first comprehensive reviews on the nature of isoenzymes and their application in forest genetics was published (FERET & BERGMANN 1976). By now, hundreds of studies have been done on trees (for recent bibliography of isoenzyme studies in forest trees see PAULE (1990). The interest and importance of these biochemical markers have not diminished although other types of gene markers, such as DNA markers, are gaining increasing popularity. Even though this type of forest genetic research is common in the world, the Czech Republic didn't have any specialized laboratory for genetic research of forest trees species until 1998.

The electroforetic laboratory for genetic research of forest tree species was built by Šumava National Park in 1998. The genesis of the laboratory was supported by GEF grant (ROUDNÁ & PRCHALOVÁ 1996). The rest of the laboratory was equipped by the support of several small grants and in-house money of Šumava National Park administration. This is how the Department of Biological Research by Šumava National Park obtained one of the first workstation where the new kind of forest research methods can be developed in the Czech Republic. The main goal of the laboratory is the identification of indigenous forest tree populations in the Šumava Mts. (MÁNEK 1998a). The results will be used as valuable information for gene conservation of autochthonous or endangered forest tree populations. In addition, we are ready for questionable forest tree hybrids identification, especially pine or birch, and the genetic verification of progeny as part of Norway spruce clonal seed orchard management (MÁNEK 1998b). The laboratory started working in the beginning of the 1999 and has made genetic analyses of more than 600 individual trees of Norway spruce. Please note that in this paper you can see the very first and preliminary results only.

Material and methods

Populations sampled

In each population, twigs with buds were collected from 100 trees for analyses. They were taken from all stands during the dormant period. Branches with dormant buds were collected by using an aluminum pole with scissors at the top. In order to minimize the sampling of relatives, spacing between sampled trees was at least 30m. All stands are considered to be autochthonous. The locations of the investigated stands and the sizes of samples are given in Table 1 and Fig. 1.

Electrophoresis

For the analysis, about 10 mg of bud tissue were extracted and homogenized in tris-glycine-PVP buffer pH 8,3 containing 0.06 (v/v) mercaptoethanol (MUONA & al. 1985) with some

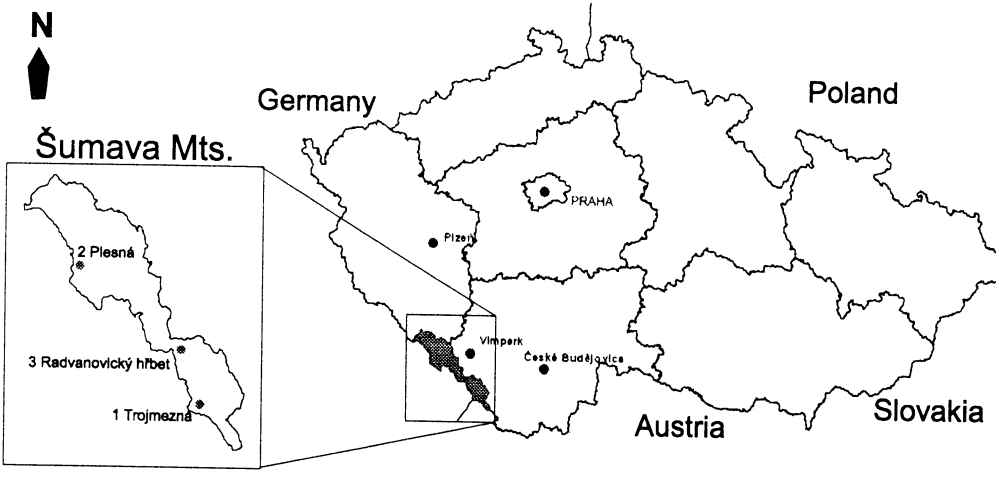

Fig. 1. – Locations of the three studied populations of *Picea abies* sampled in Šumava Mts.

Table 1. – Names and characteristics of analysed populations.

