https://doi.org/10.17221/99/2021-JFS

Genetic diversity of sessile oak populations in the Czech Republic

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Citation: Dvořák J., Korecký J., Faltinová Z., Zádrapová D. (2022): Genetic diversity of sessile oak populations in the Czech Republic. J. For. Sci., 68: 8–18.

Abstract: The sessile oak is a broadleaved tree species of great ecological and silvicultural importance. Oaks are the second most widespread deciduous tree species in the Czech Republic, and ongoing climate change negatively affects the abundant and often monocultural Norway spruce. Therefore, a proportional increase of more resilient tree species such as sessile oak has emerged. This study aimed to depict population genetic diversity when analysing 272 individuals from 10 subpopulations selected across the Czech Republic. Targeted populations were chosen based on the minimal expected human impact on the stand (presumably autochthonous stands). All individuals were genotyped using 18 polymorphic microsatellite markers (SSRs) assembled into two amplification multiplexes. The high discriminatory power of SSR markers was tested and confirmed by the probability of identity analysis. The genetic differentiation of the subpopulations was low yet significant, quantified by Wright's *F*-statistics within the range from 0.012 to 0.029. Based on discriminant analysis of principal components (DAPC), we detected two populations with geographic genetic correlation (the 15th meridian east being a north-south boundary line) and one with a distinct genetic pattern. We assume that the population might previously be established from seed sources outside the Czech Republic. Moreover, to some extent, our findings advocate the legitimacy of the legislative rules for forest reproductive material (FRM) transfer.

Keywords: *Quercus petraea* (Matt.) Liebl.; geographic genetic correlation; microsatellite markers; multiplex

Sessile oak is a woody species widely distributed across Europe. Its distribution range extends from Scotland to Turkey and from Spain to Ukraine. The distribution of *Quercus petraea* (Matt.) Liebl. is continuous, but some stands are scattered in the southern part of its occurrence (Eaton et al. 2016). In the Czech Republic, sessile oak is a species of great ecological and silvicultural importance (Kohler et al. 2020), with an upward trend of its representation (MZe 2019). In contrast to *Q. robur*, the most abundant *Quercus* species in the Czech Republic, *Q. petraea* is more resistant to drought stress (Timbal, Aussenac 1996). The environmental role is providing habitats for various insects such as moths, wood-boring beetles, and Hymenoptera. The acorns supply a valuable food source for many birds and mammals, such as jays, mice, squirrels, and wild boar (Savill 2013). Also, it provides high-

Supported by the grant "Building up an excellent scientific team and its spatiotechnical background focused on mitigation of the impact of climatic changes to forests from the level of a gene to the level of a landscape (EXTEMIT-K) at the FFWS CULS Prague" (No. CZ.02.1.01/0.0/0.0/15_003/0000433 financed by OP RDE) and project of National Agency of Agriculture research, Czech Republic (NAZV, No. QK1910480).

https://doi.org/10.17221/99/2021-JFS

quality hardwood, which is often used for the manufacture of furniture, veneers, and wine barrels, and also for fencing and special building constructions (Praciak 2013).

For many decades, silvicultural activities have been carried out without any more profound knowledge of the intrinsic genetic structure of forest reproductive material. Nevertheless, without understanding population genetic patterns, advanced genetic resource management can hardly be applied (Hedrick 2014). Therefore, revealing the genetic variability within and among populations is essential for an efficient tree breeding activity such as advanced generation seed orchard or gene reserves establishment. Genetic variability of *Quercus ssp*. has been widely studied – in *Quercus suber* (Jiménez et al. 1999), *Quercus macrocarpa* (Dow, Ashley 1996), *Quercus rubra* (Aldrich et al. 2002), *Quercus robur* (Barreneche et al. 1998), *Quercus petraea* (Le Corre at al. 1997). The population variability of sessile oak has been historically investigated using different types of markers for various traits of interest. Namely, morphological characteristics (Dupouey et al. 1993; Kleinschmit et al. 1995; Kremer et al. 2002), biochemical markers (Kremer, Zanetto 1997; Le Corre et al. 1998; Streiff et al. 1998; Finkeldey 2001; Gömöry et al. 2001) and molecular markers such as RFLP or AFLP (Dumolin-Lapègue et al. 1997; Mariette et al. 2002) were used for studies focused on *Quercus petraea*. Omitting high-throughput sequencing technologies that pose high demands on financial resources and laboratory infrastructure, microsatellite markers have been genetic markers of the best choice up today. Microsatellites, also called simple sequence repeats (SSRs), are stretches of short repetitive sequences distributed across eukaryotic genomes used to detect genetic variability of individuals and populations (Steinkellner et al. 1997). Microsatellite markers are codominant, easily genotyped, Mendelian inherited and at the same time highly polymorphic, which makes them very suitable for the study of population structure (Marwal at al. 2013; Abdul-Muneer 2014). Generally, microsatellite studies in the genus *Quercus* have revealed a high level of polymorphism (Dow, Ashley 1996; Guichoux et al. 2011) and low genetic differentiation among European oak populations, sessile oak including. These results have been observed in Ireland (Muir et al. 2004), France (Alberto et al. 2010), the Netherlands (Coart et al. 2002), Denmark (Siegismund, Jensen 2001), Italy (Bruschi et al. 2000), the Czech Republic (Dostálek at al. 2011), etc. In addition to studies at the national level, the genetic variability of sessile oak on the European level was examined (Bodénès et al. 1997; Gömöry et al. 2001).

