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Characterization of 20 new EST-SSR markers for northern red oak (*Quercus rubra* L.) and their transferability to *Fagus sylvatica* L. and six oak species of section *Lobatae* and *Quercus*

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Abstract. Northern red oak (*Quercus rubra* L.) is widely distributed in the eastern United States and southeastern Canada. It has also been introduced to Europe, where it has become an economically important plantation species now. Despite growing genomic resources, the number of available EST-SSR (expressed sequence tag – simple sequence repeat) markers for *Q. rubra* is still limited. Here, we used existing sequence information to provide a new set of EST-SSRs for northern red oak. In total, we report 20 polymorphic EST-SSRs, for which performance was evaluated in three *Q. rubra* populations from different regions in Michigan. We further tested the transferability of these markers to six additional oak species of section *Lobatae* (*Quercus ellipsoidalis* E.J. Hill, and *Quercus georgiana* M.A. Curtis) and *Quercus* (*Quercus robur* L., *Quercus alba* L., *Quercus pedunculiflora* K. Koch, and *Quercus petraea* (Matt.) Liebl.), as well as to European beech (*Fagus sylvatica* L.). The reported markers can be used in future population genetic studies.
Keywords: microsatellites, *Fagaceae*, genetic diversity

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Introduction

Northern red oak (*Quercus rubra* L.) is an important timber species that is widely distributed in the eastern United States and southeastern Canada, and grows under various environmental conditions within its distribution range (USDA NRCS 2002, Magni et al. 2005). It belongs to section *Lobatae* (red oaks), which members are native only to the Americas. Northern read oak has been introduced to Europe, where it has become an economically

important plantation species in several countries now.

Climate change is expected to significantly impact forest ecosystems in Europe, influencing the vegetation period, growth, health, and distribution of trees (European Environment Agency (EEA) 2012). Forest management can help forest ecosystems to adapt to these new conditions (Bolte et al. 2009). One component of adaptive forest management could be the promotion of non-native tree species, such as *Q. rubra*, that may be better adapted to future environmental conditions (Roloff & Grundmann 2008, Bolte et al. 2009). Currently, growing genomic and transcriptomic resources are becoming available for northern red oak (The Hardwood Genomics Project, Konar et al. 2017). Resources such as transcriptome data can be used to derive EST-SSR (expressed sequence tag – simple sequence repeat) markers. These markers usually show high transferability rates among taxa (Ellis & Burke 2007), and, due to their location in coding or closely linked regions, are candidates for the study of local adaptation. For instance, EST-SSRs can be used in genetic association studies, in which markers are identified that are significantly associated with adaptive traits or environmental variables (Balding 2006, Rellstab et al. 2015). Often these approaches are combined with outlier analyses, in which markers under selection show higher or lower differentiation among populations than expected under neutral assumptions (Helyar et al. 2011, Narum & Hess 2011). The number of available EST-SSRs for *Q. rubra*, however, is still limited (Sullivan et al. 2013).

Therefore, the aim of our study was to take advantage of available EST-SSR sequence information, and provide a new set of EST-SSR markers for northern red oak. The performance of the markers was evaluated in three *Q. rubra* populations from different regions in Michigan. In total, we report 20 markers that are polymorphic in *Q. rubra*. We further tested the transferability of these markers to six other oak species of section *Lobatae* (*Quercus ellipsoidalis* E.J. Hill, *Quercus georgiana* M.A. Curtis), and *Quercus* (*Quercus robur* L., *Quercus alba* L., *Quercus pedunculiflora* K. Koch, and *Quercus petraea* (Matt.) Liebl.), as well as to European beech (*Fagus sylvatica* L.).