No	Name	Abb.	Forest district	Latitude	Longitude	Altitude
1	**Trojmezná**	TRO	FD Stožec	48° 46′	13° 49′	1250–1320
2	**Plesná**	PLE	FD Prášily	49° 06′	13° 18′	1160–1200
3	**Radvanovický hřbet**	RAD	FD České Žleby	48° 53′	13° 48′	800–960

modification according to GÖMÖRY (pers. comm.). The crude homogenates were absorbed into Whatmann No. 3 MM filter paper wicks (10 x 3 mm). These wicks were then inserted into start place on the starch gel for electrophoretic separation. Three buffer systems were used for electrophoretic separation of 11 enzymes: **A** – continuous Tris-citrate pH 7.0, **B** – discontinuous lithium borate/ Tris-citrate pH 8.1/8.1 (ASHTON & BRADEN 1964) and **C** – discontinuous sodium borate/ Tris-citrate pH 8.0/8.7 (POULIK 1957). Electrophoresis was carried out under refrigeration at + 3°C and at 170–300V, 150mA for 4–5 hours, and in a horizontal chamber in. app. 10%, starch gel. After the electrophoresis, gels were sliced into five layers and each layer was stained for visualization and detection isoezyme bands. Horizontal electrophoresis and staining of 11 enzyme systems was performed according to CONKLE & al. (1982) and CHELIAK & PITEL (1984) with some modifications afterwards GÖMÖRY (pers. comm.). Electrophoretic patterns with bands were clasificated according to LAGERCRANTZ & al. (1988), PAULE & al. (1990), GÖMÖRY (1992b). The assayed enzyme systems, their abbreviations, enzyme commission codes and gel buffer (upon which they were run) are listed in Table 2.

Statistical analysis

For measurement of genetics diversity and differentiation in the investigated spruce populations, the used parameters were:

a) The expected proportion of heterozygotes ($\mathbf{H_e}$) at each locus was calculated according to the formula

$$\mathbf{H_e = 1 - x_i^2}$$ NEI (1978)

where x_i is the frequency of the i-*th* allele,

Table 2. – Enzyme systems assayed

Enzyme	Abbreviation	No. of scored loci	E.C. No.	Buffer system
Isocitrate dehydrogenase	IDH	2	1.1.1.42	A
Fluorescent esterase	FEST	1	3.1.1.1	B
Glutamate dehydrogenase	GDH	1	1.4.1.2	C
Glutamate-oxalacetate-transaminase	GOT	2	2.6.1.1	C
Leucine aminopeptidase	LAP	2	3.4.11.1	B
Malate dehydrogenase	MDH	2	1.1.1.37	A
Phosphoglucomutase	PGM	1	2.7.5.1	A
Phosphoglucose isomerase	PGI	2	5.31.9	B, C
Peroxidase	PX	1	1.11.1.7	B
Phosphoenolpyruvate carboxylase	PEP	1	4.1.1.31	C
Shikimate dehydrogenase	SKDH	2	1.1.1.25	A

Electrophoresis of this enzymes was conducted in three buffer systemes: A, B and C. For description see above.

b) observed heterozigosity ($\mathbf{H_o}$) was given by dividing the number of heterozygous trees by the overall trees assayed for particular locus,
c) the proportion of polymorphic loci (**PP**) was calculated by dividing the number of polymorphic loci by overall number of loci surveyed. Convention say, loci were designated polymorphic if the most common allele had frequency less than 95%,
d) the average number of alleles ($\mathbf{n_a}$) per locus was obtained by dividing the number of alleles revealed by the overall number of loci surveyed
e) the genetics differences between populations:

Populations that are geographically separated tend to accumulate different genes due to mutation, different selective forces and genetic drift (LUNDKVIST & RUDIN 1977). BERGMANN (1975) and many others had shown that differentiation in frequency of isozyme genes makes it possible to assign a sample of unknown origin to specific area or region. In this study it is used for calculation of genetics distance between populations formula proposed by NEI (1972):

Genetic distance (D) between two populations [e.g. X and Y] are calculated from formula

$$\mathbf{(D) = - \log_e I}$$

where I is normalized identity of genes between X and Y with respect to all loci and is defined as

$$\mathbf{I = J_{xy} / (J_x J_y)^{1/2}}$$

where J_x = arithmetic mean over all loci of $j_x = \mathbf{x_i^2}$ in population X
J_y = arithmetic mean over all loci of $j_y = \mathbf{y_i^2}$ in population Y
J_{xy} = arithmetic mean over all loci of $j_{xy} = \mathbf{x_i\, y_i}$ in population X and Y
x_i = frequency of the i-th allele in population X
y_i = frequency of the i-th allele in population Y