Our study aimed to depict the inter-population genetic structure of several sessile oak populations to answer the following questions: (*i*) Is there any genetic differentiation among targeted populations based on microsatellite genotyping? (*ii*) If there is, can one reveal geographic genetic patterns among the data? Both questions were answered, and a certain degree of geographically dependent genetic differentiation was detected. In addition, we identified a population probably originating from non-local forest reproductive sources.

MATERIAL AND METHODS

Plant material. The targeted populations (stands) were chosen mainly due to their phenotypic superiority and putative autochthonous origin. Samples were taken from individual trees in the form of cores from the tree trunk, extracted with a 16-mm borer targeting the cambium layer. The minimum distance between sampled individuals was 25 m to avoid sampling closely related individuals. A total of 300 individuals from 10 populations were sampled (Table 1). Due to genotyping difficulties, a total of 272 individuals (ranging from 19 to 30 individuals per population) were genotyped. The collected samples were placed into plastic bags, marked with the individual's ID and stored in a freezer at –80 °C until further processing.

DNA extraction. Tissue samples were frozen in liquid nitrogen and homogenized with Mixer Mill MM400 (Retsch, Germany). From app. 200 mg per sample, genomic DNA was extracted with Genomic DNA Mini Kit (Plant) (Geneaid Biotech, Taiwan), following the manufacturer's instructions, with minor modification – the lysis step was adjusted to 1-hour incubation at 65 °C. The DNA quality and quantity were checked with NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific, USA) and on 1% agarose gel with electrophoresis. Subsequently, DNA concentrations were standardized by dilution to 20 $ng \cdot \mu L^{-1}$.

Multiplex PCR optimization. Our study took advantage of previously published oak primer se-

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Table 1. Population parameters (locality, altitude, geographic location, and number of samples per population)

No. of the population	Locality	Average altitude (m a.s.l.)	Geographic coordinates		No. of samples
			latitude (N)	longitude (E)	
1	Křivoklát	360	50.0869300	13.8936114	30
2	Plasy	375	49.9111412	13.4383032	30
3	Hluboká	540	49.1063742	14.5035452	30
4	Jind. Hradec	488	48.9772306	14.9728822	30
5	Buchlovice	460	49.1079281	17.3189722	30
6	Luhačovice	335	49.1477714	17.6166389	30
7	Nasavrky	530	49.8302778	15.6532778	30
8	Náměšť	355	49.1510038	16.4130310	30
9	Šternberk	310	49.7235833	16.9246337	30
10	Nižbor	365	49.9992916	13.9674754	30

quences (Guichoux et al. 2011) that were assembled into two highly polymorphic multiplexes (12-plex and 8-plex). Initially, we tested the PCR amplification pattern of those primers in individual reactions. Based on agarose gel electrophoresis of PCR products and their fragment length, we selected 18 primer pairs for further evaluation. According to their compatibility and presumed product size, these selected primer pairs were partially rearranged, fluorescently labelled and assembled into two new multiplexes (10-plex and 8-plex) according to their compatibility and presumed product size (Table 2). After adjustment of the primer concentration, amplification provides highly polymorphic and reproducible profiles. The PCR amplification was performed with a Mastercycler Nexus Gradient Thermal Cycler (Eppendorf, Germany). For multiplex 1, the PCR reaction was carried out in a volume of 9.8 μL reaction mixture which contained 1 μL of template DNA (20 ng·μL^{-1}), 3.42 μL of total primer pairs, 0.48 μL of sterile water and 4.9 μL of Type-it Microsatellite PCR Kit (Qiagen, Germany). For multiplex 2, the PCR reaction was carried out in a volume of 7.8 μL reaction mixture which contained 1 μL of template DNA (20 ng·μL^{-1}), 2.58 μL of total primer pairs, 0.32 μL of sterile water and 3.9 μL of Type-it Microsatellite PCR Kit (Qiagen, Germany). The amplification conditions were identical for both multiplexes and consisted of a denaturation step of 5 min at 95 °C, followed by 35 cycles of 45 s at 95 °C, 60 s at 60 °C, 45 s at 72 °C and a final extension of 30 min at 72 °C. All PCR products were genotyped using 3500 Series Genetic Analyzer (Applied Biosystems, USA) and scored by GeneMarker genotyping software (Version 2.4.0, 2012) with manual adjustment.

