JOURNAL OF FOREST SCIENCE, *63*, 2017 (2): 53–61

doi: 10.17221/88/2016-JFS

Evaluation of the genetic diversity of selected *Fagus sylvatica* L. populations in the Czech Republic using nuclear microsatellites

Helena CVRČKOVÁ, Pavlína MÁCHOVÁ, Lucie POLÁKOVÁ, Olga TRČKOVÁ*

Forestry and Game Management Research Institute, Jíloviště-Strnady, Czech Republic

**Corresponding author: cvrckova@vulhm.cz*

Abstract

Cvrčková H., Máchová P., Poláková L., Trčková O. (2017): Evaluation of the genetic diversity of selected *Fagus sylvatica* L. populations in the Czech Republic using nuclear microsatellites. J. For. Sci., 63: 53–61.

Fagus sylvatica Linnaeus (European beech), the ecologically and economically most important broadleaved tree species in the Czech Republic, was strongly reduced in the past. Today there are efforts to increase the proportion of beech to ensure optimal forest tree species composition. When extensively reintroducing beech, it is important to acquire more detailed knowledge of genetic diversity. Thirteen important beech populations in different stands in the territory of the Czech Republic were genotyped using 12 polymorphic nuclear microsatellite markers. The genotypic data from adult trees imply genetic differences between the populations. The estimated genetic diversity expressed as Shannon's information index ranged from 1.73 to 1.92. Thirteen beech populations showed excess homozygotes, as indicated by positive fixation index (*F*) values (*F* = 0.005–0.115). The pairwise F_{ST} values indicated low genetic differentiation between the 13 Czech beech populations, because they were greater than zero, that means they confirmed the presence of population structuring in Czech European beech. Not significant linear correlations were observed between genetic and geographic distances of the 13 beech populations studied on the basis of microsatellite markers. Twelve microsatellite markers were verified as highly polymorphic and suitable for genotyping of European beech populations.

Keywords: DNA analysis; European beech populations; gene reserves; genetic distance; genetic variability; simple sequence repeats

Fagus sylvatica Linnaeus (European beech) is ecologically and economically the most important broadleaved tree in the Czech Republic, where conditions for its growth are favourable. This species is a monoecious diploid (2*n* = 24), wind-pollinated, late-successional forest tree (Oddou-Muratorio et al. 2011). It is a woody species of the European area with focus of distribution in the western, central and south-eastern parts of the continent. The Czech Republic is located within that area, so this tree species grows throughout the Central Highlands and in the Hercynian and Carpathian mountainous regions of the country. Beech often constitutes unmixed stands at altitudes of approximately 400–800 m a.s.l. It is generally associated with oak at lower natural boundaries of its occurrence and with Norway spruce or silver fir at upper boundaries (Úradníček et al. 2009). The devastation of beech stands began in the Middle Ages due to the extensive use of this species as fuel, and in the 19th century there occurred a strong decline in European beech cultivation as a consequence of artificial reforestation and silvicultural preferences of conifers, especially Norway spruce. The dissemination of beech

Supported by the Ministry of Agriculture of the Czech Republic, Resolution RO0116 (reference number 10462/2016-MZE-17011), and Project No. QJ1230334.

began in the mid-20th century again, but seeds from the Carpathian region were used for reforestation without testing the suitability of these provenances (Svoboda 1955). Recently, the main coniferous area, such as of spruce, pine and larch, has gradually decreased in the Czech Republic while growing proportions of silver fir and such deciduous trees as European beech have been planted in order to achieve optimal species composition of forests (Ministry of Agriculture of the Czech Republic 2015).

In order to reintroduce this species in larger proportions, it is important to acquire more detailed knowledge about the dynamics of genetic diversity within and among beech populations. *F. sylvatica* is one of the most thoroughly investigated European tree species, and it has been the subject of numerous genetic surveys using chloroplasts (Vettori et al. 2004), isozymes (Gömöry et al. 1998; Konnert et al. 2000; Comps et al. 2001; Dounavi et al. 2010), amplified fragment length polymorphism (AFLP) (Scalfi et al. 2004; Jump, Peñuelas 2007), single nucleotide polymorphism (SNP) markers (Seifert et al. 2012), and microsatellite markers (Pastorelli et al. 2003; Asuka et al. 2004; Vornam et al. 2004; Hasenkamp et al. 2011; Lefèvre et al. 2012; Pluess, Määttänen 2013). With their high degree of polymorphism, microsatellites provide an ideal tool for gene flow studies (Pastorelli et al. 2003). Nuclear microsatellites are codominant markers providing important information about population dynamics, spatial genetic structure, and distribution of genetic diversity (Lefèvre et al. 2012). To provide an insight into the levels of genetic variation and differentiation of 13 important, naturally regenerated European beech populations growing in different parts of the Czech Republic, we used the method of simple sequence repeats (SSR) for DNA analysis. Analyses of large populations at multiple SSR markers are expensive and time-consuming processes. Therefore, we optimized polymerase chain reaction (PCR) conditions using tested primer pairs to assemble them into multiplexes. Multiplexing consists in the amplification of several markers in a single PCR. In this study, we assembled 14 SSR markers into 3 multiplexes, and we successfully genotyped 390 individuals from the 13 beech populations.