Nei's genetic distance was used for clustering of populations by the unweighted pair-group method using arithmetic means (UPGMA; SNEATH & SOKAL 1973)

Results

Genetic structure

The genetic variation in 3 populations from Šumava Mts. were studied. Polymorphism of eleven enzyme systems with 17 loci was surveyed in the study. All allelic frequencies based on the bud diploid tissue analysis are given in Table 3. The electrophoretic analysis revealed 45 allelic variants. The schematic description of all variants is given in Fig. 2. Besides MDH-A1 allele, which is monomorphic in all studied populations, each scored loci was polymorphic in at least one population. It can be seen that most variable loci in three Šumava Mts. populations were GOT-B and PGI-B. The LAP-2 loci show an intermediate level of variability. The other loci were less polymorphic. On the basis of allelic frequencies, the main parameters of genetic variability were calculated. It is obvious, as shown in Table 4, that values of variability for each population do not differ significantly from each other.

Some minor differences were found that allow genetic differentiation of populations from the Šumava Mts. Some rare allele appertain to this indicators, which can be used as a "label" for particular populations.

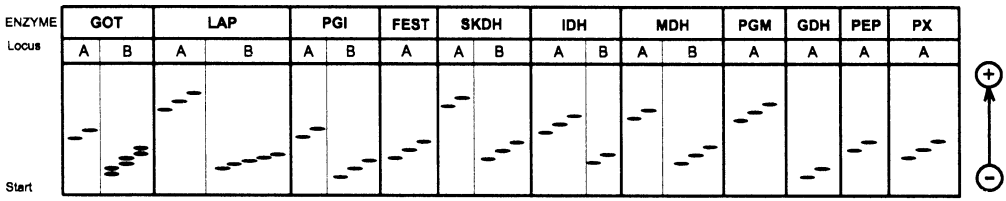

Fig. 2. – Line diagrams of isozyme staining phenotypes of *Picea abies* show the electroforetic variants of all enzymes studied. The abbreviations of enzyme name is given first, then the number of the loci, and, lastly, the designations of the bands.

In the Radvanovický hřbet population, which is observed to be slightly genetically different, was not found in the rare allele LAP-A1, PGI-B3, or PX-A1, but it was found in Tojmezná and Plesná populations. With regards to distribution of the rare alleles that are present in many loci, some of them were found only in one population studied. IDH-A3 was identified only in Radvanovický hřbet. SKDH-B3, PGI-A2, and GOT-A1 were found only in Trojmezná, while IDH-A2 was found only in the Plesná population.

Genetic diversity

Genetic characteristics are summarized in Table 4. The percentage of polymorphic loci varies considerably from 76.47 in Radvanovický hřbet population to 88.35 in Plesná. Similarly, the mean number of alleles per locus was lowest in the Radvanovický hřbet (1.94) and highest in Plesná (2.29).

Observed heterozygosity was lowest in Trojmezná (0.111). Radvanovický hřbet population had the highest value of observed heterozygosity (0.117). Only in Radvanovický hřbet population was the observed heterozygosity higher than expected.

Genetic differentiation

Genetic similarity among *P. abies* populations was quantified using Nei's genetic distance coeficient (Nei 1972). Some elementary genetic distances were found between three Norway spruce populations studied. The similarity degree of the *Picea abies* populations studied in the Šumava Mts. was visualized by dendrogram (Fig. 3), and constructed by using the unweighted pair group method of cluster analysis (UPGMA). The Nei's standard genetic distance varies from 0.001 to 0.004. This value shows that the differentiation between populations is really low.

In comparison with the Radvanovický hřbet population and according to Nei's standard genetic distance, the Trojmezná and Plesná populations are mutually more similar. Nei's genetic distance between Trojmezná and Plesná is 0.001, and 0.004 between Trojmezná and Radvanovický hřbet as well as between Plesná and Radvanovický hřbet.

