Research paper

Microsatellite analysis of genetic diversity in Czech populations of European beech (*Fagus sylvatica* L.)

Dagmar Zádrapová, Jiří Korecký*, Jakub Dvořák, Zuzana Faltinová and Jan Bílý

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Abstract. European beech (*Fagus sylvatica* L.) is one of the most important broadleaved tree species in Europe both ecologically and economically. Nowadays, in the Czech Republic, beech is underrepresented in forest tree species composition, and there are tendencies to increase its proportion. When reintroducing beech, genetic variability, along with other factors, play a key role. The main aim of this study was to evaluate the genetic diversity of ten selected indigenous beech populations across the Czech Republic. Two hundred and fifty individuals were genotyped on 21 polymorphic nuclear microsatellite markers, which were amplified using two newly assembled multiplexes. According to the results, observed heterozygosity (H_o) among populations ranged from 0.595 to 0.654 and expected heterozygosity (H_e) from 0.650 to 0.678. That is comparable with the findings in other European studies. The high discriminatory power of the assembled multiplexes was confirmed by calculating the Probability of Identity among both unrelated and related individuals. Principal Coordinate Analysis (PCoA) based on Nei's genetic distances revealed that there are genetic differences among populations resulting in three approximate clusters (geographically north, south-east, and south-west). Nevertheless, the results implicate that on a geographical scale of the Czech Republic, the distance is unlikely to be the primary driver of genetic differentiation.

Key words: multiplex SSRs PCR, genetic variation, local populations.

Authors' address: Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Prague – Suchbát, Czech Republic; korecky@fd.czu.cz

Introduction

European beech (*Fagus sylvatica* L.) is one of the most widespread broad-leaved tree species in Europe, covering circa 12 million ha of land (Buitelvelde *et al.*, 2007). It is characterized by an extensive ecological niche, high competitiveness and physiological tolerance against a wide range of environmental factors, making beech a vital tree species both ecologically and economically

(Dittmar *et al.*, 2003; Pastorelli *et al.*, 2003; Leuschner *et al.*, 2006; Lefèvre *et al.*, 2012). In many regions of Central Europe, including the Czech Republic, beech covered naturally large areas. However, due to medieval deforestation and revenue-oriented forest management, many of the natural beech stands have been replaced by spruce (*Picea*) or pine (*Pinus*) species (Paule, 1997; Hasenkamp *et al.*, 2011). In the Czech Republic, the natural representation of beech

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was 40.2% compared to its current deficient proportion of just 8.6% which represents 223,611 ha (Fulín *et al.*, 2016; Ministry of Agriculture, 2019). Nowadays, however, there are trends to put beech back into the forests, and it is frequently used together with other broadleaves for reforestation (Tomášková, 2004). The recommended proportion of beech in the tree species composition makes 18% (e.g. approximately 470,000 ha), which means a future increase of about 9.5% (Ministry of Agriculture, 2019). The preferred seed sources should provide the genetic pre-conditions for the best possible adaptation to the respective local conditions and presumed climatic shifts (Hasenkamp *et al.*, 2011; Gömöry *et al.*, 2015). Mainly because of ongoing climate change, the study of genetic variation and differentiation of beech populations is crucial for determining the adaptive potential of the species (Jump *et al.*, 2006a, 2006b; Sjölund & Jump, 2015; Kempf & Konnert, 2016). In the Czech Republic, supposedly native populations are often located in the border regions of the country. Moreover, these sources are usually under a particular degree of forest protection. Knowledge of the genetic structure of indigenous populations might reveal the significant indicator of the best-adapted populations for a given site. Therefore, efficient methods for reliable identification of potentially well-preadapted seeds are needed. In the past, beech has been studied by various genetic markers such as isozymes (Thiebaut *et al.*, 1982; Comps *et al.*, 1991; Degen & Scholz, 1998; Božič & Kutnar, 2012), Random Amplification of Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) (Scalfi *et al.*, 2004), nuclear, chloroplast and mitochondrial microsatellites (Pastorelli *et al.*, 2003; Asuka *et al.*, 2004; Vettori *et al.*, 2004; Vornam *et al.*, 2004; Magri *et al.*, 2006; Hasenkamp *et al.*, 2011; Lefèvre *et al.*, 2012; Pluess & Määtänen, 2013) and Single Nucleotide Polymorphism (SNP) (Seifert *et al.*, 2012). Out of all the markers mentioned above, nu-