Materials and methods

Marker analysis

Initially, 40 EST-SSRs were tested for amplification and polymorphism in four *Q. rubra* samples. Primer sequences were obtained from the Hardwood Genomics Project (https://www.hardwoodgenomics.org/Transcriptome-assembly/1963023?tripal_pane=group_summary_tripalpane). Successfully amplified polymorphic markers were further evaluated in 96 samples of three different *Q. rubra* populations (FC-A, HMR-IH, and MTU-1) located in different regions in Michigan. DNA extracted from leaf material of the populations was obtained from a previous study (Lind & Gailing 2013). Population FC-A was located in the Baraga Plains region (latitude: 46°39'9.407''N, longitude: 88°30'6.962''W), the population HMR-IH was located in the Huron Mountain Reserve (latitude: 46°51'12.884''N, longitude: 87°50'42.824''W), and the MTU-1 population was located in Houghton (latitude: 47°6'24.649''N, longitude: 88°32'51.209''W). Furthermore, the transferability of the markers to six other oak species and European beech was tested. The markers were tested in each five samples of the species *Q. ellipsoidalis, Q. georgiana,* and *Q. robur*, as well as in two samples of each *Q. alba, Q. pedunculiflora, Q. petraea*, and *F. sylvatica*.

For genotyping, a PCR mix was prepared consisting of 1µl DNA (ca. 0.6 ng/µl), 1.5 µl 10x reaction buffer B (Solis BioDyne, Tartu, Estonia), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM each dNTP), 0.2 µl (5 U/µL) HOT FIREPol *Taq* DNA polymerase (Solis BioDyne, Tartu, Estonia), 0.2 µl (5 picomole/µl) tailed forward primer (Schuelke 2000, Kubi-

siak et al. 2013), 0.5 µl (5 picomole/µl) PIG-tailed reverse primer (Brownstein et al. 1996), 1µl (5 picomole/µl) dye labeled (6-FAM) M13 primer, and 5.5 µl H_2O. Fragments were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) using GS 500 ROX (Applied Biosystems, Foster City, USA) as an internal size standard. Allele scoring was conducted using the GeneMapper 4.1 software (Applied Biosystems, Foster City, USA). The following touchdown PCR program was used: an initial denaturation of 95 °C for 15 min, followed by 10 touchdown cycles of 94 °C for 1 min, 60 °C (-1 °C per cycle) for 1 min, and 72 °C for 1 min, 25 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, and a final extension step of 72 °C for 20 min. For genotyping of the successfully amplified markers, the loci were combined in five different multiplexes (multiplex 1: Qr1423, Qr2927, Qr6333b, and Qr6859; multiplex 2: FS_C1702, Qr0237, Qr0332, and Qr6783; multiplex 3: FS_C2361, Qr1266, Qr3552, and Qr3173; multiplex 4: Qr0057, Qr1824, Qr5117, and Qr5623; multiplex 5: FS_C2660, FS_C2791, FS_C8183, and Qr2660).

Data analysis

The genetic diversity indices number of alleles (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) for the different markers, as well as the fixation index (F_{ST}) among populations were calculated with the GenAlEx 6.501 software (Peakall & Smouse 2006, 2012). Significant differences of N_a, H_o, and H_e among populations were tested using the Kruskal-Wallis test with multiple comparisons implemented in the R package pgirmess 1.6.7 (Giraudoux 2017). The inbreeding coefficient (F_{IS}) (Weir & Cockerham 1984) was calculated with FSTAT 2.9.3 (Goudet 1995). *P*-values (α = 0.05) were corrected for multiple comparisons using a sequential Bonferroni correction (Rice 1989). Genepop 4.7 (Rousset 2008) was used to calculate linkage disequilibrium (LD) for each pair of loci in the three populations with the following parameters for the Markov chain: 10,000 dememorisation steps, 100 batches, and 5000 iterations per batch. A Bonferroni correction on the original *p*-value (α = 0.05) was applied to correct for multiple testing. Population structure was inferred using the STRUCTURE 2.3.4 software (Pritchard et al. 2000). The admixture model and correlated allele frequencies were selected. A burn-in period of 50,000 and Markov chain Monte Carlo (MCMC) replicates of 100,000 were used. Potential clusters (*K*) from 1 to 6 were tested using 10 iterations. The ΔK method (Evanno et al. 2005) was applied to infer the most likely number of *K* with the STRUCTURE HARVESTER 0.6.94 software (Earl & vonHoldt 2012). The CLUMPAK software (Kopelman et al. 2015) was used for summation and graphical representation of the STRUCTURE results. Clustering of populations was further analyzed using a principle coordinate analysis (PCoA) with the covariance standardization method implemented in GenAlEx (Peakall & Smouse 2006, 2012). To detect potential deviations from neutral expectations, an outlier analysis was performed using the LOSITAN 1.0 software (Antao et al. 2008). LOSITAN was run with 70,000 simulations, the step-wise mutation model, and a FDR (false discovery rate) of 0.1. Annotation of the sequences, in which the SSR loci were located, was performed using similarity searches against the NCBI (National Center for Biotechnology Information) nucleotide collection (nr) database with BLASTN 2.8.0+ (Altschul et al. 1997).