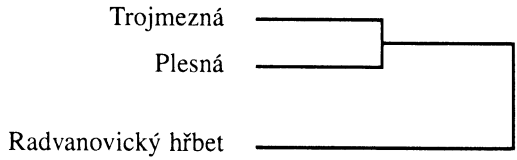

Fig. 3. – Dendrogram of Nei's genetic distances obtained with UPGMA method.

Table 3. – Allelic frequencies in individual populations.

locus	1	2	3
GOT-A			
(N)	99	97	84
1	.005	.000	.000
2	.995	1.000	1.000
GOT-B			
(N)	95	96	82
1	.447	.443	.415
2	.537	.531	.561
3	.016	.026	.024
LAP-A			
(N)	99	97	84
1	.010	.005	.000
2	.975	.990	1.000
3	.015	.005	.000
LAP-B			
(N)	99	95	84
1	.020	.032	.030
2	.157	.126	.179
3	.000	.000	.000
4	.823	.837	.792
5	.000	.005	.000
PGI-A			
(N)	99	94	84
1	.990	1.000	1.000
2	.010	.000	.000
PGI-B			
(N)	91	85	81
1	.324	.276	.414
2	.670	.718	.586
3	.005	.006	.000
FEST			
(N)	99	95	84
1	.076	.058	.077
2	.924	.942	.923
3	.000	.000	.000
SKDH-A			
	(N)	99	96
1	.010	.052	.048
2	.990	.948	.952
SKDH-B			
(N)	99	96	84
1	.924	.948	.940
2	.071	.052	.060
	3	.005	.000

locus	1	2	3
PGM			
(N)	99	95	84
1	.000	.000	.000
2	.995	.968	.988
3	.005	.032	.012
MDH-A			
(N)	99	97	84
1	1.000	1.000	1.000
2	.000	.000	.000
MDH-B			
(N)	93	97	84
1	.102	.077	.030
2	.887	.923	.964
3	.011	.000	.006
IDH-A			
(N)	99	97	84
1	.000	.005	.000
2	1.000	.995	.994
3	.000	.000	.006
IDH-B			
(N)	99	97	84
1	.990	.995	.994
2	.010	.005	.006
GDH			
(N)	99	94	83
1	.990	.968	.994
2	.010	.032	.006
PX			
(N)	99	94	84
1	.005	.016	.000
2	.970	.952	.952
3	.025	.032	.048
PEP			
(N)	99	96	82
1	.000	.010	.018
2	1.000	.990	.982

N = number of trees scored

Table 4. – Characteristics of genetic multiplicity and heterozygosity values of analyzed populations.

Number	Name	SS	PP	n_a	H_e	H_o
1	**Trojmezná**	97.9 ± 0.6	82.35	2.24 ± 0.18	0.115 ± 0.039	0.111 ± 0.037
2	**Plesná**	95.1 ± 0.7	88.35	2.29 ± 0.20	0.115 ± 0.037	0.112 ± 0.036
3	**Radvanovický hřbet**	83.5 ± 0.2	76.47	1.94 ± 0.16	0.115 ± 0.041	0.117 ± 0.042

SS mean sample size per locus
PP number of polymorphic loci (locus is considered polymorphic if more than one allele was detected)
$\mathbf{n_a}$ mean number of alleles per locus
$\mathbf{H_e}$ expected heterozygosity
$\mathbf{H_o}$ observed heterozygosity

Discussion

Species strategy (weedy-non weedy-climax) as well as environmental autocorrelations between populations play major roles in the moulding of population structure (TIGERSTEDT 1973). Forest tree species are among the most long-lived organisms and, therefore, need a high level of polymorphism for adaptation to everlasting environmental changes. Although recent genetic surveys of tree species tend to lower earlier estimates of variation (BERGMANN 1991a), the statement that genetic diversity in trees is generally twice as much as in herbaceous plant is still valid (BERGMANN 1991b). Many of the isoenzyme polymorphism studies show that most of genetics variation in tree species is localized within populations (over 90%) and only minor among them (BERGMANN 1991b). This agrees with the results of the Šumava Mts. spruce populations (variation within population – 88 %). These results confirm the fact that the genetic system of gymnosperms, and particularly of coniferous species such as *Picea abies*, is quite different from most other plants. Coniferous trees strategy seems to rely on high recombination, profuse production of zygotes tested by natural selection, and heavy gene flow between populations (TIGERSTEDT 1973).