Data analysis. For each marker, we calculated the total number of alleles (k), observed (H_o) and expected (H_e) heterozygosity and polymorphism information content (*PIC*) using the CERVUS 3.0 program (Kalinowski et al. 2007). Samples were also tested for linkage disequilibrium and departure from Hardy-Weinberg equilibrium by the Markov chain method with 10 000 dememorization steps to calculate its level of significance using the same software. The null-allele frequencies [F(Null)] were determined using the ML-NULLFREQ program (Kalinowski, Taper 2006).

The GenAlEx software (Peakall, Smouse 2012) was used for fundamental allele frequency analyses of individual populations, such as mean number of alleles (*Na*), effective number of alleles (*Ne*), Shannon genetic diversity index (I), observed heterozygosity (H_o), expected heterozygosity (H_e) and mean fixation index (F).

GenAlEx was also used for analyzing genetic structure by analysis of molecular variance (AMOVA) and estimating the discrimination power of microsatellite battery via the probability of identity analysis as described by Peakall and Smouse (2012). Finally, we applied the discriminant analysis of principal components (DAPC), a multivariate method designed to identify genetic clusters of individuals (Sokal, Michener 1958; Jombart et al. 2010). This method, implemented in the R package adegenet software (Jombart 2008), provides a visual assessment of between-population genetic structures. The number of PCA's to retain was set to 246, based on an inter-

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Table 2. Parameters of assembled multiplexes

	Locus	Primers sequences (5'-3') forward/reverse	Allele size (bp)	Motif	Fluorescent dye
Multiplex-1	PIE239[a]	TCAACAAATGGCTCAACAGTG CCCATTTGGTAGCAAAGAGTC	81–95	AT	PET
	PIE227[a]	TACCATGATCTGGGAAGCAAC AAGGGCTTGGTTGGGTTAGT	150–180	TGG	PET
	PIE271[a]	CACACTCACCAACCCTACCC GTGCGGTTGTAGACGGAGAT	181–216	TC	PET
	PIE267[a]	TCCAACCATCAAGGCCATTAC GTGCGAACAGATCCCTTGTC	81–108	AG	NED
	PIE258[a]	TTCTCGATCTCAAAACAAAACCA TTTGATTTGTTTAAGGAAAATTGGA	127–163	TC	NED
	PIE020[a]	GCAGAGGCTCTTCTAAATACAGAACT GGGAGGTTTCTGGGAGAGAT	97–113	AG	FAM
	PIE152[a]	TGTACCTCTTTCCTCTCTCTAAAACT GAATTTCTAAACCACTAGCATTGAC	234–256	TA	FAM
	QrZAG112[b]	TTCTTGCTTTGGTGCGCG GTGGTCAGAGACTCGGTAAGTATTC	80–111	GA	VIC
	PIE102[a]	ACCTTCCATGCTCAAAGATG GCTGGTGATACAAGTGTTTGG	139–169	CT	VIC
	PIE243[a]	GGGGTCAGTAGGCAAGTCTTC GAGCTGCATATTTTCCTTAGTCAG	206–226	AG	VIC
Multiplex-2	QpZAG15[c]	CGATTTGATAATGACACTATGG CATCGACTCATTGTTAAGCAC	109–155	AG	PET
	QpZAG110[c]	GGAGGCTTCCTTCAACCTACTT GATCTCTTGTGTGCTGTATTTTT	203–245	AG	PET
	QrZAG96[b]	CCCAGTCACATCCACTACTGTCC GGTTGGGAAAAGGAGATCAGA	127–182	TC	NED
	QrZAG11[b]	CCTTGAACTCGAAGGTGTCC TGGTTGACTAAAGTATGAACTGTTTG	223–287	TC	NED
	QrZAG7[b]	CAACTTGGTGTTCGGATCAA GTGCATTTCTTTTATAGCATTCAC	105–158	TC	FAM
	PIE223[a]	TAGAAGCCCAACACGGCTAC AGCAAAACACAAACGCACAA	196–228	GGT	FAM
	PIE242[a]	TGGAGGGAAAAGAACAATGC TTGCAATCCTCCAAATTTAATG	101–126	TA	VIC
	QrZAG20[b]	CCATTAAAAGAAGCAGTATTTTGT GCAACACTCAGCCTATATCTAGAA	154–195	TA	VIC

