

Genetic diversity of European beech in Poland estimated on the basis of isoenzyme analyses

Małgorzata Sulkowska¹ ✉, Dusan Gömöry² and Ladislav Paule²

¹ Forest Research Institute, Department of Sylviculture and Genetics, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland, phone: +48 22 7150461, fax: +48 22 720 0 397, e-mail: M.Sulkowska@ibles.waw.pl

² Technical University in Zvolen, Faculty of Forestry, Masaryka 24, 960 53 Zvolen, Slovakia

ABSTRACT

The aim of this study was the assessment of genetic diversity and differentiation patterns of European beech (*Fagus sylvatica* L.) populations within its natural range in Poland. These stands cover 5.2% of the forest area in Poland, and form the predominant forest tree communities throughout the Carpathians and Sudety Mountains, and the moraine landscape of the Pomeranian Lake District. Varying environmental conditions have resulted in a great number of ecotypes and populations which are characterized by various ecological requirements. Poland represents the north-eastern limit of the beech's natural distribution.

Genetic diversity and differentiation was assessed using allozyme gene markers employing 9 enzyme systems: glutamate-oxaloacetate transaminase (GOT – EC 2.6.1.1), leucine aminopeptidase (LAP – EC 3.4.11.1), isocitrate dehydrogenase (IDH – EC 1.1.1.42), malate dehydrogenase (MDH – EC 1.1.1.37), menadione reductase (MNR – EC 1.6.99.2), phosphoglucomutase (PGM – EC 2.7.5.1), phosphoglucose isomerase (PGI – EC 5.3.1.9), peroxidase (PX – EC 1.11.17) and shikimate dehydrogenase (SKDH – EC 1.1.1.25).

Beech populations occurring toward the northeast of the natural range were characterised by a decreasing the average number of alleles per locus and percentage of polymorphic loci. The highest genetic differentiation was found in the East Carpathians. The genetic differentiation levels of Polish beech populations are comparable to the other European countries.

KEY WORDS

beech, isoenzyme, genetic diversity, genetic differentiation

INTRODUCTION

The aim of the present study was the assessment of genetic diversity of European beech (*Fagus sylvatica* L.) within its natural range in Poland. Beech is one of the most important forest tree species in Poland, and beech stands account for 5.2% of the total forest area (Lasy Państwowe

2009). They constitute the most typical forest tree associations found within the lower forest belt in the Carpathians and Sudety Mountains in the south of Poland, and throughout the moraine landscape of the Pomeranian Lake District in the northern of Poland (Wojterski 1990).

The present genetic structure of beech populations in Europe was formed by many different factors, inclu-

dig anthropogenic ones. Important factors affecting the gene pool were the glacial period and the locations of few beech refugia, from which beech colonized its present range during the postglacial, as evidenced by fossil pollen analyses (Szafer 1935; Ralska-Jasiewiczowa 1983; Hazler et al. 1997; Huntley and Birks 1983). Other studies using genetic markers revealed existence of only one effective refugium (Gömöry et al. 1999), suggested as the source of beech forests in the major part of Europe except southern Italy and the Balkans on the basis of the genetic differentiation monitored by isozyme loci, which was very weak. This hypothesis was confirmed later by Magri et al. (2006) using a combination of genetic markers (isozymes and chloroplast DNA) and paleobotanical analyses (fossil pollen and macrofossils). Investigations of beech variation in Poland performed with isoenzyme studies (Sułkowska 2002; Gömöry et al. 2003) showed high genetic diversity, similar to other neighboring European populations. Slight decrease of average number of alleles per locus and lower level of differentiation towards the northern natural range limit generally confirm the migration path after the glacial period.

Varying environmental conditions resulted in a great number of ecotypes and populations reflecting various ecological requirements (Dzwonko 1990; Giertych 1990; Sułkowska et al. 2008). In Poland the beech attains its north-eastern distribution limit (Szafer and Pawłowski 1972; Boratyńska and Boratyński 1990). The geographic range of beech is limited by climate continentality, soil conditions, winter temperatures and air humidity (Sławiński 1947; Jedliński 1922; Boratyńska

and Boratyński 1990). The growth of beech stands outside the natural beech limit indicates that the species possesses potentially wider range (Brzeziecki 1995; Tarasiuk 1999).

