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# Genetic and Ecotypic Characterization of European Beech (*Fagus sylvatica* L.) in Poland

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**Abstract –** European beech (*Fagus sylvatica* L.) is one of the most important forest tree species in Poland and it covers 5.2% of forest area. Present genetic structure of beech populations has been formed within the last few thousand years and influenced by many different factors, not only of environmental (postglacial) and genetic origin, but also by anthropogenic ones. In Poland, beech attains its north-eastern limit of natural range, and is limited by continental climate, winter temperatures, air humidity and soil conditions. The growth of beech stands outside the natural beech limit indicates that the species possesses a potentially wider range.

Based on their phytosociological characteristics, nine beech experimental plots of one hectare area were established in selected seed stands, representing the typical plant associations and the most important beech provenance (seed) regions. The genetic analyses were performed using isoenzyme electrophoresis for seven loci (GOT, LAP, MDH, MNR, PGM, PGI, SKDH) and DNA markers using RAPD primers. The following genetic parameters were calculated: average number of alleles per locus, percentage of polymorphic loci and heterozygosity (on the basis of isoenzyme analysis). Dendrograms based on genetic distances were constructed.

There is a slight decrease of genetic variation of beech populations towards the north of Poland, which can be explained by migration paths and selection after the glacial period. The genetic differentiation of beech in Poland does not allow distinguishing provenance regions. The data show a mosaic character of species differentiation and an ecotypic variation.

**isoenzyme analysis / DNA-RAPD markers / genetic variation / genetic differentiation / progeny differentiation**

## 1 INTRODUCTION

Beech stands cover 5.2% of the forest area in Poland (Lasy Państwowe, 2009). They dominate the typical forest associations at the lower forest belt in the Carpathians and Sudety Mountains in South of Poland and in the moraine landscape of the Pomeranian Lake District of North Poland (Wojterski, 1990).

In Poland beech attains its north-eastern limit of the natural range (Boratyńska – Boratyński 1990). The geographic range of beech is limited by the continental climate, winter temperatures, air humidity and soil conditions (Jedliński 1953, Boratyńska – Boratyński 1990). The growth of beech stands outside the natural beech limit indicates that the species possesses potentially a wider range (Brzeziecki 1995, Tarasiuk 1999).

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Present genetic structure of beech populations in Poland was formed by many different factors, among them not only environmental and genetic but also anthropogenic ones. Basic factors affecting the gene pool were the glacial epoch, the location of beech refugia, and postglacial migration paths of the species (Szafer W. 1935, Huntley – Birks 1983, Ralska – Jasiewiczowa 1983, Hazler *et al.* 1997).

Variability of environmental conditions resulted in great number of ecotypes and populations (Dzwonko 1990), resulting in a differentiation of ecological requirements, which was confirmed by provenance experiments (Giertych 1990). Some genotypes are favourable in specific environmental conditions such as certain peroxidase enzyme alleles in relation to climate continentality (Cugen et al. 1985), while non-tolerant organisms are eliminated during natural selection process (Müller – Starck 1985, Starke et al. 1996). The occurrence of beech soil ecotypes was confirmed for German and Romanian provenances where survival was found correlated with LAP A2 allele frequency in seedlings planted in open-air and in green-house conditions (Kim 1985).

Recent investigations in Poland (Sułkowska 2002, Gömöry *et al.* 2003) have confirmed high genetic diversity of beech, similar to other neighbouring European populations, with a slight decrease of average number of alleles per locus and level of differentiation towards the North of the natural range limit, which generally confirms the migration paths after glaciations but does not provide a basis to distinguish geographic regions. The differentiation of selected stands and their progenies for genetic parameters and on the basis of soil characteristics of their habitats were also studied (Sułkowska *et al*. 2008).

## 2 METHODS

Based on their phytosociological characteristics, nine beech experimental plots of one hectare area were established in selected seed stands, representing the following plant associations *(Table 1)*: *Galio-odorati-Fagetum* (Gryfino and Kartuzy), *Dentario glandulosae-Fagetum* (Lutowiska and Łosie), *Luzulo-luzuloides-Fagetum* (Miechów, Suchedniów, Tomaszów, Zwierzyniec) and *Dentario enneaphyllidis-Fagetum* (Zdroje). The stands are located in compact forest areas mainly in the most important beech provenance (seed) regions *(Figure 1).* A data base was established covering climate, geology and plant association data of the stands. The selected stands were practically homogenous related to site conditions, which were classified ecologically according to Zarzycki et al. (2002) as shown in *Table 1.* The largest differences were in moisture and acidity of soils. Most stands were characterised by good stem quality and crown shape.