Results

Of the 40 initially tested EST-SSRs, 20 markers were polymorphic, 5 were monomorphic (Table 1), and 15 markers did not amplify (Table S1 - Supporting Information). The 20 polymorphic markers were used to genotype 96 individuals of three *Q. rubra* populations

Table 1 Characteristics of EST-SSRs that amplified in *Q. rubra*

Marker	[a]Contig name	[a]Forward primer sequence (5'-3')	[a]Reverse primer sequence (5'-3')	[a]Repeat motif	Observed size (bp)	[b]E value	Annotation
FS_C1702	RO454_contig11702_v2	CTACCCTGTTGCCTCCTCTG	GAGGCCTCATCACATGGACT	ACA	209-227	3.00E-118	Mucin-2
FS_C2361	RO454_contig12361_v2	AGGTCCTTCAGTTTGGGAGC	ATTCCCATGCATCAAAATCC	GAA	193-196	9.00E-113	Light-harvesting complex-like protein OHP2
FS_C2660	RO454_contig12660_v2	AGCAGAATTCGCCAAGTGAT	TGCCTTTGCATTCTCCTCTT	GAG	217-232	6.00E-172	Eukaryotic translation initiation factor 5B-like
FS_C2791	RO454_contig12791_v2	CGAAACAGAGAGAACCCAAGA	CTTCAAACATCCAGCGTTGA	GA	287-302	1.00E-155	50S ribosomal protein L13, chloroplastic
FS_C8183	RO454_contig18183_v2	TATTCAACCACAGCTGCCTG	ACAGCTGCCTCTGTGGATCT	AGC	172-216	0	Auxin response factor 19-like
Qr0044	RO454_contig1044_v2	TACCAACCAAGTGGCATTCA	ATTTGCCTTCCATGAGCATC	TGGA	301	0	Abscisic stress-ripening protein 3-like
Qr0057	RO454_contig257_v2	CCGACCTTGTTGATTGTTCC	TATTGATCCTATCGGAGGCG	AAG	128-156	0	Vacuolar protein sorting-associated protein 60.1
Qr0237	RO454_contig10237_v2	GTCTGTTGCTGTTGGGGAAT	ATCGCAGAGCAGGTTATCGT	CGC	119-143	1.00E-85	Phosphoribosylamine--glycine ligase
Qr0332	RO454_contig10332_v2	AATATCAAATCGGCCAGCAG	GTGGTGGACCTGTGCCATAC	CCT	154-169	5.00E-166	DEAD-box ATP-dependent RNA helicase 20
Qr1146	RO454_contig21146_v2	TGTTGCATCAATCCTTGGAA	TGACTGTGCTACTTGGGCTG	CAG	208	0	Protein TIME FOR COFFEE-like
Qr1183	RO454_contig11183_v2	GAGAAAAACGAACCCAACCA	GCGATGGAAGAAGAAAATGG	AAC	182	5.00E-173	*Quercus suber* uncharacterized LOC112013054
Qr1266	RO454_contig11266_v2	CCTTGAGGTTGAGAGGCTTG	TTCCCATTCCCATTCACATT	GAT	252-267	5.00E-179	Universal stress protein PHOS34-like
Qr1423	RO454_contig21423_v2	TCCCTTCTCGTTTCACCATC	TGCACCATACGGATTGAAAG	CAC	276-282	7.00E-152	Galactan beta-1,4-galactosyltransferase GALS3-like
Qr1824	RO454_contig11824_v2	GCTTCAGTGCCAGATCCTCT	TCTCATCAGGAGACGAGGGT	TTA	302-328	0	26S proteasome non-ATPase regulatory subunit 2 homolog A

Table 1 (continuation)