METHODS

In this study 26 populations were sampled, representing the best preserved selected seed stands, as well as a number of other natural beech stands (Tab. 1). Many of the beech populations chosen were located in the Carpathian Mountains, where beech is the most important forest tree species.

Twigs and dormant buds were collected from 50 trees in each population. Proteins from buds and cambium were extracted using 0.1 M Tris-HCl buffer pH 7.0. Starch gel electrophoresis was applied to genotype trees at allozyme loci. Histochemical staining and interpretation of zymograms followed Thiébaud et al. (1982), Merzeau et al. (1989), and Müller-Starck and Starke (1993). Nine enzyme systems were analysed: glutamate-oxaloacetate transaminase (GOT – EC 2.6.1.1 – *Got-2*), leucine aminopeptidase (LAP – EC 3.4.11.1 – *Lap-1*), isocitrate dehydrogenase (IDH – EC 1.1.1.42 – *Idh-1*), malate dehydrogenase (MDH – EC 1.1.1.37 – *Mdh-1*, *Mdh-2*, *Mdh-3*), menadione reductase (MNR – EC 1.6.99.2 – *Mnr-1*), phosphoglucosmutase (PGM – EC 2.7.5.1 – *Pgm-1*), phosphoglucose isomerase (PGI – EC 5.3.1.9 – *Pgi-2*), peroxydase (PX – EC 1.11.17 – *Px-1*, *Px-2*) and shikimate dehydrogenase (SKDH – EC 1.1.1.25 – *Skdh-1*). Allelic frequen-

Tab. 1. Geographic location of Polish beech populations studied

No.	Provenance	Geographic region	Altitude (m. a.s.l.)	Geographic coordinates (N/E)
1	2	3	4	5
1	Cisna	South (Mountains)	900	49° 11' 22° 18'
2	Moczarne	South (Mountains)	800	49° 06' 22° 29'
3	Caryna	South (Mountains)	950	49° 08' 22° 46'
4	Rozsypaniec	South (Mountains)	950	49° 05' 22° 50'
5	Nad Sanem	South (Mountains)	750	49° 15' 22° 42'
6	Hulskie	South (Mountains)	650	49° 12' 22° 38'
7	Wielka Rawka	South (Mountains)	950	49° 05' 22° 30'
8	Łosie 1	South (Mountains)	850	49° 35' 21° 04'
9	Łosie 2	South (Mountains)	500	49° 35' 21° 05'

1	2	3	4	5
10	Folusz	South (Mountains)	650	49° 34' 21° 24'
11	Krynica	South (Mountains)	700	49° 21' 20° 58'
12	Zakopane	South (Mountains)	700	49° 16' 19° 57'
13	Gorce Mount.	South (Mountains)	900	49° 32' 20° 05'
14	Świętokrzyskie Mount.	Central (Mountains)	500	50° 48' 20° 48'
15	Narol	Central (Uplands)	345	50° 20' 23° 20'
16	Staszów	Central (Uplands)	200	50° 30' 21° 20'
17	Łągów	Central (Mountains)	450	50° 50' 21° 10'
18	Brzeziny	Central (Lowlands)	200	51° 50' 19° 40'
19	Wielkopolski PN	Central (Lowlands)	140	52° 40' 17° 32'
20	Kartuzy	North (Lowlands)	200	54° 15' 18° 10'
21	Młynary	North (Lowlands)	120	54° 00' 20° 00'
22	Świerczyna	North (Lowlands)	180	53° 25' 16° 15'
23	Gryfino	North (Lowlands)	95	53° 20' 14° 45'
24	Świebodzin	Central (Lowlands)	170	52° 20' 15° 20'
25	Śnieżka	South (Mountains)	755	50° 50' 15° 40'
26	Łądek	South (Mountains)	965	50° 15' 16° 50'