The genetic analyses of the plant material were performed using isoenzyme electrophoresis for the following loci: glutamate-oxaloacetate transaminase (GOT – EC 2.6.1.1 – *Got-2*), leucine amino peptidase (LAP – EC 3.4.11.1 – *Lap-1*), malate dehydrogenase (MDH – EC 1.1.1.37 – *Mdh-1*, *Mdh-2*, *Mdh-3*), menadione reductase (MNR – EC 1.6.99.2), phosphoglucomutase (PGM – EC 2.7.5.1), phosphoglucose isomerase (PGI – EC 5.3.1.9 – *Pgi-2*), shikimate dehydrogenase (SKDH – EC 1.1.1.25) and DNA markers using RAPD primers H02, H12, P06, W09, W11.

*Table 1. Localization and characteristics of investigated European beech populations in Poland*

| Forest Division | Forest Association | Soil | Average Ecological Index* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | L | T | W | Tr | R | D | H |
| Gryfino | *Galio-odorati-Fagetum* | Brown acid | 2.60 | 3.05 | 3.07 | 3.31 | 3.56 | 3.82 | 3.01 |
| Kartuzy | *Galio-odorati-Fagetum* | Typical brown | 2.85 | 2.88 | 2.97 | 2.83 | 3.02 | 3.58 | 2.80 |
| Lutowiska | *Dentario glandulosae-Fagetum* | Brown acid | 2.54 | 3.10 | 3.04 | 3.28 | 3.65 | 3.84 | 2.94 |
| Losie | *Dentario glandulosae-Fagetum* | Brown acid | 2.52 | 3.29 | 3.23 | 3.72 | 4.08 | 4.06 | 2.91 |
| Miechow | *Luzulo-luzuloides-Fagetum* | Rendzina | 2.77 | 3.32 | 3.33 | 3.82 | 4.07 | 3.82 | 3.02 |
| Suchedniow | *Luzulo-luzuloides-Fagetum* | Brown acid | 2.68 | 3.03 | 3.50 | 3.23 | 3.48 | 4.23 | 3.23 |
| Tomaszow | *Luzulo-luzuloides-Fagetum* | Typical brown | 2.83 | 3.18 | 3.29 | 3.53 | 3.98 | 3.79 | 2.96 |
| Zdroje | *Dentario enneaphyllidis-Fagetum* | Typical brown | 2.53 | 3.26 | 3.28 | 3.65 | 3.96 | 3.79 | 3.04 |
| Zwierzyniec | *Luzulo-luzuloides-Fagetum* | Grey brown | 2.54 | 3.22 | 3.14 | 3.52 | 3.79 | 3.83 | 2.93 |

*Ecological Index (Zarzycki *et al.* 2002): Climate indices: L – light, T – thermal;
Edaphic indices: W – wet, Tr – trophic, R – acid,
D – mechanical soil components, H – humus

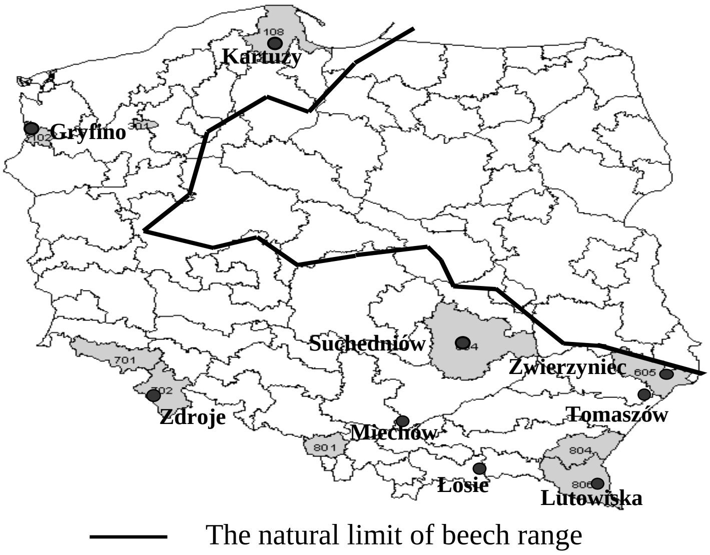

*Figure 1. Location of studied populations in Poland on the map of beech seed regions*

Proteins from buds were extracted with 0.1 M Tris-HCl buffer pH 7.0. Based on the results of starch gel electrophoresis, genetic diversity and differentiation were estimated following Thiebaut *et al.* (1982), Merzeau *et al.* (1989) and Muller – Starck & Starke (1993). Gene diversity (expected heterozygosity) was calculated according to Nei (1978) using BIOSYS 1 program (Swoford – Selander 1981). Dendrograms were constructed based on Nei's (1972) genetic distances.