MATERIAL AND METHODS

Sampling was carried out during 2012–2015 from 13 populations growing mostly in gene reserves (gene conservation units), protected landscape areas, or national nature reserves. The sample sites were selected to cover the most important stands of naturally growing *F. sylvatica* populations in the Czech Republic (Fig. 1). The distance between the individually sampled adult trees was approximately 100 m, their average age was about 120 years. Localities of the populations are illustrated in Fig. 2 and their designations, geographic coordinates, altitudes and natural origin are recorded in Table 1. Total genomic DNA was extracted while following the manufacturer's instructions using a DNeasy Plant Mini Kit (Qiagen, Germany) from 20 mg dry or 100 mg fresh young leaves or buds collected from 390 *F. sylvatica* adult individuals. Liquid nitrogen was used for disrupting the plant material. DNA concentrations and purity were measured spectrophotometrically using a NanoPhotometer (Implen, Germany). The SSR method based on PCR with specific primers was used to screen the selected beech populations. We assessed 17 SSR loci. The oligonucleotide sequence primer pairs had been searched in the literature (Pastorelli et al. 2003; Asuka et al. 2004; Vornam et al. 2004; Lefèvre et al. 2012; Pluess, Määttänen 2013). In order to optimize the amplification conditions, different concentrations of primers, concentrations of the deoxynucleotide (dNTP) solution, and annealing temperature were tested. The quality of PCR products was checked on 2% agarose gels stained with GelRed (Biotium, USA). Fourteen specific primer pairs producing variable, well-scorable bands with the expected product size were used for fragmentation analyses. They were labelled fluorescently using FAM, VIC, PET and NED dyes and then assembled into three multiplexes (A, B, C) from the viewpoint of the targeted allele sizes: multiplex A

Fig. 1. View of the *Fagus sylvatica* Linnaeus population at BK08 (Loučná nad Desnou)

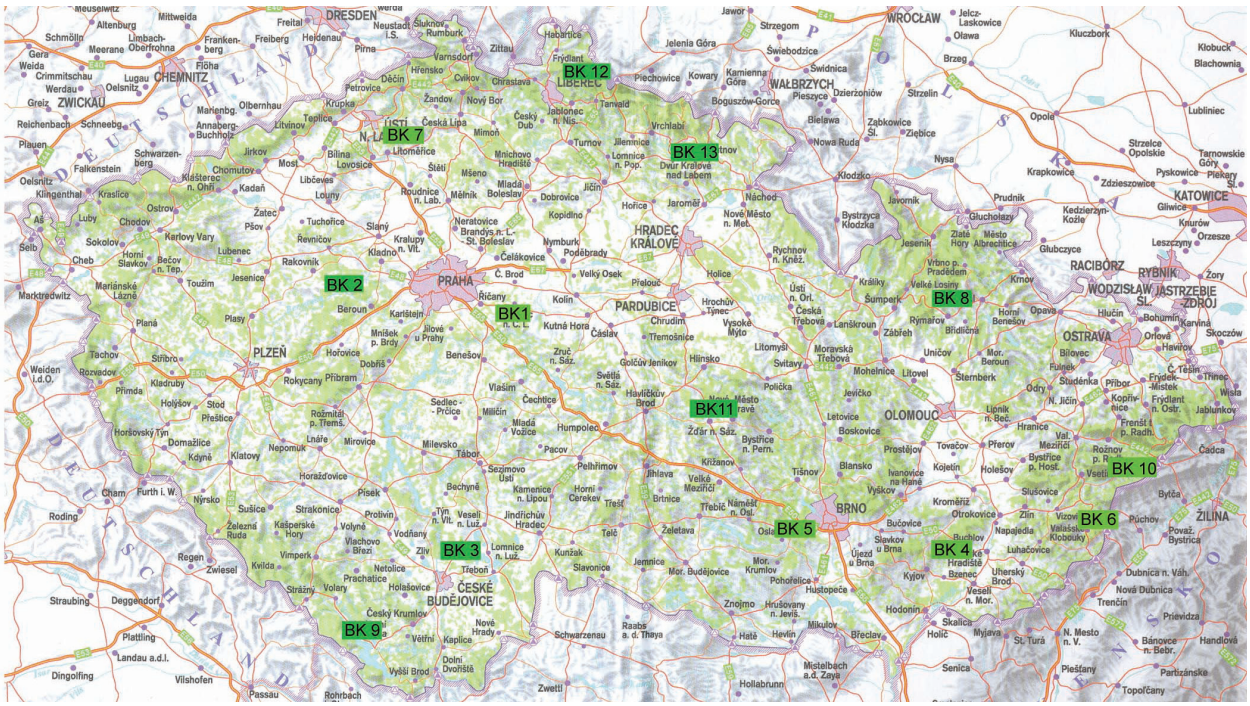

Fig. 2. Localities of the *Fagus sylvatica* Linnaeus populations (for details see Table 1)

with loci mfc 11, FS1-03, FS1-15, Fagsyl-000905, and Fagsyl-001018; multiplex B with loci FS1-11, mfc 7, csolfagus-31, and sfc0036; and multiplex C with loci Fagsyl-002929, Fagsyl-004597, csolfagus-29, FS4-46, and mfc 5. Amplification reaction conditions for each sample were in a final volume of 15 µl containing 1 µl of template DNA (≈ 20 ng·µl^{-1}), 1.5 µl of 10× PCR buffer (Mg-free), 0.067 mM of dNTP mixture (Takara Bio, Japan), 2 mM $MgCl_2$, 0.37 U of Platinum® Taq DNA polymerase (Invitrogen by Thermo Fisher Scientific, USA), and primer combinations of the forward and reverse primers in three multiplex reactions. The concentration of each primer was 0.1 µM. The reaction mixtures were supplemented with sterile water for molecular biology (Sigma-Aldrich, USA). The PCR profile was as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 s,