Marker	[a]Contig name	[a]Forward primer sequence (5'-3')	[a]Reverse primer sequence (5'-3')	[a]Repeat motif	Observed size (bp)	[b]E value	Annotation
Qr2927	RO454_ contig12927_v2	CTCGAGCGAGCGAGATAGAT	CCAGAGTGGATCGTTGAGGT	CGT	210-219	2.00E-139	Selenocysteine methyltransferase-like
Qr3173	RO454_ contig13173_v2	GAGAATGAGGAACAATCGGC	CATCGAAACCCGTGAAGAAT	AAC	461-482	0	*Quercus suber* uncharacterized LOC111985897
Qr3552	RO454_ contig13552_v2	TGTCCTCTTCTTCCTTCCCA	GGATCTCCTGCAAAACTTCG	AAGA	322-389	7.00E-57	F-box/kelch-repeat protein At3g23880-like
Qr5117	RO454_ contig25117_v2	TGCAAGGAATGAACTCCCTC	CAAGAACACGAAGGGATGGT	AAC	238-253	0	Transcription factor bHLH66-like
Qr5426	RO454_ contig25426_v2	TGGTCCCTTTCCTTCTCCTT	CCGCCATTAACTCTCCAAAA	GAAA	288	0	Fructose-bisphosphate aldolase 6
Qr5439	RO454_ contig15439_v2	CCATGCTGTTGTTGTTGCTC	TATGGAGGTGGAGAAGTGGG	CCA	197-215	8.00E-139	Transcription factor UNE12-like
Qr5623	RO454_ contig25623_v2	CACCAACACCAACATCAAGC	TTTGGATTGTGGGGGTTCTA	CTT	258-278	0	Serine/threonine-protein phosphatase PP1 isozyme 3-like
Qr6333b	RO454_ contig26333_v2	GCCGACGACGACTCTATCTC	GCTATGGCGGTGTGACTCTC	GT	114-136	4.00E-79	Abscisic acid receptor PYL8
Qr6783	RO454_ contig26783_v2	GAGAGCCCTGTTATCCTCCC	AATGAGTCTCAAAGCGGTGG	GTT	227-279	9.00E-157	Superoxide dismutase [Cu-Zn] 2
Qr6859	RO454_ contig26859_v2	CGCTGAGTGTTGAAGTTGGA	CAGACCCGACAAATCAAACA	TTG	255-267	5.00E-109	GDP-mannose 4,6 dehydratase 1
Qr7748	RO454_ contig17748_v2	CAAAGCTCCTCCACAAAGAGA	TGAGCATCATGGTGGTCTTG	GGT	376	6.00E-83	*Quercus suber* uncharacterized LOC112028246

Note. [a]Contigs, primer sequences, and the repeat motifs were obtained from the *Q. rubra* transcriptome assembly conducted within the Hardwood Genomics Project (https://www.hardwoodgenomics.org/Transcriptome-assembly/1963023?tripal_pane=group_summary_tripalpane). [b]Expect value (describes the number of matches, which can be expected by chance, when searching a database of a particular size.

from different regions in Michigan. The number of alleles (N_a) for the different loci ranged from 2 to 13 in population FC-A (mean N_a: 5.0), from 2 to 13 in population HMR-IH (mean N_a: 5.8), and from 2 to 15 in population MTU-1 (mean N_a: 5.5) (Table 2). The observed heterozygosity (H_o) ranged from 0.147 for marker Qr2361 in population MTU-1 to 0.939 for marker Qr6783 in the same population. The mean values of H_o over all loci were 0.483 for FC-A, 0.620 for HMR-IH, and 0.566 for MTU-1. The expected heterozygosity (H_e) ranged from 0.136 for marker Qr2361 in population MTU-1 to 0.907 for marker Qr6783 in population HMR-IH. The mean values of H_e were 0.597 for FC-A, 0.623 for HMR-IH, and 0.618 for MTU-1 (Table 2). All genetic diversity indices were not significantly different among populations. The F_{IS} value was significantly different from zero only for marker Qr5623 in population MTU-1. Over all loci, the F_{IS} values of the populations FC-A and MTU-1 were significantly different from zero (Table 2). Genetic differentiation (F_{ST}) among populations ranged from 0.002 for marker Qr5623 to 0.127 for marker Qr1266. The mean differentiation among populations was 0.041 ($p<0.05$) (Table 2). No F_{ST}- outlier loci were detected. Differentiation among populations was also detected with the STRUCTURE and principle coordinate analyses. The STRUCTURE analysis revealed a most likely number of clusters of $K = 2$ (Fig. S1 - Supporting Information). Population FC-A was clearly separated from HMR-IH and MTU-1 (Fig. 1). A separation of FC-A from the other two populations was also revealed by the PCoA, albeit less pronounced (Fig. 2). Significant