Total genomic DNA from buds was extracted using the isolation DNeasy Plant Kits (QIAGEN). After DNA electrophoresis the qualitative and quantitative analysis of DNA was performed. On the basis of RAPD primers differentiation was estimated for chosen genetic parameters similar to isoenzyme loci (percentage of polymorphic loci, average number of alleles per locus and observed heterozygosity) and dendrograms of genetic distances were constructed using average frequencies of alleles.

## 3 RESULTS

Localization and description of the selected stands, representing the typical plant associations and the most important beech provenance (seed) regions, are presented in Table 1.

The genetic diversity estimated on the basis of izoenzyme and DNA analysis revealed a high variation of investigated populations (*Tables 2, 3*).

*Table 2. Genetic diversity of populations on the basis of isoenzyme analysis*

| Provenence | Average number of alleles per locus | | Percentage of polymorphic loci | | Heterozygosity | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Observed | | Expected | |
| | M | P | M | P | M | P | M | P |
| Gryfino | 2.0 | 2.7 | 77.8 | 88.9 | 0.245 | 0.237 | 0.209 | 0.221 |
| Kartuzy | 1.9 | 2.4 | 66.7 | 88.9 | 0.201 | 0.229 | 0.194 | 0.197 |
| Lutowiska | 2.2 | 2.6 | 77.8 | 88.9 | 0.226 | 0.223 | 0.212 | 0.204 |
| Łosie | 2.0 | 2.3 | 66.7 | 66.7 | 0.195 | 0.240 | 0.216 | 0.221 |
| Miechów | 2.3 | 2.6 | 77.8 | 88.9 | 0.208 | 0.241 | 0.221 | 0.207 |
| Suchedniów | 2.0 | 2.6 | 66.7 | 88.9 | 0.236 | 0.225 | 0.213 | 0.202 |
| Tomaszów Lub. | 2.1 | 2.4 | 77.8 | 88.9 | 0.233 | 0.223 | 0.205 | 0.213 |
| Zdroje | 2.1 | 2.4 | 77.8 | 88.9 | 0.216 | 0.238 | 0.202 | 0.208 |
| Zwierzyniec | 1.9 | 2.3 | 66.7 | 66.7 | 0.238 | 0.219 | 0.178 | 0.200 |

M – mother stands, P – progeny

*Table 3. Genetic diversity of populations on the basis of DNA analysis*

| Provenance | Average number of alleles per locus | Effective number of alleles per locus | Percentage of polymorphic loci | Heterozygosity observed |
|---|---|---|---|---|
| Gryfino | 2.000 | 1.549 | 100.00 | 0.334 |
| Kartuzy | 1.818 | 1.469 | 81.82 | 0.268 |
| Lutowiska | 1.954 | 1.528 | 95.45 | 0.314 |
| Łosie | 2.000 | 1.503 | 100.00 | 0.305 |
| Miechów | 1.818 | 1.455 | 81.82 | 0.269 |
| Suchedniów | 1.909 | 1.541 | 90.91 | 0.317 |
| Tomaszów Lub. | 1.954 | 1.484 | 95.45 | 0.300 |
| Zdroje | 2.000 | 1.559 | 100.00 | 0.329 |
| Zwierzyniec | 1.954 | 1.514 | 95.45 | 0.309 |

The dendrograms *(Figure 2*) constructed on the basis of Nei's (1972) genetic distances do not offer possibilities to distinguish beech regions similar to the European beech differentiation pattern. The provenances originating from fertile sites were characterised by higher differentiation value of genetic parameters, as e.g. Miechów provenance. For the mother stand of provenance Miechów (South Poland) average number of alleles per locus (isoenzyme markers) was estimated at 2.3, while percentage of polymorphic loci was 77.8% and for its progeny 2.6 and 88.9% respectively. The lowest average number of alleles per locus (1.9) was found for the Zwierzyniec mother stand (South-East Poland, at the natural range border), with 66.7% polymorphic loci and for its progeny the values were 2.3 and 66.7% respectively. The mother stand of provenance Kartuzy (North Poland) was also characterised by low values of analysed genetic parameters: average number of alleles per locus 1.9, with a percentage of polymorphism 66.7%, but for the progeny the values were higher: 2.4 and 88.9% respectively.