Table 1. Geographic coordinates of the *Fagus sylvatica* Linnaeus populations

Population	Geographic coordinates		Altitude (m a.s.l.)	Natural origin
	north latitude	east longitude		
BK01 – Kostelec nad Č. lesy	49°57'39"–49°57'54"	14°47'18"–14°48'12"	350–500	national nature reserve, gene conservation unit
BK02 – Křivoklát	49°56'22"–50°0'0.28"	13°47'3.0"–13°51'39"	380–500	protected landscape area, gene conservation unit
BK03 – Hluboká	49°4'16"–49°7'9.12"	14°27'24"–14°32'39"	370–550	gene conservation unit
BK04 – Buchlovice	49°5'39.7"–49°6'29.8"	17°12'30"–17°15'15"	320–510	
BK05 – Náměšť nad Oslavou	49°8'8.3"–49°10'17"	16°24'35"–16°25'38"	330–440	
BK06 – Vsetín	49°21'43"–49°22'43"	18°9'43"–18°11'59"	575–840	
BK07 – Děčín	50°40'2.9"–50°41'35"	14°13'19"–14°14'12"	270–680	
BK08 – Loučná nad Desnou	50°3'21.6"–50°6'49.9"	17°11'42"–17°17'57"	800–1,135	
BK09 – Kaplice	48°36'43"–48°39'39"	14°40'19"–14°42'59"	780–935	
BK10 – Luhačovice	49°2'32.7"–49°3'16.7"	18°3'21"–18°4'23"	460–650	
BK11 – Žákova hora, Cikháj	49°38'60"–49°39'35"	15°59'2.2"–15°59'54"	740–810	protected landscape area, national nature reserve
BK12 – Frýdlant v Čechách	50°51'44"–50°52'11"	15°6'19.836"–15°9'19"	460–520	national nature reserve, gene conservation unit
BK13 – Trutnov, Hrádeček	50°34'54"–50°35'40"	15°49'39"–15°51'7.6"	425–560	gene conservation unit

annealing temperature at 58°C for 45 s and extension at 72°C for 55 s, with a final extension step at 72°C for 15 min. Amplifications were carried out in a Veriti Thermal Cycler (Applied Biosystems, USA). PCR products were separated by capillary electrophoresis using the Applied Biosystems 3500 genetic analyser. As size standard, we used GeneScan™-600LIZ® (Applied Biosystems, USA). PCR amplifications and sizing of amplification products were repeated twice to confirm the fragment lengths. Alleles were sized using the GeneMapper® software (Version 4.1, 2009). The majority of genetic diversity parameters: number of alleles, Shannon's information index, observed heterozygosity, expected heterozygosity, fixation index (F_{ST}) values, Nei's genetic distance were calculated using the statistical program GenAlEx (Version 6.501, 2013) (Peakall, Smouse 2012). The fixation index (F) was calculated according to Wright (1965), as Eq. 1:

$$F = 1 - \left(H_o / H_e \right) \quad (1)$$

where:
H_o – observed heterozygosity,
H_e – expected heterozygosity.

The genetic divergence F_{ST} and Nei's genetic distance (Nei 1972) between populations were estimated by computing a pairwise population matrix. Pairwise F_{ST} was calculated by the GenAlEx software as the analysis of molecular variance based on 999 permutations (Peakall, Smouse 2012). Chi-squared test for the Hardy-Weinberg equilibrium for codominant genotypes at single loci and for single populations was used. The number of private alleles is the number of alleles unique to a single population. It was calculated as the mean value from all studied loci. The frequencies of null alleles were estimated using the CERVUS program (Version 3.0.7, 2007) (Kalinowski et al. 2007). The Micro-Checker software (Version 2.2.3, 2003) was used for identifying and correcting genotyping errors in microsatellite data (Van Oosterhout et al. 2004). The dendrogram was produced by the unweighted pair group method with arithmetic mean clustering technique using NCSS statistical software (Version 8.0, 2012) (Hintze 2012). Cophenetic correlation coefficient and delta parameters at distances 0.5 ($\Delta_{0.5}$) and 1 (Δ_1) were used to select the method providing maximum goodness of fit for hierarchical clustering. The centroid method (unweighted pair group) was chosen as the best. The statistical evaluation methods used were in accordance with Meloun and Militký (2011).