clear microsatellite markers (SSRs, Simple Sequence Repeats) represent an ideal tool for studying genetic diversity, considering their high abundance in genomes, a high degree of polymorphism, neutrality and locus-specificity (Pastorelli *et al.*, 2003). In the past, several studies used multiplexed microsatellite loci to determine genetic variability on a population level (Tanaka *et al.*, 1999; Pastorelli *et al.*, 2003; Asuka *et al.*, 2004; Lefèvre *et al.*, 2012; Plues & Määtänen, 2013).

This paper presents the results of nuclear-microsatellite analyses of beech populations from ten regions in the Czech Republic. To evaluate their population genetics parameters, we used two newly modified multiplexes (13- and 8-plex). We focused on the following questions: (i) Are there significant differences between local beech populations across the Czech Republic? (ii) Can we eventually identify substantial differences among the targeted population? (iii) What is the discrimination power of our marker assemblage?

Material and Methods

Study site and sampling

We identified ten beech stands across the Czech Republic (Table 1, Figure 3) aiming at populations that are considered to be locally adapted and superior under local conditions. Each of the selected populations was represented either by 20 or 30 mature individuals according to the site characteristics, which turned into 250 tested individuals in total. Sampling was carried out in 2017. The minimal distance between sampled trees was 30 m to avoid sampling among closely related individuals (von Wühlisch, 2008). For each individual, we collected unburst buds and extracted the vascular cambium with a hole punch. Plant tissues were labeled appropriately and placed in plastic bags with silica gel. Then they were long-term stored in a freezer.

Table 1. Stand-specific data of the samples.

No. of Population	Provenance Name	Average Altitude (m a.s.l.)	Geographic Coordinates		No. of Samples
			latitude	longitude	
1	Frýdlant	441	50°52'24.1"N	15°06'36.1"E	20
2	Kladská	755	50°00'11.8"N	12°38'55.5"E	20
3	Litvínov	794	50°36'46.8"N	13°23'14.5"E	20
4	Boubín	916	49°09'05.5"N	13°40'03.2"E	20
5	Nové Hradý	863	48°39'46.6"N	14°40'51.0"E	20
6	Hluboká	538	49°06'24.3"N	14°29'51.6"E	30
7	Jeseník	652	50°11'34.1"N	17°13'55.0"E	30
8	Frýdek Místek	839	49°31'44.7"N	18°28'28.6"E	30
9	Luhačovice	479	49°03'06.0"N	18°04'19.0"E	30
10	Lanškroun	573	49°56'07.4"N	16°30'33.2"E	30

DNA extraction

Initially, both the vascular cambium and leaf buds were tested for DNA extraction. Even though both types of tissues provided identical genetic profiles, we decided to extract the DNA from the buds, because of its higher purity and yield. From each individual, approximately 2 or 3 unsterilized fresh buds were cut into small pieces and placed into 2 mL safe-lock tubes (Eppendorf, Germany). Two 3 mm tungsten beads were added in each tube and frozen in liquid nitrogen before grinding (1 min, 30 Hz) using a mixer mill MM400 (Retsch, Haan, Germany). Genomic DNA was extracted with the Geneaid® Genomic DNA Mini Kit (Plant) according to the manufacturer's instructions with a minor modification in the lysis step (60 min of incubation phase). The DNA quantity and quality were measured with a NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The quality of the DNA was verified using 1.5% agarose gel electrophoresis. For subsequent Polymerase Chain Reaction (PCR) steps, we diluted all DNA samples to a working concentration of 20 ng/μL.

