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# GENETIC STRUCTURE OF EUROPEAN BEECH OF MOTHER AND PROGENY STANDS IN POLAND ON THE BASIS OF DNA CHLOROPLAST MARKERS

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## *Abstract*

European Beech (*Fagus sylvatica* L.) is one of the main forest tree species in Poland and covers 5.2% of the forest area. The most typical forest tree associations are formed at the lower forest belt in Carpathians and Sudety Mountains on the South of Poland and at the moraine landscape of Pomeranian Lake District of the northern Poland. In Poland, beech attains its north-eastern limit of natural range. The investigated six beech populations were classified according to phytosociogical characteristics to the following plant associations: *Galio-odorati-Fagetum* (Gryfino and Kwidzyn), *Dentario glandulosae-Fagetum* (Bieszczadzki National Park), *Luzulo-luzuloides-Fagetum* (Suchedniów, Tomaszów), *Dentario enneaphyllidis-Fagetum* (Zdroje). The genetic structure of these populations was analyzed. Thirty individuals per one generation (mother and progeny stands) in every provenance were investigated. The genetic variation and differentiation of mother stands and their open-pollinated progeny were characterized on the basis of DNA chloroplast markers: ccmp4, ccmp7 and ccmp10. Parameters of genetic diversity ($H_S$ and $H_T$) and differentiation ($G_{ST}$) were counted and compared between mother and progeny generation.

**Key words:** *Fagus sylvatica*, genetic variation and differentiation, heterozygosity level.

## Introduction

European beech (*Fagus sylvatica* L.) is one of the most important forest tree species in Poland and covers 5.2% of the forest area in Poland (Lasy Państwowe 2009). Present genetic structure of beech population was formed within last few thousand years influenced by many different factors, not only environmental (glacial epoch) nor genetic (selection) but also anthropogenic (Szafer 1935, Huntley and Birks 1983, Ralska-Jasiewiczowa 1983, Hazler et al. 1997). In Poland, beech attains its north-eastern limit of natural range, which is limited by: continental climate, soil conditions, winter temperatures and air humidity (Sławiński 1947, Jedliński 1953, Boratyńska and Boratyński 1990). The growth of beech stands outside the natural beech limit indicates that this species possess potentially wider range (Brzeziecki 1995, Tarasiuk 1999). Recent investigations

of beech variation in Poland performed with isoenzyme study (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 North of the natural range limit, which generally confirm the migration paths after glaciations. The present paper describes the genetic structure within one generation, i.e. mother and progeny beech stands in Poland assessed with chloroplast cpDNA markers.

## Methods

The investigated nine beech populations were classified according to phytosociological characteristics to the following plant associations: *Galio-odorati-Fagetum* (Gryfino and Kwidzyn), *Dentario glandulosae-Fagetum* (Bieszczadzki National Park), *Luzulo-luzuloides-Fagetum* (Suchedniów, Tomaszów), *Dentario enneaphyllidis-Fagetum* (Zdroje) and were located within the natural range of beech distribution in Poland (Fig. 1). The genetic structure of these populations was analyzed. Thirty individuals per one generation (mother, progeny stands) in every provenance were investigated. The extraction of total DNA from the leaves was performed using Qiagen DNeasy™ Plant Minikit according to the manufacturer instruction (Qiagen). The quality and purity of DNA were analyzed on 1% agarose gel electrophoresis and via absorption in 230, 260 and 280 nm in NanoDrop® spectrophotometer (Wilmington, USA). The genetic variation and differentiation of mother stands and their open-pollinated progeny were characterized on the basis of three DNA microsatellite chloroplast markers: ccmp4, ccmp7 and ccmp10 according to Grivet et al. (2001) and Weising and Gardner (1999). DNA samples were analyzed in 8% acrylamide gel using automatic sequencer ALFexpress II (Amersham Pharmacia Biotech). Parameters of genetic diversity ($H_S$ and $H_T$) and differentiation ($G_{ST}$) were counted and compared between mother and progeny generation according to Nei (1972, 1978) in PopGene 1.32 software (Yeh and Boyle 1997). The obtained results were elaborated with ALFwin Fragment Analyser™ 1.0 software.