RESULTS

The 13 *F. sylvatica* populations were evaluated separately to acquire genetic characteristics. Clear, reproducible PCR products were produced for the 14 microsatellite loci. All of the SSR markers have dinucleotide repeats except that the markers FS4-46, Fagsyl-000905, and Fagsyl-002929 have trinucleotide repeats. Their primer pairs provided good patterns with the expected product size and so they were used for the genetic characterization. Two loci were subsequently eliminated: Fagsyl-004597 due to stuttering in six beech populations which might have resulted in scoring errors as was checked using the Micro-Checker software (Van Oosterhout et al. 2004) and csolfagus-29 due to a low level of polymorphism. The primer sequences of the 12 ultimately selected nuclear microsatellite markers and their genetic diversity parameters estimated using GenAlEx and CERVUS are recorded in Table 2. There were 209 different alleles detected at 12 loci in 390 individuals of European beech. The number of alleles ranged between 9 and 43, with an average of 17.4 alleles per locus. H_e ranged from 0.61 (mfc 7) to 0.88 (mfc 5) with a mean value of 0.76 while the mean value of H_o was 0.70 and ranged from 0.41 (mfc 11) to 0.99 (FS1-11). Shannon's information index calculated for allelic and genetic diversity ranged from 1.25 at locus mfc 7 to 2.40 at locus mfc 5. These values do not correspond to the highest and lowest number of different alleles at the same loci, because this estimation also depends on the evenness of allele frequencies. Fixation index values varied from –0.391 (FS1-11) to 0.390 (mfc 11). Most of the loci exhibited homozygote excess with positive F values. F values with significant deviations ($P < 0.001$) from the Hardy-Weinberg equilibrium (HWE) were at loci mfc 5, mfc 11, FS1-03, FS4-46, and Fagsyl-002929 as was calculated by CERVUS (Kalinowski et al. 2007). The loci mfc 7, FS1-11, Fagsyl-000905, Fagsyl-001018, and sfc0036 with negative fixation indexes reflect excesses of heterozygotes. Although no deviations from HWE were observed at FS1-15 locus across all 390 beech trees, significant ($P < 0.001$) deviations were identified in the BK08 and BK09 populations by the GenAlEx program (Peakall, Smouse 2012). The frequencies of null alleles at the studied loci were estimated by CERVUS (Kalinowski et al. 2007) and their values are reported in Table 2.

The Micro-Checker software was used for identifying and correcting genotyping errors in microsatellite data (Van Oosterhout et al. 2004).

Table 2. Characteristics of selected nuclear microsatellite loci across 13 investigated *Fagus sylvatica* Linnaeus populations

Locus	Primer sequence (5'-3')	PCR product size range (bp)	N_a	I	H_o	H_e	F	F (Null)
mfc 5	F: ACT GGG ACA AAA AAA CAA AA R: GAA GGA CCA AGG CAC ATA AA	277–321	22	2.40	0.62	0.88	0.302	0.1928
mfc 7	F: AAA ATA CAC TGC CCC AAA A R: CAG GTT TTG GTT TCT TAC AC	100–128	13	1.25	0.63	0.61	–0.028	–0.0018
mfc 11	F: ACA GAT AAA AAC AGA AGC CA R: TTT GGT TTT GTT GAG TTT AG	310–330	11	1.49	0.41	0.67	0.390	0.2539
FS1-03	F: CAC AGC TTG ACA CAT TCC AAC R: TGG TAA AGC ACT TTT TCC CAC T	76–126	23	1.76	0.63	0.72	0.130	0.0816
FS1-11	F: TGA ATT CAA TCA TTT GAC CAT TC R: GGA AGG GTG CTT CAA TTT GG	90–120	16	1.68	0.99	0.72	–0.391	–0.2131
FS1-15	F: TCA AAC CCA GTA AAT TTC TCA R: GCC TCA ATG AAC TCA AAA AC	92–136	16	1.85	0.75	0.80	0.062	0.0437
FS4-46	F: GCA GTC CTC CAC CAT TAC TA R: TAC AAC AGC AGG CTA TCC AT	213–372	43	2.30	0.62	0.85	0.273	0.1685
Fagsyl-000905	F: GAT CAT AGC GCC GGA ATT GG R: GGT CCT CCT CCT GGT ACA AC	146–168	12	1.40	0.73	0.68	–0.080	0.0266
Fagsyl-001018	F: CGA GAT GGA CTT CTA AGT TTT ATT TGC R: CGA GAT GGA CTT CTA AGT TTT ATT TGC	96–120	13	1.57	0.76	0.75	–0.024	–0.0013
Fagsyl-002929	F: GCG GCG ACT GGA ATA ATA GC R: CAA TCA CAC GCT GCA CAA AC	143–200	19	1.94	0.72	0.81	0.118	0.0796
csolfagus-31	F: TCT ATT GAC ACA AGA ATA AGA ACA CC R: CTT GGC AAG AAA AGG GGA TT	106–128	12	2.06	0.83	0.84	0.011	0.0173
sfc0036	F: CAT GCT TGA CTG ACT GTA AGT TC R: TCC AGG CCT AAA AAC ATT TAT AG	98–114	9	1.60	0.74	0.73	–0.011	0.0075

N_a – number of different alleles, I – Shannon's information index, H_o – observed heterozygosity, H_e – expected heterozygosity, F – fixation index, F (Null) – estimated null allele frequency

No evidence was found for large allele dropout at any loci. At the four loci FS1-11, Fagsyl-001018, Fagsyl-002929 and csolfagus-31, no evidence was found for a scoring error due to stuttering, nor was there any evidence of null alleles for any population. At other loci and in some populations – namely mfc 5 (in 7 populations), mfc 7 (in 1 population), mfc 11 (in 11 populations), FS1-03 (in 6 populations), FS1-15 (in 3 populations), FS4-46 (in 11 populations), Fagsyl-000905 (in 2 populations), and sfc0036 (in 2 populations) – the analysis indicated that homozygote excess and null alleles may be present at these loci.