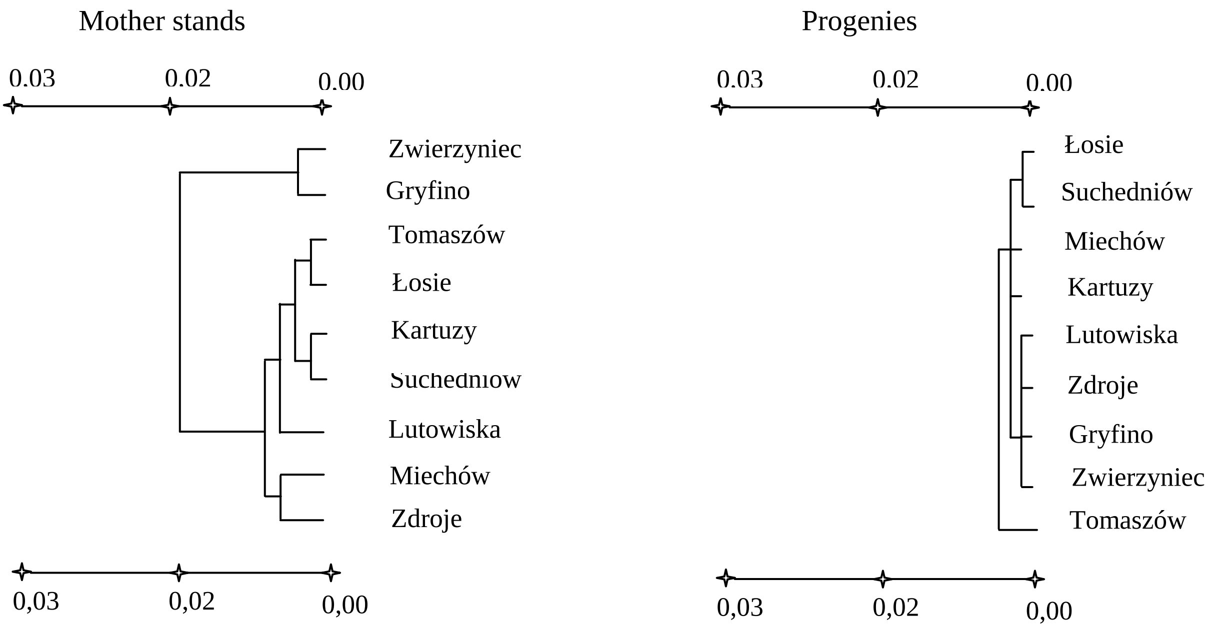

*Figure 2. Genetic differentiation of mother stands and their progeny on the basis of average frequency of alleles*

## 4 DISCUSSION

Geographical locations are not in accordance with genetic diversity patterns of investigated beech populations. This result corresponds with recent investigations of beech diversity in Poland (Sułkowska 2002, Gömöry *et al.* 2003, Sułkowska *et al.* 2008). Low range of values of genetic differentiation of investigated beech populations point toward homogenous characteristics of ecological conditions as expressed by indices (Zarzycki *et al.* 2002).

The slight decrease in the average number of alleles per locus and in the level of differentiation towards the natural range limits confirms the effect of migration after the last glaciation. The Kartuzy and Zwierzyniec provenances, located close to the border of natural beech range were characterized by the lowest values of genetic variation for isoenzyme markers.

On the basis of isoenzyme markers, estimated values of the expected and observed heterozygosity do not show differences. This is typical for genetically stable populations with a random mating system (Hartl 1988) – except the populations Gryfino and Zwierzyniec. The high value of observed heterozygosity of Gryfino, a fertile Pomeranian beech site, can be explained by the origin of the stand (stand of probably artificial origin). The difference between observed and expected heterozygosity in population Gryfino and Zwierzyniec (the latter located close to the natural border of the range) can be caused by selection processes occurring in the stands.

On the basis of DNA-RAPD markers, slight decrease of average number of alleles per locus and level of differentiation towards the North of Poland was observed, but the trend was not clear. The results indicate the ecotypic character of genetic variation of European beech in Poland which may be related to site differentiation. So, using of local European beech ecotypes and taking into account its plasticity seems to be the best advice for success in forest management.

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