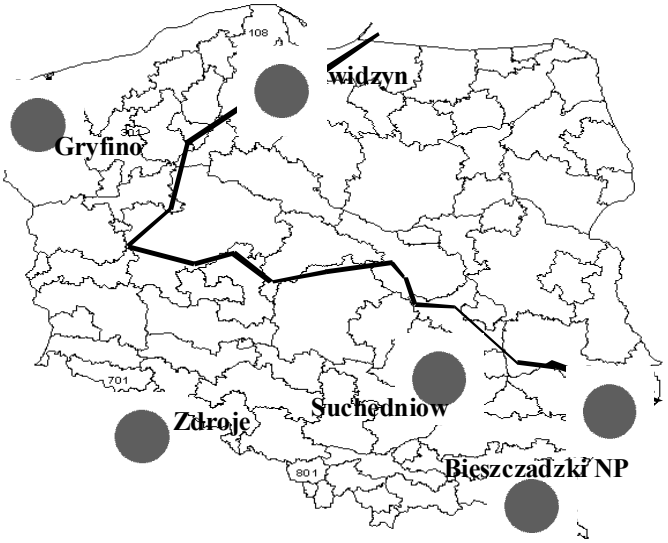



**Fig. 1. Localization of investigated Polish European beech populations.**

## Results and Discussion

Quality and quantity of the analyzed DNA

The very high quality and purity of DNA were assessed on the basis of the ratio of absorbance at 260 and 280 nm. A ratio about ~1.8 was typical for most of the samples (Fig. 2), and proved a high purity of the extracted DNA. A ratio ~2.0 is generally accepted as „pure" for RNA. If the ratio is lower in either case, it indicates the presence of contaminants. The good quality of isolated DNA from samples was confirmed also by measurement of absorbance at 230 nm wavelength. The quantity of the genomic DNA samples balanced between 35 up to 160 ng.$\mu l^{-1}$ and was fully appreciated to perform next steps of the DNA analysis procedure.

Genetic structure based on cytoplasmic DNA markers

As far as ccmp4 and ccmp10 microsatellite markers of Polish beech stands were concerned, different fragment-variants of haplotypes, i.e. from 116 to 152 base-pair size were distinguished. The ccmp7 locus was the most polymorphic, i.e. exhibited 8 allele variants: 144, 145, 147, 148, 149, 150, 151 and 152 bp. Nevertheless, some populations e.g. Bieszczadzki NP showed less polymorphism in the locus ccmp7, with only one 147 bp variant present, similarly to the previous study performed on Polish beech (Nowakowska and Oszako 2008). Another two loci, ccmp4 and ccmp10, shared the same range of 5 allele-size variants of 116, 117, 118, 119 and 120 bp. Generally, mother beech trees had more variable allele, than the

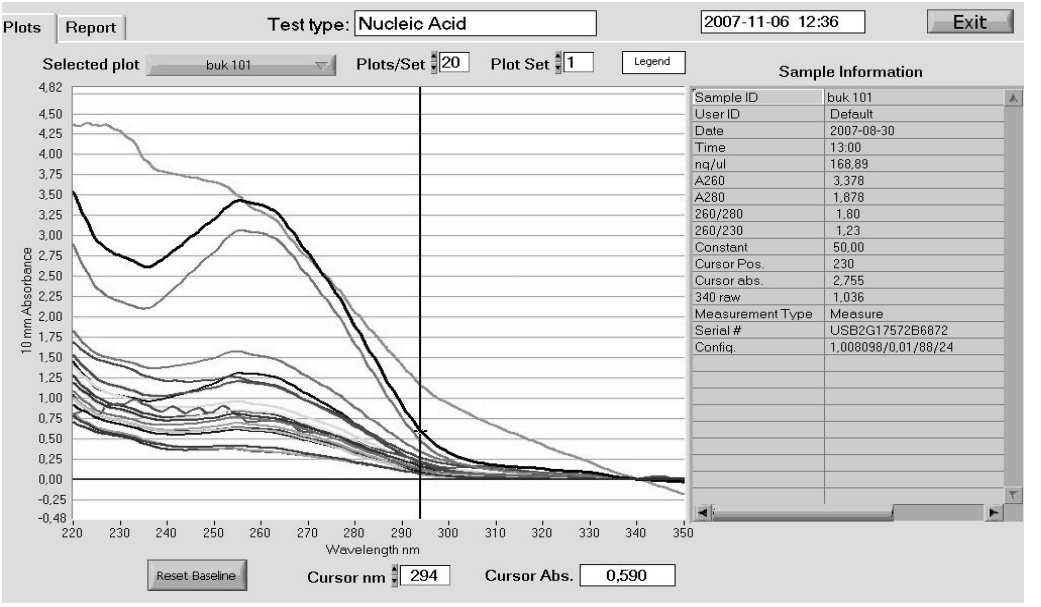
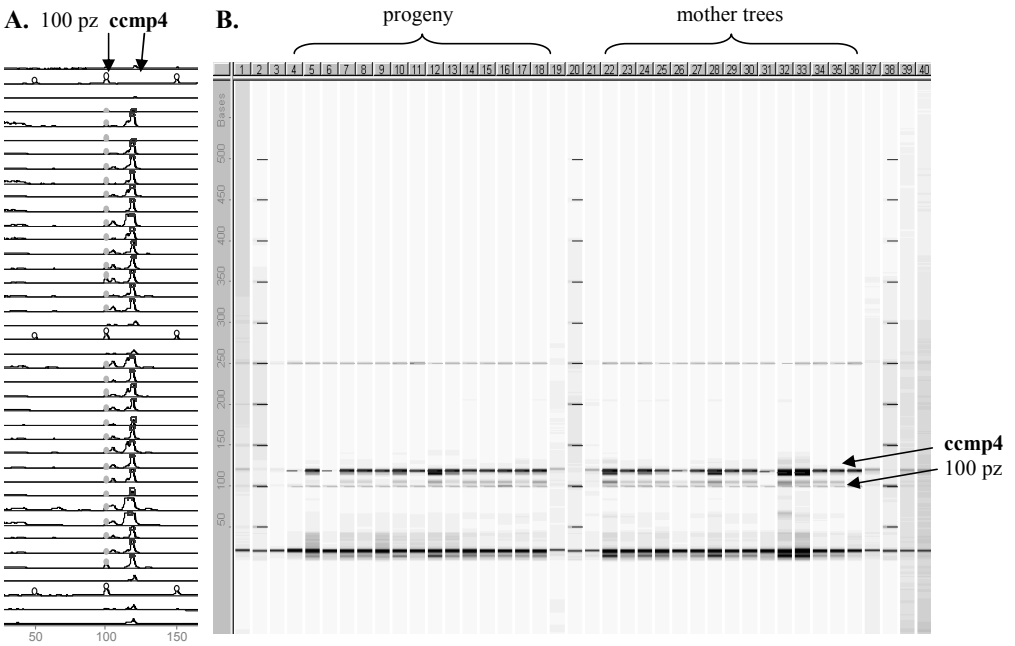