The number of alleles varied by as many as 8 among populations at individual loci, but the mean numbers of alleles per population from all loci did not differ remarkably (Table 3). Significant differences were found in the allelic frequencies across the 13 studied beech populations at individual loci. At each locus, certain alleles were changing their representation in the populations by as much as 30% and more. For example, allele 156 at the locus Fagsyl-002929 varied in frequency from 8 to 43% (Fig. 3). Additional characteristics of genetic diversity within populations are given in Table 3. The estimated genetic diversity expressed as Shannon's information index in the studied populations ranged from 1.73 to 1.92. Beech populations showed positive values of fixation index (0.005–0.115), thus indicating heterozygote deficiencies relative to the expected fraction under random mating. There were no significant differences between the populations in the values of observed (H_o = 0.664–0.754) and expected (H_e = 0.734–0.791) heterozygosity. The number of private alleles was the highest at FS4-46 locus, where the private alleles appeared in 8 populations. The highest number of private alleles was found in BK04 population. There were not any private alleles at loci sfc0036, mfc 11, csolfagus-31. Estimates of differentiation between populations are presented in Table 4. F_{ST} values ranging from 0.001 to 0.053 indicated low genetic differentiation. Most of the pairwise F_{ST} values were significantly greater than zero, thus confirming the presence of a slight, but significant amount of population structuring in Czech European beech.

Table 3. Mean values of genetic characteristics of 13 investigated *Fagus sylvatica* Linnaeus populations (N = 30) from 12 selected nuclear microsatellite loci

Population	N_a	N_e	I	No. of private alleles	H_o	H_e	F
BK01	10	5	1.81	0.15	0.667	0.766	0.113
BK02	10.9	5.2	1.84	0.23	0.672	0.762	0.099
BK03	9.4	5.1	1.75	0.23	0.664	0.752	0.108
BK04	10.2	5.3	1.84	0.46	0.721	0.780	0.061
BK05	10.8	5.1	1.84	0.15	0.705	0.769	0.073
BK06	10.4	4.3	1.73	0.23	0.728	0.734	0.005
BK07	10.2	4.7	1.78	0.23	0.708	0.755	0.054
BK08	11	5.7	1.92	0.31	0.713	0.791	0.093
BK09	11.2	5.6	1.90	0.15	0.697	0.779	0.098
BK10	10.7	5	1.82	0.23	0.754	0.765	0.012
BK11	10.2	4.6	1.78	0.15	0.673	0.757	0.098
BK12	10.8	5.2	1.87	0.39	0.695	0.772	0.099
BK13	10.8	5.2	1.83	0.08	0.667	0.762	0.115
Mean	10.5	5.1	1.82	0.23	0.697	0.765	0.079

for localities of the populations see Table 1, N – sample size, N_a – number of different alleles, N_e – number of effective alleles, I – Shannon's information index, H_o – observed heterozygosity, H_e – expected heterozygosity, F – fixation index

Genetic distances between populations were calculated based on Nei's standard genetic distance (Nei 1972). The longest Nei's genetic distance (0.260) appeared between the BK03 (Hluboká) and BK12 (Frýdlant v Čechách) populations. The closest Nei's genetic distance (0.062) was between the BK11 (Žákova hora, Cikháj) and BK12 (Frýdlant v Čechách) populations. Nei's standard genetic distances between the 13 Czech populations are graphically illustrated in a dendrogram (Fig. 4). The Mantel test was used for comparing the Nei's genetic distance matrix with the geographic distance matrix to find relationships between the geographic and genetic distances. No significant linear correlations appeared between the genetic and geographic distances of 13 beech populations (R^2 = 0.0047).

DISCUSSION

European beech is a tree species occurring in various forest ecosystems that covers a large and continuous geographic range within Europe. For the conservation and utilization of its genetic resources, it is important to acquire fundamental knowledge about the distribution of its genetic variation. Sufficiently high levels of genetic diversity constitute a prerequisite for the ability of a long-lived organism to survive in forest populations, and particularly in view of the recently very often varying environmental conditions, and to preserve their adaptation potential for future generations (Vornam et al. 2004).

The aim of this study was to assess the levels of genetic diversity of important *F. sylvatica* populations situated at different localities across the Czech

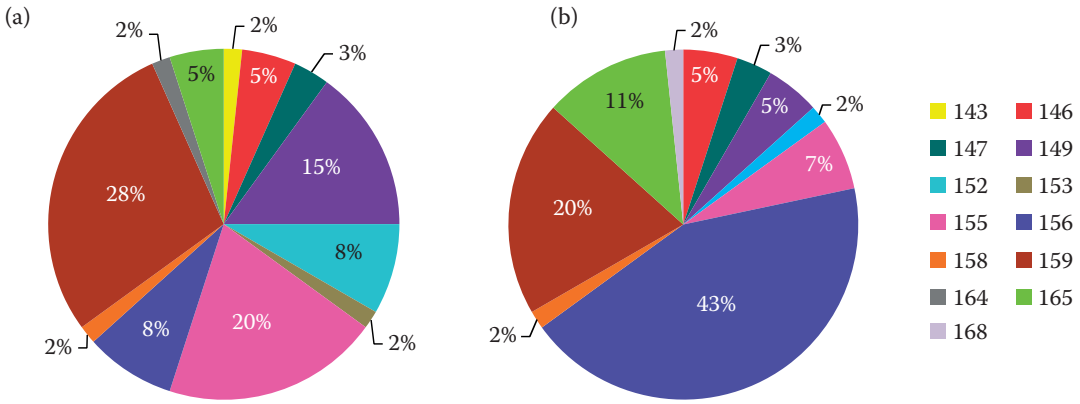

Fig. 3. Example of different allele frequencies at the locus Fagsyl-002929 in the populations BK01 – Kostelec nad Černými lesy (a), BK10 – Luhačovice (b)