[a]Durand et al. (2010); [b]Kampfer et al. (1998); [c]Steinkellner et al. (1997)

nal optimization algorithm. This study determined the number of clusters (*k*) using the clustering procedure – Bayesian information criterion (BIC) value – implemented in the R package adegenet software (Jombart 2008) using 30 clusters as a starting point. Finally, to determine whether there is an overall correlation between geographic distance and genetic divergence (isolation by distance), the Mantel test was performed as described by Smouse et al. (1986), using the GenAlEx software (Peakall, Smouse 2012).

https://doi.org/10.17221/99/2021-JFS

RESULTS

Allelic patterns and statistical parameters of individual loci. The analyzed set of samples (N = 272) showed a notable level of genetic diversity with a low allelic dropout rate (1.83×10^{-2}). A total of 321 different alleles were detected across all loci. The number of different alleles detected per locus spanned between 7 and 32, with a mean of 17.833 and standard deviation (SD) = 6.858 (Table 3). The highest number of detected alleles was found at the locus PIE_258 (32), while the lowest at the locus PIE_239 (7).

The values of observed heterozygosity ranged between 0.035 and 0.878. The lowest values of observed heterozygosity were detected at loci PIE_239 (0.035) and QrZAG_112 (0.363). In contrast, the highest values were found at loci QrZAG_96 (0.878) and PIE_242 (0.859). *PIC* values were calculated for all studied loci. The mean PIC value for both investigated multiplexes was 0.751, with a standard deviation of 0.179. The PIC value exceeded the value of 0.85 in total for six loci – PIE_258, PIE_102, QrZAG_96, QrZAG_7, PIE_242 and QrZAG_20. The locus PIE_239 exhibits a high level of potential null allele occurrence [*F*(Null) = 0.317]. Therefore we performed intra- and interpopulation genetic diversity characterization omitting this locus, but for all downstream analyses, we decided not to omit any loci, consistently with studies cited and discussed in that loci with a high probability of null allele occurrence were not excluded from the analyses.

Table 3. Statistical parameters of individual loci

Locus	*K*	H_o	H_e	*PIC*	*HW*	*F*(Null)
PIE_239	7	0.035	0.288	0.276	***	0.317
PIE_227	12	0.657	0.674	0.621	NS	0.024
PIE_271	13	0.784	0.790	0.757	NS	0,004
PIE_267	13	0.788	0.816	0.790	NS	0.001
PIE_258	32	0.659	0.936	0.931	***	0.162
PIE_020	9	0.661	0.691	0.650	NS	0.022
PIE_152	14	0.560	0.819	0.794	***	0.183
QrZAG_112	11	0.363	0.371	0.348	NS	0.001
PIE_102	24	0.758	0.866	0.854	***	0.049
PIE_243	11	0.721	0.748	0.704	NS	0.005
QpZAG_15	24	0.547	0.818	0.797	***	0.176
QpZAG_110	20	0.812	0.857	0.845	NS	0.019
QrZAG_96	25	0.878	0.914	0.906	NS	0.020
QrZAG_11	23	0.724	0.782	0.753	NS	0.033
QrZAG_7	26	0.770	0.942	0.937	NS	0.100
PIE_223	15	0.588	0.796	0.770	***	0.129
PIE_242	23	0.859	0.915	0.908	***	0.024
QrZAG_20	19	0.840	0.889	0.878	NS	0.035
Mean	17.833	0.667	0.773	0.751	–	–
SD	6.858	0.199	0.174	0.179	–	–

k – number of alleles at each locus; H_o – observed heterozygosity; H_e – expected heterozygosity; *PIC* – polymorphic information content; *HW* – deviations from Hardy-Weinberg equilibrium; level of significance: ***α = 0.001; *α = 0.05; NS – not significant; *F*(Null) – estimation of null allele frequency; SD – standard deviation

Probability of identity. To verify the discrimination power of selected markers, we performed the probability of identity analysis (*PI*). Figure 1 shows the probability of determining two random genotypes as identical depending on the cumulative number of randomly selected markers. When the examined individuals are not related, the sufficient number of markers used to distinguish them was considered to be 4 ($PI = 0.1 \times 10^{-3}$). If there is a possibility for examined individuals to be closely related, the sufficient number of markers was 7 ($PI = 0.1 \times 10^{-2}$). For an entire set of 18 loci, PI values are 4.7×10^{-0} for unrelated individuals and 1.3×10^{-6} for the scenario when the relatedness of individuals is expected. Therefore, for the applied genotyping tool, we confirmed highly robust discriminative ability, even for related individuals.