Tab. 2. Genetic diversity of Polish beech populations

No.	Region	Average number of individuals	Average number of alleles per locus	Average effective number of alleles per locus	Percentage of polymorphic loci	Average heterozygosity		Fixation index
						Observed	Expected	
1	2	3	4	5	6	7	8	9
1	Cisna	49.8	2.1	1.5	83.3	0.250	0.249	-0.004
2	Moczarne	59.7	2.5	1.5	83.3	0.241	0.255	0.055
3	Caryna	49.7	2.4	1.5	91.7	0.249	0.278	0.104
4	Rozsypaniec	49.4	2.2	1.5	91.7	0.248	0.254	0.024
5	Nad Sanem	57.5	2.3	1.5	83.3	0.265	0.269	0.015
6	Hulskie	58.2	2.3	1.5	83.3	0.242	0.250	0.032
7	Wielka Rawka	50.9	2.3	1.4	83.3	0.223	0.244	0.086
8	Łosie 1	49.3	2.3	1.5	83.3	0.241	0.262	0.080
9	Łosie 2	45.5	2.1	1.4	91.7	0.230	0.229	-0.004
10	Folusz	45.8	2.3	1.5	91.7	0.290	0.308	0.058
11	Krynica	48.2	2.2	1.5	83.3	0.266	0.280	0.050
12	Zakopane	49.9	2.4	1.5	91.7	0.268	0.274	0.022
13	Gorce	59.7	2.3	1.4	91.7	0.218	0.231	0.056
14	Świętokrzyskie	47.4	2.1	1.5	83.3	0.271	0.268	-0.011
15	Narol	48.4	2.2	1.5	83.3	0.259	0.272	0.048
16	Staszów	49.8	2.0	1.5	75.0	0.242	0.255	0.051
17	Łągów	49.7	2.1	1.5	75.0	0.263	0.271	0.030
18	Brzeziny	49.4	2.1	1.5	91.7	0.233	0.264	0.117

1	2	3	4	5	6	7	8	9
19	Wielkopolski PN	50.5	2.1	1.4	75.0	0.223	0.237	0.059
20	Kartuzy	48.6	2.0	1.4	75.0	0.224	0.236	0.051
21	Młynary	49.3	1.9	1.5	66.7	0.242	0.269	0.100
22	Świerczyna	49.8	1.9	1.4	75.0	0.236	0.249	0.052
23	Gryfino	46.8	2.3	1.5	83.3	0.246	0.268	0.082
24	Świebodzin	48.6	2.0	1.5	66.7	0.222	0.258	0.140
25	Śnieżka	48.8	2.3	1.5	91.7	0.254	0.262	0.031
26	Łądek	47.8	2.2	1.5	83.3	0.270	0.277	0.025

Tab. 3. Variation of analysed genetic parameters within different European regions

No	Region	Average number of individuals	Average number of alleles per locus	Percentage of polymorphic loci	Average heterozygosity		Fixation index
					Observed	Expected	
27	West Slovakia	657.3	2.8	91.7	0.269	0.280	0.039
28	East Slovakia	424.3	2.8	91.7	0.258	0.263	0.019
29	Romania	615.1	3.3	100.0	0.246	0.263	0.065
30	Ukraine	123.8	2.4	91.7	0.251	0.285	0.119
31	Czech + Moravia	272.1	2.5	91.7	0.254	0.271	0.063
32	Poland	1310.3	3.1	100.0	0.246	0.262	0.061

Tab. 4. Characteristics of investigated alleles in Poland and neighboring regions in Europe

Alleles	Region	
	Observed	Not observed
<i>Lap-1A</i>	Sudety Mts, West Pomerania	Carpathians, Uplands of Central Poland
<i>Got-2C</i>	Carpathians (Bieszczady), Romania, Gryfino	Sudety, Uplands of Central Poland
<i>Got-2D</i>	Carpathians (Bieszczady), West Slovakia	Other regions
<i>Idh-C</i>	Carpathians	Other regions
<i>Mdh-1B</i>	Świętokrzyskie Mts., Romania,	Other regions
<i>Mdh-1D</i>	Sudety Mts.	Other regions
<i>Mdh-2B</i>	Other regions	Sudety
<i>Mdh-2D</i>	Romania, Czech + Moravia	Poland, Slovakia
<i>Mdh-2E</i>	Ukraine	Other regions
<i>Mdh-3C</i>	Carpathians, Romania	Other regions
<i>Mdh-3D</i>	Romania	Other regions
<i>Pgi-1A</i>	Carpathians, West Slovakia, Czech, Ukraine	Sudety, Uplands of Central Poland, Pomerania
<i>Pgi-1D</i>	Carpathians (Bieszczady), West Slovakia	Other regions
<i>Pgm-A</i>	All other regions, Carpathians (Bieszczady)	Other regions of Poland
<i>Skdh-A</i>	Carpathians	Other regions
<i>Skdh-D</i>	Slovakia	Other regions