Table 2 Genetic diversity indices based on EST-SSRs for the populations

		FC-A					HMR-IH					MTU-1				
Marker	F_{ST}	N	N_a	H_o	H_e	F_{IS}	N	N_a	H_o	H_e	F_{IS}	N	N_a	H_o	H_e	F_{IS}
FS_C1702	0.038*	28	5	0.500	0.679	0.281	32	5	0.750	0.693	-0.067	34	6	0.647	0.593	-0.077
FS_C2361	0.108*	26	2	0.538	0.473	-0.118	33	2	0.303	0.257	-0.164	34	2	0.147	0.136	-0.065
FS_C2660	0.062*	28	5	0.500	0.582	0.158	33	5	0.818	0.732	-0.103	35	5	0.771	0.769	0.011
FS_C2791	0.018	28	2	0.286	0.245	-0.149	33	3	0.424	0.404	-0.033	35	2	0.400	0.382	-0.033
FS_C8183	0.011	28	5	0.571	0.582	0.036	33	6	0.758	0.697	-0.071	35	5	0.600	0.638	0.073
Qr0057	0.022	26	6	0.462	0.652	0.310	33	5	0.758	0.647	-0.155	34	7	0.706	0.743	0.064
Qr0237	0.051*	23	7	0.391	0.494	0.230	33	8	0.788	0.793	0.022	34	8	0.735	0.730	0.007
Qr0332	0.046*	26	5	0.577	0.708	0.204	33	6	0.727	0.733	0.023	34	6	0.765	0.716	-0.053
Qr1266	0.127*	26	3	0.385	0.508	0.261	33	3	0.424	0.430	0.028	34	4	0.382	0.544	0.310
Qr1423	0.025	27	3	0.185	0.497	0.639	33	3	0.273	0.330	0.189	34	3	0.353	0.424	0.182
Qr1824	0.045*	24	9	0.583	0.857	0.338	33	11	0.788	0.801	0.031	34	11	0.824	0.846	0.042
Qr2927	0.013	21	3	0.381	0.439	0.156	32	4	0.531	0.482	-0.087	34	3	0.382	0.436	0.139
Qr3173	0.073*	26	6	0.462	0.511	0.116	32	8	0.906	0.805	-0.111	32	7	0.781	0.811	0.053
Qr3552	0.028	20	3	0.200	0.515	0.627	32	3	0.156	0.330	0.537	33	3	0.333	0.417	0.216
Qr5117	0.033	26	3	0.462	0.607	0.257	33	6	0.545	0.625	0.143	34	5	0.765	0.730	-0.033
Qr5439	0.037	28	4	0.607	0.654	0.089	33	6	0.636	0.718	0.128	35	6	0.743	0.762	0.040
Qr5623	0.002	28	4	0.321	0.601	0.479	33	5	0.424	0.579	0.281	35	4	0.286	0.565	0.505*
Qr6333b	0.031*	28	8	0.857	0.835	-0.008	32	9	0.906	0.867	-0.030	34	10	0.765	0.859	0.125
Qr6783	0.041*	23	13	0.826	0.875	0.078	33	13	0.879	0.907	0.047	33	15	0.939	0.888	-0.043
Qr6859	0.020	28	4	0.571	0.635	0.118	33	4	0.606	0.629	0.053	35	4	0.543	0.698	0.236
Mean/ all loci	0.041*	25.9	5	0.483	0.597	0.210*	32.8	5.8	0.620	0.623	0.020	30.9	5.5	0.566	0.618	0.079*

Note. Abbreviations: F_{ST} - fixation index, N - number of individuals, N_a - number of alleles, H_o - observed heterozygosity, H_e - expected heterozygosity, F_{IS} - inbreeding coefficient, *F_{IS} or F_{ST} significantly different from zero after Bonferroni correction.