Intra- and interpopulation genetic diversity. The mean number of alleles (*Na*) in a single population ranged between 9.059 (Hluboká) and 11.941 (Šternberk) with a mean of 10.788 (Table 4). The effective number of alleles (*Ne*) varied between 5.343 (Hluboká) and 6.598 (Náměšť). The values of Shannon's information index (*I*) representing population genetic diversity ranged between 1.785 (Hluboká) and 1.972 (Luhačovice). The values representing expected heterozygosity for each population are greater than the values of observed heterozygosity, which results in positive fixation indices caused by heterozygote deficiency. The fixation indices ranged from 0.042 (Šternberk) to 0.146 (Jindřichův Hradec).

To measure the genetic distances between populations, the pairwise population F_{ST} values and the pairwise population matrix of Nei's genetic distance were calculated. The F_{ST} population pairwise values ranged between 0.012 and 0.029 with

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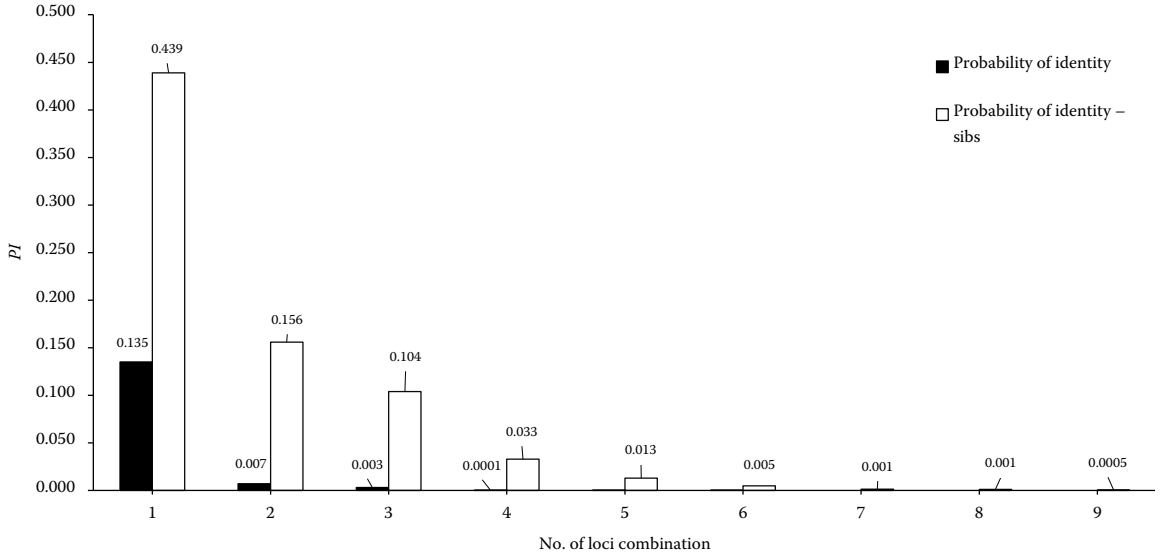

Figure 1. Discrimination power of the used loci – probability of identity (*PI*)