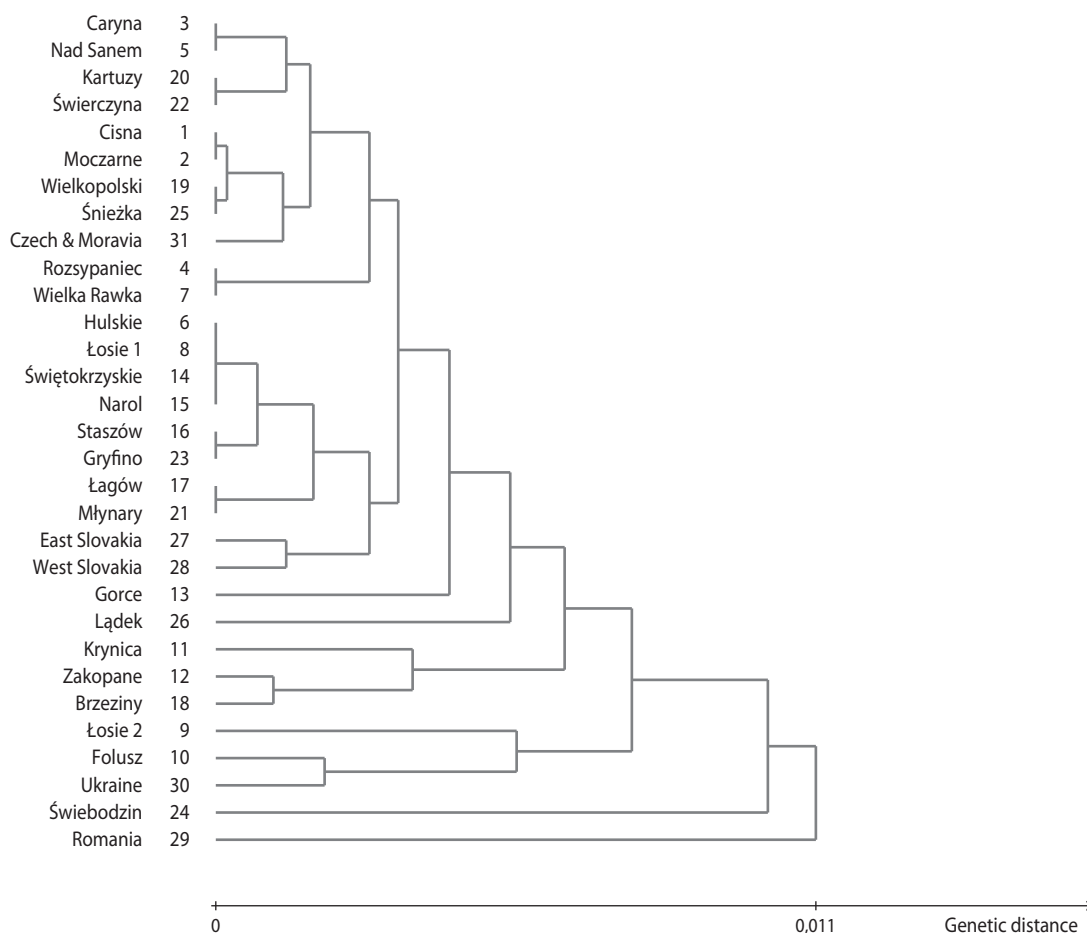


Fig. 1. Dendrogram based on Nei (1972) genetic distances

cies and observed heterozygosities (Tab. 2) were calculated based on diploid genotypes. Gene diversities (expected heterozygosities) were calculated according to Nei (1974) using BIOSYS 1 program (Swofford and Selander 1981). Effective number of alleles per locus (Crow and Kimura 1970) and fixation index (Wright 1965) were also calculated to estimate deviation from panmictic equilibrium in each population. To assess genetic differentiation patterns, dendrogram based on Nei (1972) genetic distances was constructed (Fig. 1). The results were compared to the genetic diversity parameters of beech populations in the neighbouring European countries (Tab. 3, Tab. 4) as published by Gömöry et al. (1995, 1998, 2003).