Table 4. Pairwise population F_{ST} values determining genetic differentiation between populations with values of probability

Population	BK01	BK02	BK03	BK04	BK05	BK06	BK07	BK08	BK09	BK10	BK11	BK12	BK13
BK01	0.000	0.261	0.243	0.414	0.017	0.027	0.030	0.015	0.008	0.001	0.001	0.001	0.005
BK02	0.002	0.000	0.059	0.190	0.016	0.004	0.169	0.002	0.021	0.001	0.001	0.001	0.027
BK03	0.002	0.006	0.000	0.007	0.002	0.010	0.001	0.002	0.002	0.001	0.001	0.001	0.001
BK04	0.001	0.003	0.012	0.000	0.315	0.068	0.223	0.061	0.029	0.010	0.001	0.001	0.042
BK05	0.009	0.009	0.016	0.001	0.000	0.013	0.122	0.002	0.005	0.002	0.001	0.001	0.001
BK06	0.008	0.014	0.013	0.006	0.010	0.000	0.035	0.012	0.012	0.015	0.001	0.001	0.008
BK07	0.007	0.003	0.017	0.002	0.004	0.009	0.000	0.002	0.036	0.003	0.001	0.001	0.013
BK08	0.009	0.015	0.019	0.006	0.014	0.012	0.013	0.000	0.221	0.026	0.001	0.001	0.025
BK09	0.010	0.008	0.014	0.008	0.014	0.009	0.007	0.002	0.000	0.036	0.001	0.001	0.136
BK10	0.017	0.028	0.026	0.013	0.017	0.011	0.018	0.009	0.007	0.000	0.001	0.001	0.001
BK11	0.038	0.037	0.051	0.031	0.036	0.030	0.035	0.025	0.028	0.032	0.000	0.390	0.011
BK12	0.037	0.037	0.053	0.032	0.038	0.036	0.036	0.023	0.031	0.039	0.001	0.000	0.015
BK13	0.012	0.008	0.019	0.007	0.018	0.011	0.009	0.008	0.004	0.020	0.009	0.009	0.000

for localities of the populations see Table 1, F_{ST} values below diagonal, P based on 999 permutations above diagonal

Republic. It examined genetic variation of naturally regenerated Czech beech populations using nuclear microsatellite markers. Genetic variation of 17 European beech populations distributed in the Czech Republic had previously been studied by isozyme loci (Gömöry et al. 1998). The populations analysed in this study showed important amounts of genetic diversity, as estimated by means of variation scored at 12 nuclear microsatellite loci. Detected were 9–43 alleles per locus and values of observed heterozygosity ranged from 0.41 to 0.99. The number of alleles at individual loci varied in observed populations and averaged 10.5 alleles per population from all studied loci. The number of alleles obtained for our beech populations at loci FS1-03, FS1-11, FS1-15, mfc 5, mfc 11, csolfagus-31, and sfc0036 is approximately comparable with the numbers reported in the literature (Pastorelli et al. 2003; Vornam et al. 2004; Hasenkamp et al. 2011; Lefèvre et al. 2012). Pluess and Määttänen (2013) reported for the loci Fagsyl-000905, Fagsyl-001018, and Fagsyl-002929 lower numbers of alleles from 60 individuals of *F. sylvatica* originating from three regions of Switzerland. In Czech beech populations, this comparable level of variability was identified despite the occurrence of deviations from HWE and which alternated at individual loci for different populations. One possible explanation for deviations from the Hardy-Weinberg expectation with an excess of homozygotes may be due to the presence of null alleles and population differentiation (Asuka et al. 2004). Another reason could also be due to lower quality of some DNA samples.

The observed heterozygosity of the 13 studied populations ranged from 0.664 to 0.754. Buiteveld et al. (2007) found similar small differences in observed heterozygosity (from 0.560 to 0.721) using microsatellite markers within beech stands in five European countries (Austria, France, Germany, Italy, and the Netherlands). These stands showed small but significant differentiation (F_{ST} = 0.058). The F_{ST} value across the 13 Czech beech populations was determined to be 0.017 ($P < 0.001$). When considering the shorter geographical distances between Czech populations, their differentiation is not inconsiderable and indicates the population structuring of Czech European beech populations. Pairwise comparison of F_{ST} values between Czech populations ranged from 0.001 to 0.053, being comparable with F_{ST} values of beech stands in Austria (F_{ST} = 0.0085), France (F_{ST} = 0.0535), Germany

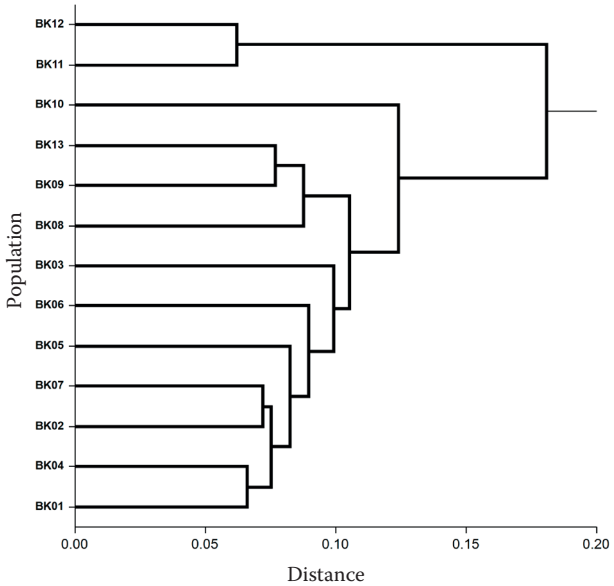

Fig. 4. The dendrogram based on Nei's genetic distances between *Fagus sylvatica* Linnaeus populations (for details see Table 1)

(F_{ST} = 0.0295), Italy (F_{ST} = 0.0168), and the Netherlands (F_{ST} = 0.0207) (Buiteveld et al. 2007).