Table 4. Statistical parameters of individual populations

Population		*N*	*Na*	*Ne*	*I*	H_o	H_e	*F*
Křivoklát	mean	30	10.824	5.735	1.861	0.702	0.770	0.087
	SE		1.154	0.765	0.131	0.035	0.032	0.027
Plasy	mean	30	10.588	5.806	1.868	0.689	0.773	0.098
	SE		1.004	0.709	0.128	0.037	0.033	0.043
Hluboká	mean	19	9.059	5.343	1.785	0.697	0.771	0.102
	SE		1.005	0.570	0.119	0.046	0.029	0.041
Jin. Hradec	mean	20	10.294	5.691	1.910	0.680	0.797	0.146
	SE		0.852	0.607	0.090	0.033	0.019	0.037
Buchlovice	mean	30	11.529	6.020	1.931	0.722	0.785	0.071
	SE		1.189	0.665	0.130	0.034	0.034	0.034
Luhačovice	mean	29	11.647	6.485	1.972	0.747	0.797	0.056
	SE		1.197	0.871	0.127	0.026	0.027	0.028
Nasavrky	mean	30	10.471	5.926	1.855	0711	0.767	0.072
	SE		1.179	0.823	0.141	0.045	0.037	0.037
Náměšť nad Oslavou	mean	30	11.647	6.598	1.930	0.675	0.775	0.114
	SE		1.323	1.043	0.156	0.046	0.042	0.044
Šternberk	mean	29	11.941	6.423	1.964	0.745	0.785	0.042
	SE		1.086	0.851	0.133	0.036	0.035	0.032
Nižbor	mean	25	9.882	5.662	1.804	0.657	0.762	0.128
	SE		1.153	0.719	0.140	0.040	0.038	0.038
Total	mean	27.2	10.788	5.969	1.888	0.702	0.778	0.091
	SE		0.351	0.240	0.040	0.012	0.010	0.012

N – sample size; *Na* – mean number of alleles; *Ne* – effective number of alleles; *I* – Shannon genetic diversity index; H_o – observed heterozygosity; H_e – expected heterozygosity; *F* – mean fixation index

https://doi.org/10.17221/99/2021-JFS

a mean of 0.018 (SD = 0.004). The pairwise population Nei's genetic distance values ranged between 0.168 and 0.065 with a mean of 0.107 (SD = 0.026).

The analysis of molecular variance (AMOVA) shows that 84% of the total variance is within individuals, 15% among individuals and just 1% of total genetic variance is distinguishable among populations.

Discriminant analysis of principal components (DAPC) was applied to visualize the genetic diversity of populations (Figure 2). The scatterplot demonstrates the clustering of populations and the individuals in populations.

The outputs of the DAPC analysis were transferred to a cartographic layer. The scatterplot indicates that the populations formed, to a certain extent, three geographically different groups. Namely, the western group A (including populations 1, 2, 3 and 10), eastern group B (populations 5, 6, 7, 8 and 9) and group C, consisting of only population 4. Due to the apparent genetic distinctiveness of that population, we anticipated that population 4 (Jindřichův Hradec) was artificially established with seed sources of foreign origin. Nevertheless, the Mantel randomization test showed no significant relationship between geographic and genetic distance (R^2 = 0.0041, P = 0.360).

DISCUSSION

In our study, a total of 272 individuals at 18 loci were analyzed. According to Dostálek at al. (2011), 50 individuals per population is a lower boundary for intrapopulation studies, nevertheless, the number close to 30 individuals per population was proved to be sufficient to inspect interpopulation differentiation. Higher numbers of analyzed individuals within population might help reveal intrapopulation spatial variability, but this was not the aim of this study. All targeted populations exhibited high levels of genetic diversity across all loci, with the value of expected heterozygosity ranging from 0.288 to 0.942 and the mean of 0.773. These results are directly comparable with the findings of other authors. For instance, Alberto et al. (2010), with a very similar experimental design, observed H_e = 0.822. Novotný et al. (2016) carried out a comparable study in the conditions of the Czech Republic with the mean value of H_e = 0.771.

When comparing the allelic richness of individual loci, it was also found that genomic markers (gSSRs) are more polymorphic than EST-derived markers (eSSRs) with the mean allelic richness of 21.1 for gSSRs and 15.7 for eSSRs. These results are consistent with a previous study where the