Multiplex PCR optimization

After amplification in single reactions, from preselected microsatellite loci, we excluded those which were difficult to evaluate or of low polymorphism (< 4 alleles), with poor amplification quality or low signal intensity. This led to a total number of 21 highly validated fluorescently labeled genomic markers – mfc5, mfc7 a mfc11 (Tanaka *et al.*, 1999), FS1-03 (Pastorelli *et al.*, 2003), sfc0036 (Asuka *et al.*, 2004), concat14_A_0, EMILY_A_0, csolfagus_05, csolfagus_06, csolfagus_19, csolfagus_29, csolfagus_31, DE576_A_0, ERHBI_A_0, EEU75_A_0, DZ447_A_0 a DUKCT_A_0 (Lefèvre *et al.*, 2012), Fagsyl_002929, Fagsyl_003849, Fagsyl_001018 and Fagsyl_003093 (Pluess & Määttänen, 2013) as presented in Table 2. The final loci selection was divided into two multiplexes (13- and 8-plex) according to their size ranges and PCR amplification compatibility. An issue of different annealing temperatures among selected primers was considerably decreased when using Type-it® Microsatellite PCR kit (Qiagen, Hilden, Germany) for PCR amplification. Both multiplexes showed clear patterns and consistent amplification profiles.

Table 2. Features of microsatellite primers assembled into multiplexes (SSR: simple sequence repeats).

SSR Locus	Allele Size Range (bp)*	Primer Sequences 5' – 3'		Repeats	Fluorescent Dye	Source Reference	Primers Volume F + R [μL]**
		F	R				
Multiplex 1	concat14_A_0	TGAAGAAATTCACACCCAACA	GGGTGTTTACGATGGTGA	(TC) ₉	PET	1	0.6
	EMILY_A_0	GACCCCAAGGTACAGTGCT	CGTACAATTGCACCCACATC	(GA) ₁₁	PET	1	0.36
	csolfagus_31	TCTATTGACACAAGAAATAAGAACACC	CTTGCGAAGAAAAGGGGATT	(AG) ₁₂	PET	1	0.45
	mfc11	ACAGATAAAAACAGAAGCCA	TTTGTTTTTTGTTAGTTAG	(AG) ₁₀	NED	2	0.45
	DE576_A_0	TCTCTTAGATCCACAATCACA	AGCTCTTCATTGCTCAGAACG	(CAA) ₁₀	NED	1	0.9
	csolfagus_05	GGTTTCTAGCAAAATTGGCATT	CCCAAAAGGCCCTACTACAA	(GA) ₁₀	NED	1	0.36
	FS1-03	CACAGTTTGACACATTCCAAC	TGGTAAAGCACTTTTCCCACT	(GA) ₁₈	NED	3	0.24
	mfc5	ACTGGGACAAAAAACAATAA	GAAGGACCAAGGCACATAAA	(AG) ₁₀	FAM	2	0.6
	csolfagus_19	TGCCATGAGTTTGTATCA	GCCGAATAAACCCAGAAAACA	(TC) ₁₃	FAM	1	0.24
	csolfagus_06	GTTGTGTCTACAGCAGTCG	AGCTTGGTCTCTTCTGCAC	(AG) ₁₃	FAM	1	0.24
Multiplex 2	sfc0036	CATGCTTGACTGACTGTAAGTTC	TCCAGGCCCTAAAAACATTATAG	(TC) ₂₃	FAM	4	0.3
	mfc7	AAATACACTGCCCCCAAAA	CAGGTTTTGGTTTCTTACAC	(GA) ₉	VIC	2	0.3
	ERHBI_A_0	TGCAACAACCTAGCACTTTGA	GCGTGGCTTATCCAAAAT	(AG) ₉	VIC	1	0.12
	EEU75_A_0	TTCAAACCAACCCCTTTATCC	GACGGAGATTGAGGAAGAACA	(CT) ₁₀	PET	1	0.3
	Fagsyl_002929	GCGGGACTGGAAATAATAGC	CAATCACACGCTGCACAAAC	(TTG) _x	NED	5	0.3
	Fagsyl_003849	GCTTGTTCTTCGGCATCTC	AATAGCACAAATAGCCCGCC	(CT) _x	NED	5	0.21
	DZ447_A_0	GGTGCAATACTTCACTTTAGGACA	ATAGGAGTGGGACGGCTAGG	(TC) ₁₀	FAM	1	0.3
	csolfagus_29	CACAACCTGCATCCCTTTC	GTTTGGCACTTTGGCTTGT	(CT) ₁₁	FAM	1	0.18
	Fagsyl_001018	AATATCAGGGAGGCAGCAC	CGAGATGGACTCTTAAGTTTATTTC	(CA) _x	FAM	5	0.21
	DUKCT_A_0	GCCTCTCGCAGCTCCTATAA	GATCTAATGTGGGTTTGTTTTG	(AC) ₁₄	FAM	1	0.18
	Fagsyl_003093	TCATCACCGAGACAAGGGAC	ATGTTGGTGTGGAAGCTAGG	(CA) _x	VIC	5	0.18