Based on the data obtained in this study, high genetic similarity between Šumava Mts. populations surveyed must be mentioned (According to NEI's coefficient (1972) genetic distances are not higher 0.004). But considering the fact that pollen dispersal and gene flow in *Picea abies* is considerably larger than in most wind-pollinated herbaceous species, the small genetic distance between Šumava Mts. populations is not a surprise. Moreover, the Šumava Mts. area is not so large as to find some significant correlations between geographic and genetic distances (the total area is app. 70 000 square hectares, Norway spruce covered app. 80%). On the whole, values of genetic similarity obtained in this study are typical of very closely related, geographically connected populations of spruce. On the basis of data obtained in this study, I can say that more than 80 % of loci in *Picea abies* populations of Šumava Mts. are polymorphic (Table 4). Regarding the expected and obtained heterozygosity values, these appear to agree with LAGERCRANTZ & RYMAN (1990) which reported for populations from Czechoslovakia, observed heterozygosity in range from 0.089 to 0.109 (after my results H_o vary from 0.111 to 0.117). Heterozygosities calculated from the results of my study are lower in comparison with other authors. For instance H_o obtained by GÖMÖRY (1992a) in 14 Norway spruce population from Slovakia (H_o = 0.225 – 0.376). Significantly higher H_o were reported by LUNDKVIST (1979) in a study of four *P. abies* populations from Sweden based on analysis of 11 loci (H_o = 0.361 – 0.389). BERGMANN & GREGORIUS (1979) found in a study of 21 populations from all over Europe based on 7 loci heterozygosity even 41%. But these high values are apparently due to a large contribution of allozyme variation of highly polymorphic loci, coding such enzymes as esterase, leucin aminopeptidase and acid phosphatase (LUNDK-

VIST 1979, BERGMANN & GREGORIUS 1979). On the other hand, LANGERCRANTZ & RYMAN (1990) reported the heterozygosity of *Picea abies* populations from Central Europe in a range of 7.6 – 12.4%. They analyzed 22 loci, and, in my opinion, their results are representative for European Norway spruce populations. As can be seen from GONCHARENKO & al. (1995) results, genetic variation in Norway spruce populations from the Baltic countries, Byelorussia and Russia is significantly greater then in Central Europe. They reported H_0 reached 17%. LANGERCRANTZ & RYMAN (1990) believe that *P. abies* populations in Central Europe lost their diversity during the last glaciation. Regarding Goncharenko's results, my data from this short study confirm Langercrantz & Ryman hypothesis of a lower level of *Picea abies* variation in Central Europe compared to other regions of its natural distribution.

The most different population relative to the others is Radvanovický hřbet. There is no large distance between Šumava Mts. populations surveyed. Nevertheless, some differences can be due to inheritance. Radvanovický hřbet population belongs to the 5 and 6 Forest vegetation zones (it means beech-fir and beech-spruce zone), while the others are climatic spruce forest and belong to the 8 Forest vegetation zone (spruce zone). Accommodation to different natural conditions might be the reason for the different genetic structure. The environmental conditions can cause the differentiation of Radvanovický hřbet population from others. Other causes of some differentiation may be the forest management of *P. abies* in the Šumava Mts. region during the last 200 years (reforestation with nonindigenous material). This all indicates the existence of genetic differentiation of spruce populations in the Šumava Mts. region. The above results showed that the differentiation between populations was very small. Explanation of genetic differentiation can be much more elementary. It may be simply due to the natural variation of spruce.

Finally, it must be mentioned that the sample size of material in this analysis is very small. Therefore, as stated in the Introduction of this paper, all presented parameters of genetic variation and differentiation between populations should be considered with caution and treated as preliminary results. In order to explain more precisely the small genetic differentiation of Norway spruce that I observed in the Šumava Mts., further investigations are needed. They should include a large number of populations from different places with different management (e.g. naturally regenerated primeval forest vs. logged forest with artificial regeneration).

Acknowledgement. My sincere thanks are due to Ms. Lenka Tučková, University of New Mexico – Albuquerque, for revision of English. This research is partly supported with Research project No. VaV/610/1/99 by Ministery of Environment of the Czech Republic.

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