In the Czech beech populations, significant genetic variations in the allelic frequencies of microsatellite markers were found. Considerable variation of allelic frequencies was also reported in previous studies which examined European beech in the Czech Republic based on isozyme gene markers (Gömöry et al. 1998). The genetic diversity expressed by Shannon's information index ranged from 1.73 to 1.92 in the studied beech populations. We previously estimated the genetic diversity of important silver fir and Norway spruce populations growing in the various regions of the Czech Republic. We obtained similar values of Shannon's information index for silver fir at 1.63–1.91, and higher values of 1.9–2.3 for Norway spruce (Čáp et al. 2015; Fulín et al. 2016).

Fixation index values of some loci used in this study with a higher proportion of putatively homozygous genotypes suggest the presence of null allele corresponding with those reported in literature for the loci mfc 5 (Vornam et al. 2004; Chybicki et al. 2009; Piotti et al. 2012), FS4-46 (Buiteveld et al. 2007), mfc 11 (Tanaka et al. 1999) and FS1-15 (Piotti et al. 2012). Another explanation for inbreeding would be self-fertilization of mother trees (Chybicki et al. 2009). All 13 beech populations showed positive values of fixation index (0.05–0.115), which indicates a reduction in heterozygosity when compared to the Hardy-Weinberg expectation of genotype frequencies. Higher values (0.139–0.320) of inbreeding coefficient were observed in 10 beech stands using microsatellites from Austria, France, Germany, Italy, and the Netherlands (Buiteveld et al. 2007).

A possible effect of human activities, such as medieval devastation due to an extensive use of beech wood for fuel, and later forest management strategies, especially the 19th century preference of coniferous monocultures, could have affected the genetic structures of beech populations. Not significant linear correlations were observed between genetic and geographic distances of the 13 beech populations studied on the basis of microsatellite markers. Using isozyme markers, Gömöry et al. (1998) did not observe any clear continuous correlation between European beech populations distributed in the Czech Republic. The mosaic character of the genetic variations is therefore assumed to be caused by the influence of human activities as a consequence of artificial reforestation.

For more extensive reforestation aiming to achieve a more natural forest tree species composition, it is important to use suitable propagation material. This means that large amounts of forest reproductive material, and especially of seeds, are needed. High genetic diversity and origin must be considered to ensure the best possible adaptation to the respective local conditions (Hasenkamp et al. 2011). The presence and maintenance of genetic variation in genes controlling adaptive traits are important for the stability of long-lived forest trees. Microsatellites as neutral markers are mostly located in noncoding regions, thus they are the most suitable tool to examine genetic diversity and then proceed to the SNP method which has a sufficient potential for adaptation analyses. Recently, SNP was developed in order to analyse genes involved in adaptation to drought stress and bud phenology in beech (Seifert et al. 2012). In order to verify the most suitable reproductive material, it will be necessary to continue in genetic studies of adaptive traits.

In conclusion, this study shows the distribution of gene diversity in selected Czech populations of European beech and provides information essential for the management and conservation of genetic variability in European beech populations. Twelve microsatellite markers were successfully used for genotyping of beech trees and they were assembled into three multiplexes to achieve time and cost savings. The genotypic data from adult trees implies genetic differences between the studied populations. One of the priority tasks of the national forest policy and an international obligation of the Czech Republic are to conserve biodiversity in forest ecosystems and promote the principles of sustainable management. Given the prevailing artificial reforestation, knowledge based on DNA analyses regarding the variability of genetic resources will contribute to the quality of the reproduction material and to creating optimal species composition in forests.

References

Asuka Y., Tani N., Tsumura Y., Tomaru N. (2004): Development and characterization of microsatellite markers for *Fagus crenata* Blume. Molecular Ecology Notes, 4: 101–103.

Buiteveld J., Vendramin G.G., Leonardi S., Kamer K., Geburek T. (2007): Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. Forest Ecology and Management, 247: 98–106.

Čáp J., Novotný P., Cvrčková H., Máchová P., Fulín M., Frýdl J., Dostál J., Buriánek V., Beran F., Lefnar R., Poláková L., Malá J. (2015): Genetická charakterizace významných regionálních populací smrku ztepilého v České republice. Specializovaná mapa s odborným obsahem. Jíloviště-Strnady, VÚLHM: 32.

Chybicki I.J., Trojankiewicz M., Oleksa A., Dzialuk A., Burczyk J. (2009): Isolation-by-distance within naturally established populations of European beech (*Fagus sylvatica*). Botany, 87: 791–798.

Comps B., Gömöry D., Letouzy J., Thiébaut B., Petit R.J. (2001): Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. Genetics, 157: 389–397.