¹Lefèvre *et al.*, 2012, ²Tanaka *et al.*, 1999, ³Pastorelli *et al.*, 2003, ⁴Asutka *et al.*, 2004, ⁵Pluess & Määtänen, 2013.

* Allele Size Range stands for minimum and maximum lengths of PCR products detected for a locus (in base pairs).

** Final concentration in each primer premix forward + reverse.

PCR

Amplification was performed in two multiplex polymerase chain reactions (Mastercycler® nexus, Eppendorf, Germany). The PCR mixture for multiplex 1 was performed in a total volume of 11.4 µL containing 1 µL template DNA ($c = 20 \text{ ng}/\mu\text{L}$), 5.7 µL of Type-it® solution (Qiagen, Hilden, Germany), 0.26 µL sterile water and a total volume of 4.44 µL primer premix. The PCR for multiplex 2 was performed in a total volume of 6 µL, consisting of 1 µL template DNA ($c = 20 \text{ ng}/\mu\text{L}$), 3 µL of Type-it® solution (Qiagen, Hilden, Germany), 0.14 µL of sterile water and 1.86 µL of primer premix. Concentrations and label dyes for each primer pair in the final primer premix are shown in Table 2. Both multiplexes had the same PCR conditions as follows: an initial incubation (5 min at 95 °C) was followed by 33 cycles consisting of the denaturation (45 s at 95 °C), annealing (45 s at 60 °C) and extension phase (60 s at 72 °C). Amplification was terminated with the final extension step (30 min at 72 °C). The fluorescently labelled PCR products, together with an internal size standard (GMC-GT500 LIZ) were processed on a 3500 Series Genetic Analyzer® (Applied Biosystems, Foster City, CA, USA).

Data analysis

Allele identification and genotyping were done using the GeneMarker® Fragment Analysis software (SoftGenetics, State College, PA, USA). All alleles were subsequently checked manually to minimize genotyping errors. Allele frequency analysis was performed using CERVUS v. 3.0.7 (Kalinowski *et al.*, 2007) in order to identify the number of alleles per locus. Also, summary statistics were calculated for each locus, such as observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC) and fixation index (F_{IS}).

For the analysis of population genetic parameters, we used software GenAIE

v. 6.5 (Peakall & Smouse, 2006, 2012). To group populations according to their pairwise distances in a distance matrix, a Principal Coordinate Analysis (PCoA) was carried out. The distribution of total variance at different hierarchical levels was quantified with an Analysis of Molecular Variance (AMOVA). We also evaluated the discrimination power of assembled multiplexes. Therefore, the Probability of Identity (PI) for unrelated and related (PI_{sibs}) individuals was calculated, which represents a measure for the statistical power of individual identification with the chosen marker combination (Hartl & Clark, 2007).