RESULTS

Polish populations were characterized by a slight decrease in the average number of alleles per locus and percentage of polymorphic loci towards the north-eastern natural range of beech (Tab. 2). The highest average numbers of alleles per locus were found in the mountain populations from the Sudety and the Carpathians Mountains (south Poland): Moczarne (2), Caryna (3), and Zakopane (12). Low levels of allelic richness were characteristic for the lowlands (in north, west and central Poland). The highest percentage of polymorphic loci was found in southern Poland for populations Caryna (3), Rozsypaniec (4), Łosie 2 (9) in the Carpathians and Śnieżka (25) in the Sudety Mountains. Unexpectedly high polymorphism was observed in Brzeziny (18), located on the border of the

natural beech limit in Poland, while the lowest value was found in Młynary (21), and in the probably planted population Świebodzin (24).

The highest values of observed and expected heterozygosity were recorded in the Carpathian populations Folsz (10), and Gorce (13). The differences between expected and observed heterozygosity were the highest in the populations Brzeziny (18), and Świebodzin (24).

The fixation index was found to be negative in beech populations from Cisna (1), Łosie 2 (9), and the Świątokrzyskie Mountains (14), which can be explained by the excess of heterozygotes in the populations and some disturbances of typical mating system for balanced populations in Hardy – Weinberg equilibrium. A comparison of genetic parameters from different European countries is shown in Tab. 3. The level of genetic variation and differentiation estimated in Poland was comparable to other regions of Central Europe. Differences in the average number of alleles per locus, and average heterozygosity were negligible between regions. Analysis of the frequency distribution of different alleles allowed for the division of regions characterized by the presence or absence of certain alleles (Tab. 4). This distribution of alleles might reflect the migration paths during the Holocene.

DISCUSSION

The genetic differentiation of Polish beech populations is comparable to those in other neighbouring European countries including Slovakia, the Czech Republic, Ukraine, and even Romania, which was reported previously (Paule et al. 1995).

Generally, alleles can be divided due to the range of alleles of large-scale example *Mdh-1* as well as genes encoding peroxidases: *Px-1* and *Px-2*. Some alleles were locally or regionally specific, namely *Lap-1A* (Sudety Mountains, Pomerania), *Got-2C* (Bieszczady Mountains, Gryfino, Romania), *Mdh-1B* (Świątokrzyskie Mountains, Romania), *Mdh-3D* (Romania), *Mdh-3D* (Ukraine), *Pgi-1D* (Bieszczady Mountains, West Slovakia), *Skdh-D* (Slovakia).

The dendrogramme (Fig. 1) constructed based on the Nei's (1972) genetic distances does not allow discrimination of regional beech populations. The dendro-

gramme indicates small genetic differentiation between populations geographically located far from each other such as Caryna (3) and Nad Sanem (5), compared with Kartuzy (20) and Młynary (21).

The highest genetic variation between populations was found in the East Carpathians. There is a slight decrease in genetic polymorphism to the north of Poland, which can be explained by postglacial migration paths from south to north (Szafer 1935; Ralska-Jasiewiczowa 1983; Hazler et al. 1997 as well as Gömöry et al. 1999, and Magri et al. 2006).

It turns out that it is difficult to find specific markers for beech provenances that allow for discrimination between geographical regions, due to the observed small genetic differentiation. This may be explained by the restricted occurrence of this species in small refugia during the glacial period with subsequent expansion into the currently occupied range during a short time (about 5.000 years). High genetic variability and the presence of alleles *Lap-1A* and *Mdh1D* not occurring in the Carpathians, but only in Sudety populations might confirm this opinion, which may support the observed expansion of beech to Polish territories from one refugium (Magri et al. 2006).

Beech from the north could slightly increase its range and connect to that migrating from the south. Ralska-Jasiewiczowa (1983) indicates that about 1.000 BP it was noticed the significantly large participation of beech in the Świątokrzyskie Mountains in comparison to surrounding regions. The combination of beech refugium from the south and migrating from north along the lake may have occurred just in the region, which may explain the rare occurrence of allele *Mdh-1B* in the region.

CONCLUSIONS

- Polish beech populations are characterized by high genetic diversity, similar to other European beech populations.
- Towards the north of the natural range limit, the beech populations are poorer in allelic diversity and are genetically less well differentiated than those in southern regions.
- The highest genetic differentiation was found in the East Carpathians (Bieszczady Mountains). The

analysis of paths of beech migration after the glacial period from one refugium for Central Europe could be an explanation of this phenomenon.

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