Dounavi A., Koutsias N., Ziehe M., Hattemer H.H. (2010): Spatial patterns and genetic structures within beech populations (*Fagus sylvatica* L.) of forked and non-forked individuals. European Journal of Forest Research, 129: 1191–1202.

Fulín M., Čáp J., Cvrčková H., Novotný P., Máchová P., Dostál J., Frýdl J., Beran F. (2016): Genetická charakterizace významných regionálních populací jedle bělokoré v České republice. Specializovaná mapa s odborným obsahem. Jíloviště-Strnady, VÚLHM: 42.

Gömöry D., Hynek V., Paule L. (1998): Delineation of seed zones for European beech (*Fagus sylvatica* L.) in the Czech Republic based on isozyme gene markers. Annals of Forest Science, 55: 425–436.

Hasenkamp N., Ziegenhagen B., Mengel C., Schulze L., Schmitt H.P., Liepelt S. (2011): Towards a DNA marker assisted seed source identification: A pilot study in European beech (*Fagus sylvatica* L.). European Journal of Forest Research, 130: 513–519.

Hintze J.L. (2012): NCSS 8. Kaysville, NCSS: 2823.

Jump A.S., Peñuelas J. (2007): Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the wind-pollinated tree, *Fagus sylvatica*. Molecular Ecology, 16: 925–936.

Kalinowski S.T., Taper M.L., Marshall T.C. (2007): Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16: 1099–1106.

Konnert M., Ziehe M., Trober U., Mauer W., Janßen A., Sander T., Hussendörfer E., Hertel H. (2000): Genetische Variation der Buch (*Fagus sylvatica* L.) in Deutschland: Gemeinsame Auswertung genetischer Inventuren über verschiedene Bundesländer. Forts und Holz, 55: 403–408.

Lefèvre S., Wagner S., Petit R.J., de Lafontaine G. (2012): Multiplexed microsatellite markers for genetic studies of beech. Molecular Ecology Resources, 12: 484–491.

Meloun M., Militký J. (2011): Statistical Data Analysis, A Practical Guide with 1250 Exercises and Answer Key on CD. New Delhi, Woodhead Publishing India: 1600.

Ministry of Agriculture of the Czech Republic (2015): Information on forests and forestry in the Czech Republic by 2014. Available at http://eagri.cz/public/web/file/433136/ZZ2014AJ_16112015.pdf (accessed June 27, 2016).

Nei M. (1972): Genetic distance between populations. American Naturalist, 106: 283–392.

Oddou-Muratorio S., Klein E.K., Vendramin G.G., Fady B. (2011): Spatial vs. temporal effects on demographic and genetic structures: The roles of dispersal, masting and differential mortality on patterns of recruitment in *Fagus sylvatica*. Molecular Ecology, 20: 1997–2010.

Pastorelli R., Smulders M.J.M., Van't Westende W.P.C., Vosman B., Giannini R., Vettori C., Vendramin G.G. (2003): Characterization of microsatellite markers in *Fagus sylvatica* L. and *Fagus orientalis* Lipsky. Molecular Ecology, 3: 76–78.

Peakall R., Smouse P.E. (2012): GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics, 28: 2537–2539.

Piotti A., Leonardi S., Buiteveld J., Geburek T., Gerber S., Kramer K., Vettori C., Vendramin G.G. (2012): Comparison of pollen gene flow among four European beech (*Fagus sylvatica* L.) populations characterized by different management regimes. Heredity, 108: 322–331.

Pluess A.R., Määttänen K. (2013): Characterization of eighteen novel microsatellite markers and multiplex PCR protocol for *Fagus sylvatica*. Conservation Genetics Resources, 5: 311–314.

Scalfi M., Troggio M., Piovani P., Leonardi S., Magnaschi G., Vendramin G.G., Menozzi P. (2004): A RAPD, AFLP and SSR linkage map, and QTL analysis in European beech (*Fagus sylvatica* L.). Theoretical and Applied Genetics, 108: 433–441.

Seifert S., Vornam B., Finkeldey R. (2012): DNA sequence variation and development of SNP markers in beech (*Fagus sylvatica* L.). European Journal of Forest Research, 131: 1761–1770.

Svoboda P. (1955): Lesní dřeviny a jejich porosty. Část II. Prague, Státní zemědělské nakladatelství v Praze: 573.

Tanaka K., Tsumura Y., Nakamura T. (1999): Development and polymorphism of microsatellite for *Fagus crenata* and the closely related species, *F. japonica*. Theoretical and Applied Genetics, 99: 11–15.

Úradníček L., Maděra P., Tichá S., Koblížek J. (2009): Dřeviny České republiky. Kostelec nad Černými lesy, Lesnická práce, s.r.o.: 366.

Van Oosterhout C.V., Hutchinson W.F., Wills D.P.M., Shipley P. (2004): Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology, 4: 535–538.

Vettori C., Vendramin G.G., Anzidei M., Pastorelli R., Paffetti D., Giannini R. (2004): Geographic distribution of chloroplast variation in Italian populations of beech (*Fagus sylvatica* L.). Theoretical and Applied Genetics, 109: 1–9.

Vornam B., Decarli N., Gailing O. (2004): Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. Conservation Genetics, 5: 561–570.

Wright S. (1965): The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution, 19: 395–420.

Received for publication July 7, 2016
Accepted after corrections December 9, 2016