Results

Allele frequency analysis

All samples ($n = 250$) were genotyped on 21 SSR loci grouped into two multiplexes (Table 3). The number of genotyped individuals on each locus (N) varies because some of the samples did not show any apparent alleles on a particular locus. We did not detect vegetative copies among the samples. We observed the most significant dropout of samples (56 individuals) on locus Fagsyl_003093.

The total amount of 208 alleles was detected. The number of alleles per locus (k) ranged from 4 to 22 with a mean value of 9.905 ± 4.098 . The highest count of alleles per locus (22) was found at mfc5 where we also observed significant deviation from Hardy-Weinberg equilibrium. The expected heterozygosity (H_e) ranged from 0.436 (csolfagus_29) to 0.906 (mfc5), with an average of 0.685 ± 0.134 . The observed heterozygosity (H_o) ranged from 0.216 (Fagsyl_003093) to 0.859 (csolfagus_06) with an average of 0.625 ± 0.183 . The mean polymorphic information content (PIC) across all loci was 0.649 ± 0.146 . Most loci did not show a significant deviation from the expected Hardy-Weinberg frequencies, except 5 loci (mfc11, FS1-03, mfc5, Fagsyl_001018, Fagsyl_003093). We also

detected 6 loci with a significant occurrence of null alleles ($\alpha = 0.05$, marked bold in Table 3).

Private alleles were found in all analyzed populations except populations 5 and 10. The frequencies of rare alleles were

generally low and ranged between 0.017 and 0.077, with an average of 0.033 ± 0.019 . The highest amount of private alleles was observed in populations 1 and 9. Private alleles with the highest frequencies were found in population 2.

Table 3. Locus-specific parameters.

Locus	<i>k</i>	<i>N</i>	<i>H_o</i>	<i>H_e</i>	<i>PIC</i>	<i>HW</i>	<i>F (Null)</i>
concat14_A_0	4	249	0.474	0.494	0.461	NS	0.0229
EMILY_A_0	6	249	0.763	0.770	0.731	NS	0.0009
csolfagus_31	13	249	0.855	0.858	0.842	NS	-0.0005
mfc11	9	249	0.353	0.616	0.594	***	0.2575
DE576_A_0	7	248	0.710	0.669	0.613	NS	-0.0325
csolfagus_05	8	249	0.675	0.679	0.639	NS	0.0068
FS1-03	14	246	0.528	0.622	0.594	**	0.088
mfc5	22	249	0.669	0.906	0.897	**	0.1504
csolfagus_19	13	249	0.803	0.844	0.824	NS	0.0262
csolfagus_06	12	248	0.859	0.867	0.851	NS	0.0042
sfc0036	8	248	0.786	0.772	0.744	NS	-0.0114
mfc7	9	249	0.578	0.596	0.559	NS	0.134
ERHBI_A_0	6	249	0.514	0.530	0.491	NS	0.0163
EEU75_A_0	11	245	0.808	0.805	0.777	NS	-0.0040
Fagsyl_002929	11	247	0.765	0.729	0.693	NS	-0.0269
Fagsyl_003849	14	247	0.814	0.808	0.783	NS	-0.0034
DZ447_A_0	4	245	0.592	0.593	0.545	NS	0.0051
csolfagus_29	8	248	0.435	0.436	0.400	NS	-0.001
Fagsyl_001018	9	246	0.390	0.656	0.608	***	0.2696
DUKCT_A_0	8	246	0.537	0.569	0.449	NS	0.0291
Fagsyl_003093	12	194	0.216	0.571	0.530	***	0.4491
Mean	9.905	245	0.625	0.685	0.649		
<i>SD</i>	4.098		0.183	0.134	0.146		
<i>SE</i>	0.894		0.040	0.029	0.032		

Number of allele per locus (*k*), number of individuals genotyped on given locus (*N*), observed heterozygosity (*H_o*), expected heterozygosity (*H_e*), polymorphic information content (*PIC*), deviation from Hardy-Weinberg equilibrium (*HW*), level of significance: *** $\alpha = 0.001$, ** $\alpha = 0.01$, NS - not significant), estimation of null allele frequency (*F (Null)*), significant estimates are marked bold), standard deviation (*SD*), standard error (*SE*).