**Fig. 2. Quantity and quality of the extracted DNA from 10 mother and 10 progeny beech trees from Bieszczadzki NP stand, analyzed in NanoDrop® ND-1000 spectrophotometer.**




A: picks observed after automatic sequencer analysis. B: Electrophoresis on the acrylamide gel. 100 bp – size reference, ccmp4 – chloroplast locus with variable allele size around 119 base pairs. Lines 4–18: progeny trees, lines 22–36: mother trees.

**Fig. 3. Amplification profile of the locus ccmp4 in Bieszczadzki NP stand.**

progeny trees of the same provenance. For instance, mother trees presented alleles from 117 to 119 bp, comparatively to only one allele of 119 bp found in progeny from Bieszczadzki NP population (Fig. 3). Mean gene diversity among and within all studied mother stands (Table 1) were slightly higher ($H_T$ = 0.4916, $H_S$ = 0.3606 respectively) than mean genetic diversity in the progeny stands ($H_T$ = 0.4600, $H_S$ = 0.3375). The gene diversity level of all mother and progeny stands

**Table 1. Genetic variation parameters of microsatellite loci analyzed in *Fagus sylvatica* L.**

| Locus | Mother Stands | | | Progeny Stands | | |
|---|---|---|---|---|---|---|
| | $H_T$ | $H_S$ | $G_{ST}$ | $H_T$ | $H_S$ | $G_{ST}$ |
| ccmp4 | 0.5053 | 0.3058 | 0.3947 | 0.3957 | 0.3042 | 0.2313 |
| ccmp7 | 0.3228 | 0.2667 | 0.1738 | 0.4650 | 0.2767 | 0.4050 |
| ccmp10 | 0.6468 | 0.5092 | 0.2128 | 0.5193 | 0.4317 | 0.1688 |
| Mean | 0.4916 | 0.3606 | 0.2666 | 0.4600 | 0.3375 | 0.2663 |
| St. Deviation | 0.0264 | 0.0169 | – | 0.0038 | 0.0068 | – |

were almost at the same level: $G_{ST}$ = 0.2666 and $G_{ST}$ = 0.2663 respectively (Table 1). The overall haplotypic differentiation among studied Polish *Fagus sylvatica* populations was quite low ($G_{ST}$ = 0.016), which means that most genetic diversity resides within the stands. More cpDNA variation in the chloroplast ccmp4, ccmp7 and ccmp10 loci ($G_{ST}$ = 0.810) was reported for other 400 European beech populations (Magri et al. 2006).

High forest genetic diversity has a crucial role in maintaining biological diversity at species and ecosystem level, in the way of better forest trees survival, adaptation and evolution under changing climatic conditions. Facing several biotic and abiotic threats, variable gene pool may ensure the vitality of forests and better resistance against drought, pests, diseases and inappropriate use of forest reproductive material. Our study demonstrated almost unchanged gene pool between mother and progeny *Fagus* Polish stands, which means stable gene transmission between one generation analyzed.

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