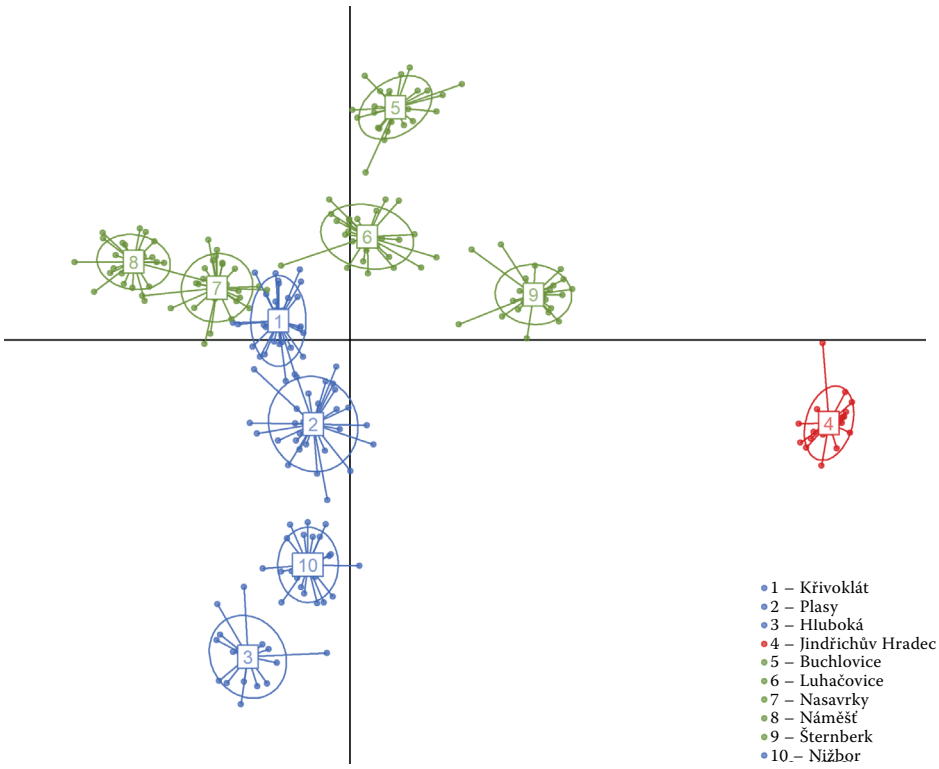

Figure 2. Discriminant Analysis of Principal Components (DAPC, R package: Adegenet)

https://doi.org/10.17221/99/2021-JFS

mean allelic richness for gSSRs was 16.9 and for eSSRs it was 10.3 (Guichoux et al. 2011). Alberto et al. (2010) inspected a total of 16 nuclear microsatellite markers in his study and observed the mean allelic richness of just 8.400. On the opposite scale, Novotný et al. (2016) published the mean value of allelic richness equal to 23.3.

The mean value of observed heterozygosity (H_o = 0.667) is comparable with that found in previous studies. Using 11 loci, Novotný et al. (2016) reported H_o = 0.719. For example, in a study using an identical set of loci, the mean value of observed heterozygosity was found to be slightly higher (H_o = 0.713) (Guichoux et al. 2011). Another study from the area of the Czech Republic determined a mean value of H_e = 0.808, based on five populations and 10 loci (Dostálek at al. 2011). In our study, the F_{ST} population pairwise values ranged between 0.012 and 0.029 with a mean of 0.018. These values are very similar to those found for sessile oak populations on the northern side of the Pyrenees Mountains, where the mean F_{ST} values for two separate regions were 0.018 and 0.028 (Alberto et al. 2010). Slightly lower values of pairwise F_{ST} were published by Novotný et al. (2016), where the values for ten Czech populations spanned between 0.008 and 0.022 with the mean of 0.013. While comparing natural and artificial forest stands, Dostálek et al. (2011) observed F_{ST} values ranging from 0.0065 to 0.0509 with a mean value of 0.0281.

The analysis of molecular variance (AMOVA) shows that 84% of the total variance is within individuals, 15% among individuals, and 1% is distinguishable among populations. This result is also in agreement with previous studies. Gugerli et al. (2007) observed 91% of the intrapopulation variation within and among individuals and 9% between observed regions. Another study from the Apennine Peninsula found a distribution of variance between 80% within populations and 20% among populations (Bruschi et al. 2003).

The Mantel test results are very similar to those obtained by Bruschi et al. (2003) (R^2 = 0.0047, P = 0.405). A slightly higher relationship between geographic and genetic distance (R^2 = 0.0578) was observed by Novotný et al. (2016).

Although the Mantel test did not confirm statistical significance, the genetic-geographic correlation among the targeted populations is visually apparent (Figures 2 and 3). Based on the DAPC scatterplot, populations could be grouped into three clusters (A, B and C). The first group (A) consists of four populations in the western part of the country, the eastern group (B) consists of five