The discriminatory power of multiplexed SSRs markers

In order to evaluate the discriminatory power of used multiplexes, the Probability of Identity (*PI*, GenAlEx v. 6.5) was carried out. Figure 1 shows the decreasing probability of identity with an increasing

number of markers used (e.g., the effect of cumulative markers).

In our study, we also evaluated a modified version *PI_{sib}* for estimating the probability of identity when related individuals are included in the sample. When using 5 randomly selected loci, the probability of

statistically significant distinction was for unrelated individuals $PI = 7.2 \times 10^{-4}$ and for related individuals $PI_{sib} = 0.04$. With 8 loci used the probability decreases to $PI = 3.13 \times 10^{-7}$ and $PI_{sib} = 2.2 \times 10^{-2}$. With 15 loci the probability further declines to $PI = 4.97 \times 10^{-15}$ and $PI_{sib} = 3.78 \times 10^{-6}$. For an entire set of 21 loci, the observed PI for un-

related individuals was $PI = 1.85 \times 10^{-20}$ and for related individuals $PI_{sib} = 2.77 \times 10^{-8}$. These values confirm a high discriminatory power of used multiplexes even when family structures are expected among samples and the discriminatory power of loci is therefore significantly lower.

Figure 1. The discriminatory power of loci expressed by Probability of Identity (PI) for unrelated individuals (orange line) and related individuals (green line).

Intra and interpopulation genetic diversity

The parameter of observed heterozygosity (H_o) among populations ranged from 0.595 (Pop. 3) to 0.654 (Pop. 9) with the mean value of 0.625 ± 0.014 . Expected heterozygosity (H_e) ranged from 0.651 (Pop. 6) to 0.678 (Pop. 8) with the mean value of 0.664 ± 0.010 .

The Principal Coordinate Analysis was performed based on genetic distances between the populations (e.g. the pairwise population matrix of Nei's genetic distance, Table 5). The result of PCoA is presented in Figure 2. The first two coordinates explain 35% of the total variation. PCoA sorted the populations in three groups, which can be

simplified as south-west (Pops. 5, 4, 2, 3 and 7), north (Pops. 1 and 10), and south-east (Pops. 6, 9 and 8). Population 9 was the most distant from the others. The approximate regionalization of the populations is shown in Figure 3. However, in the scale of the Czech Republic, the geographical distance cannot be seen as the main driver of the genetic variation between the tested indigenous populations. AMOVA indicated that 98% of total molecular variance occurs within populations. Only 2% of the variation can be observed on an interpopulation level. It corresponds with the previous findings that a significant part of the genetic variability of forest trees is attributable to the intrapopulation level.

Table 4. Estimations of population parameters.

Population		N	N_a	N_e	I	H_o	H_e	F
1	Mean	19.524	6.905	3.925	1.447	0.635	0.674	0.074
	SE	0.290	0.581	0.522	0.102	0.046	0.030	0.042
2	Mean	19.571	6.476	3.583	1.414	0.629	0.662	0.072
	SE	0.335	0.550	0.339	0.093	0.057	0.034	0.063
3	Mean	19.381	6.238	3.427	1.363	0.595	0.650	0.089
	SE	0.288	0.530	0.335	0.095	0.047	0.033	0.054
4	Mean	19.667	6.571	3.627	1.410	0.623	0.667	0.069
	SE	0.159	0.500	0.364	0.091	0.049	0.032	0.054
5	Mean	19.810	6.667	3.625	1.416	0.632	0.664	0.045
	SE	0.190	0.618	0.383	0.095	0.048	0.032	0.054
6	Mean	29.381	6.905	3.549	1.393	0.607	0.651	0.082
	SE	0.288	0.589	0.417	0.098	0.044	0.032	0.042
7	Mean	29.857	7.238	3.792	1.439	0.623	0.667	0.064
	SE	0.143	0.617	0.419	0.104	0.044	0.035	0.046
8	Mean	29.667	7.381	3.879	1.484	0.630	0.678	0.064
	SE	0.333	0.674	0.502	0.099	0.039	0.030	0.046
9	Mean	29.190	6.905	3.501	1.415	0.654	0.670	0.041
	SE	0.190	0.625	0.290	0.088	0.043	0.028	0.038
10	Mean	29.143	6.905	3.648	1.419	0.619	0.662	0.062
	SE	0.469	0.625	0.419	0.098	0.042	0.032	0.046
Total	Mean	24.519	6.819	3.655	1.420	0.625	0.664	0.066
	SE	0.352	0.185	0.126	0.030	0.014	0.010	0.015