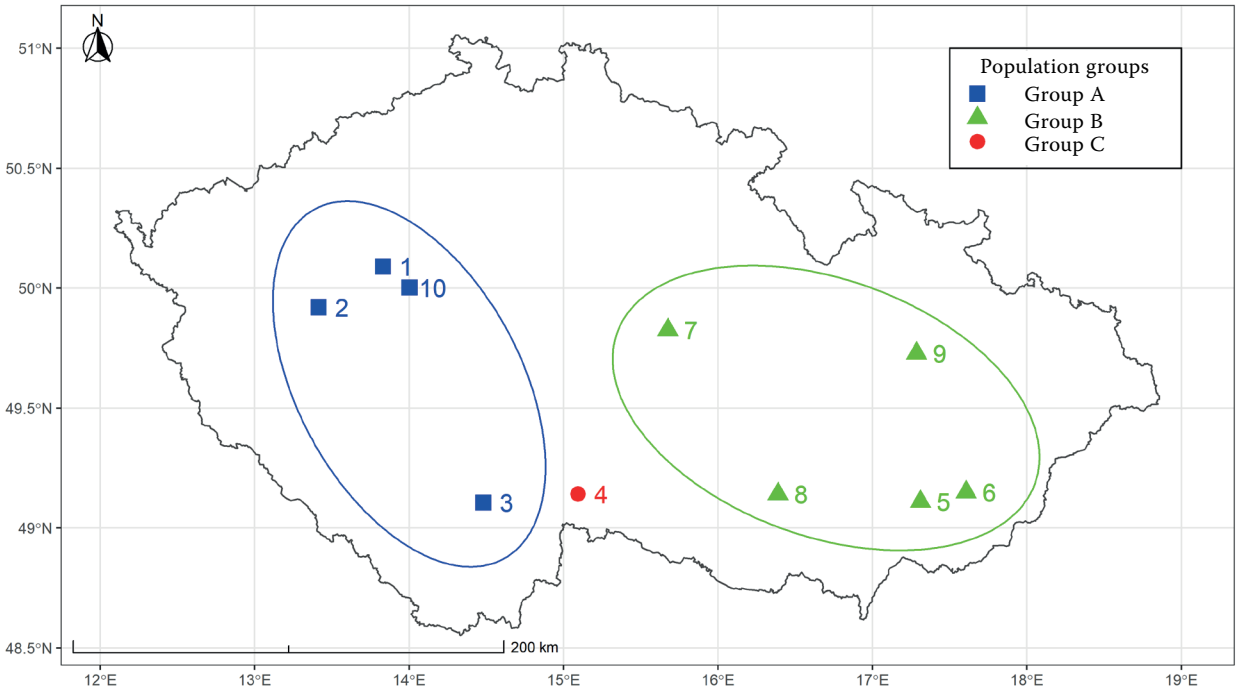

Figure 3. Scatterplot of population clusters

https://doi.org/10.17221/99/2021-JFS

populations. The 15th meridian represents the approximate north-south borderline between these clusters. Population 4 (Jindřichův Hradec) is genetically different from all others.

The partition of examined populations into two separate clusters is explainable by the postglacial development of vegetation. Petit et al. (2002) focused on identifying glacial refugia and natural routes of recolonization using chloroplast DNA analysis. The most perspicuous refugia of sessile oak for Central Europe were located on the Iberian Peninsula, Apennine Peninsula and Balkan Peninsula. Based on the analysis of linkage groups, the presumed migration routes of sessile oak merge in the area of the Czech Republic. The direct source of recolonization cannot be determined by the biparentally inherited genomic markers applied in our study due to the generally high level of gene flow and their codominant character. Still, indirect indices support our hypothesis of the diverse postglacial origin of Czech sessile oak populations.

Having detected the apparent genetic distinctiveness of population 4, we searched for a historical context which would help to elucidate our findings. In general, neutral microsatellite markers do not usually detect any apparent differentiation among geographically closed populations if these populations are autochthonous with non-restricted gene flow. We do not assume distinctiveness of population 4 due to any natural barriers causing gene-flow reduction, but we thought of probable historical artificial translocation of gene sources from geographically more distant regions. The forest stands in this area were historically the property of the Austrian aristocratic family. Due to disastrous windthrow damage in the first half of the 19th century, the area had to be reforested artificially. From the historical forestry records, it is evident that foreign seed sources were frequently used (Jansen, Geburek 2016). In the 19th century, it was customary that reproductive material, especially for border areas, was imported from all of Austria or Germany, as aristocratic owners had access to such reproductive material sources. Therefore, our findings imply a relatively high probability of the long-distant origin of this population.

CONCLUSION

This study took advantage of previously developed SSRs multiplexes being adjusted for the genetic analysis of Czech sessile oak populations. Although we analyzed a moderate number of individuals per population, we were able to explore the interpopulation structure identifying two distinctive geographic genetic groups, and one stand very probably originating from the geographically more distant seed source. We, therefore, confirmed that at least partial regionalization of seed zones within the Czech Republic is worth maintaining, and geographic genetic variation was detected to some extent.

Acknowledgement: We thank Jan Bílý for his contribution to genotyping protocol optimization. We also thank Oldřich Hrdlička and Michal Remeš (LČR, s.p.), who sellected target sessile oak stands for this study.

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Received: August 6, 2021
Accepted: December 17, 2021