Sample size (N), mean number of alleles per locus (N_a), effective number of alleles (N_e), mean Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), mean fixation index (F).

Principal Coordinates (PCoA)

Figure 2. Principal Coordinate Analysis (PCoA) of the populations 1–10 (further described in Table 1).

Table 5. Nei's genetic distances between the provenances (Nei, 1972, 1987)

1	2	3	4	5	6	7	8	9	10	
0.000										1
0.067	0.000									2
0.071	0.078	0.000								3
0.064	0.061	0.069	0.000							4
0.078	0.061	0.064	0.049	0.000						5
0.062	0.070	0.076	0.061	0.077	0.000					6
0.064	0.059	0.053	0.051	0.062	0.067	0.000				7
0.082	0.062	0.064	0.062	0.067	0.056	0.049	0.000			8
0.079	0.075	0.088	0.081	0.091	0.056	0.074	0.057	0.000		9
0.055	0.065	0.074	0.065	0.072	0.079	0.060	0.061	0.087	0.000	10

Figure 3. Populations 1–10 divided into three regions by PCoA (populations further described in Table 1).

Discussion

The mean value of observed heterozygosity (H_o) among all 21 loci was 0.625 ± 0.183 , expected heterozygosity (H_e) was 0.685 ± 0.134 . These values resemble other authors – Vornam *et al.* (2004) who observed $H_o = 0.572$ and $H_e = 0.765$ on 4 loci, Kempf & Konnert (2016) $H_o = 0.698$ and

$H_e = 0.701$ on 10 loci, Hasenkamp *et al.* (2011) $H_o = 0.663$ and $H_e = 0.750$ and for the other study site $H_o = 0.667$ and $H_e = 0.776$ on 6 loci, Dounavi *et al.* (2016) $H_o = 0.578$ and $H_e = 0.604$ on 9 loci and Pluess & Määttänen (2013) $H_o = 0.564$ and $H_e = 0.616$ on 18 loci. Slightly higher values observed Pastorelli *et al.* (2003) $H_o = 0.727$ and $H_e = 0.752$ on 7 loci and Lefèvre *et al.* (2012) $H_o = 0.727$ and

$H_e = 0.695$ on 16 loci. A similar study in the Czech Republic was carried out by Cvrčková *et al.* (2017). They evaluated 390 beech individuals from 13 populations on 12 SSR markers where seven loci were identical with the ones we used in our study. They observed $H_o = 0.702$ and $H_e = 0.755$.

The most variable locus was *mfc5*, as previous studies have shown (Vornam *et al.*, 2004; Hasenkamp *et al.*, 2011). However, we also observed a deviation from Hardy-Weinberg equilibrium on this locus as well as a higher rate of null alleles occurrence ($F(\text{Null}) = 0.1504$). Null alleles above the threshold $\alpha = 0.05$ were also observed on loci *mfc11*, *FS1-03*, *mfc7*, *Fagsyl_001018* and *Fagsyl_003093*. As confirmed in a review assembled by Janković *et al.* (2019), in many studies, null alleles were commonly observed on both *mfc5* and *FS1-03*. Besides those, one study observed null alleles also on loci *csolfagus_06*, *csolfagus_19* and *csolfagus_31* where we failed to do so. Loci *mfc11*, *FS1-03*, *mfc5*, *Fagsyl_001018* and *Fagsyl_003093* exhibited high F_{is} values. During scoring locus *csolfagus_05* exhibited split peaks and locus *Fagsyl_003093* created an artefact allele. Loci *sfc0036* (Asuka *et al.*, 2004), *mfc5*, *mfc7* and *mfc11* (Tanaka *et al.*, 1999) were originally developed for related species *Fagus crenata* Fagus Blume and successfully used also for *F. japonica* Maxim. and *F. sylvatica* L.. It can be due to the fact that species within the genus *Fagus* are very closely related (Tanaka *et al.*, 1999).

PI and PI_{sib} analysis confirmed the high discriminatory power of both newly assembled multiplexes, and their ability to identify particular individuals. In analogous studies mentioned above, between 4 and 18 loci were used to genotype the individuals. In their study, Lefèvre *et al.* (2012) estimated PI for 16 loci 2.1×10^{-8} and stated that this probability of finding two randomly matching genotypes is extremely low. In our study, we reached this level of PI by using nine loci for unrelated and 21 loci for related individuals.

The PCoA analysis clustered studied indigenous populations into three approximate regions, geographically roughly corresponding to north, south-east and south-west. However, on the scale of the Czech Republic, distance cannot be considered as a significant criterion of population clustering. Long-term local adaptive processes are more likely to cause it.

Significant differences in observed heterozygosity within populations have not been detected. In general, the genetic diversity of the investigated populations was similar to populations across Central Europe. In our study H_o range was 0.595–0.654 and H_e range was 0.650–0.678 which is comparable with the results of Dounavi *et al.* (2016) who observed the mean values of $H_o = 0.578$ and $H_e = 0.604$ among stands in southern Germany (Bavaria), Croatia, Bulgaria and Greece. Slightly higher values observed Kempf & Konnert (2016) among stands in Poland where northern ecotypes exhibited $H_o = 0.697$ and $H_e = 0.706$, southern ecotypes $H_o = 0.705$ and $H_e = 0.701$ and overall $H_o = 0.701$ and $H_e = 0.704$. In the Czech Republic, Cvrčková *et al.* (2017) observed values of $H_o = 0.697$ and $H_e = 0.765$. In all these studies, differences between the observed and expected values of heterozygosity of studied populations were rather small. Across the studied populations, the highest number of private alleles was found on locus *Fagsyl_003093*, followed by loci *Fagsyl_003849*, *mfc5* and *csolfagus_29*. Cvrčková *et al.* (2017) observed the highest number of private alleles in a population which was geographically very close to population 9 in our study and which also exhibited the highest number of private alleles. This may indicate a bigger genetic differentiation of beech individuals in this region which is also shown in the PCoA analysis (Figure 2).

AMOVA showed that 98% of total variability is observed on the intrapopulation level, only 2% occurs among the populations. This result is in agreement with previous studies as well, for example,

Kempf & Konnert (2016) observed 93% of total variation within populations and 5% among them and 2% between observed regions. As previous findings suggest, little differentiation on interpopulation level might be due to an extensive gene flow between beech populations over long distances (Wang, 2004; Piotti *et al.*, 2012).

Conclusions

The results presented in this paper offer an insight into the genetic diversity of indigenous populations of European beech on the territory of the Czech Republic where only a few such studies have been done so far. Although the majority of detected variance is not attributable to a particular population, the evaluation of allelic parameters (heterozygosity, allelic diversity) is crucial and should not be omitted. Genetic variability, especially during artificial reforestation and the ongoing effort to increase the percentage of beech in the forests, should be carefully considered. It gains even more importance in the context of the continuing climate change, which is highly challenging for the adaptive and survival potential of species. In general, maximizing genetic diversity during artificial reforestation might prevent gene pool reduction and thus indirectly maintain allelic forms with higher fitness levels under shifted climatic conditions. Moreover, we confirmed that a well-optimized set of nuclear microsatellite markers is a useful and robust tool for the examination of genetic structure.

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