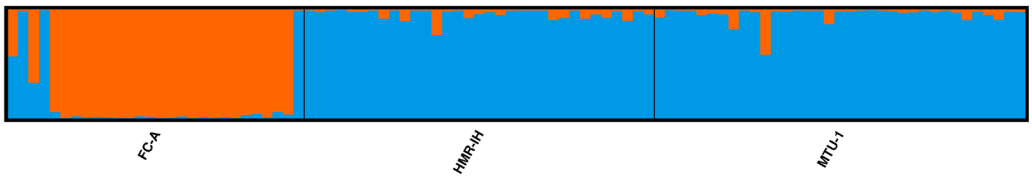

Figure 1 Clustering of individuals of the three populations

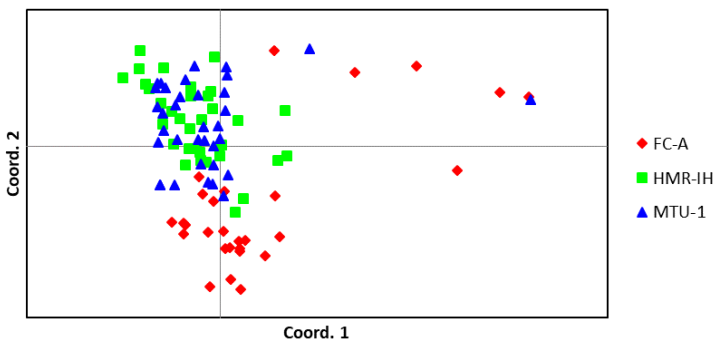

Figure 2 Principal coordinates analysis

LD was only observed between the markers Qr2791 and Qr3173 in population FC-A, and between markers Qr2927 and Qr6783 in population HMR-IH.

The transferability of the 20 polymorphic markers in *Q. rubra* to six other oak species and European beech was successfully tested (Table 3). All loci, except for marker Qr3552 to *Q. robur*, were successfully transferred to all tested oak species (*Q. ellipsoidalis*, *Q. georgiana*, *Q. robur*, *Q. alba*, *Q. pedunculiflora*, and *Q. petraea*), and most of the markers were polymorphic. In total, 13 of the 20 markers (65 %) were transferable to *F. sylvatica*, and five of them were polymorphic. Markers FS_C1702, FS_C2361, FS_C2660, FS_2791, and FS_8183 have been tested for amplification in *F. sylvatica* before. Like in our study, all of these markers amplified in *F. sylvatica*, but only FS_C2361 was polymorphic.

Discussion

The performance of 20 successfully amplified and polymorphic EST-SSR markers was evaluated in three *Q. rubra* populations from different regions in Michigan. A high genetic diversity was revealed for all populations (Table 2). Other studies, however, showed slightly higher genetic diversity estimates for the same and other *Q. rubra* populations. For instance, based on seven EST-SSRs, Lind & Gailing (2013) reported a mean number of alleles per locus of 11, a mean observed heterozygosity of 0.610, and a mean expected heterozygosity (unbiased expected heterozygosity (Peakall & Smouse 2006) of 0.700. Based on 18 EST-SSRs, also Sullivan et al. (2013) reported slightly higher values (mean N_a: 7, mean H_o: 0.700, mean H_e: 0.700). Hence, the EST-SSRs tested in our study, seem on average to be slightly less variable than previously reported EST-SSRs for *Q. rubra*, but variable enough for future population genetic studies. Over all loci, the F_{IS} values of the populations FC-A and MTU-1 were significantly different from zero. Nevertheless, since all markers (except Qr5623 for MTU-1) showed F_{IS} values not different from zero, the populations are likely not affected by inbreeding. A low but significant genetic differentiation among populations was detected (F_{ST}: 0.041, $p<0.05$). This is in agreement with other studies in forest tree species including *Q. rubra*

Table 3 Transferability of the tested EST-SSRs

Marker	Species	Number of alleles	Size (bp)	Amplification success	Marker	Species	Number of alleles	Size (bp)	Amplification success
	Quercus ellipsoidalis	3	212-224	4/5		*Quercus ellipsoidalis*	5	302-314	5/5
	Quercus georgiana	4	215-227	4/5		*Quercus georgiana*	7	302-329	5/5
	Quercus robur	5	206-218	3/5		*Quercus robur*	3	305-320	3/5
FS_C1702	*Quercus alba*	3	206-218	2/2	Qr1824	*Quercus alba*	2	293-296	2/2
	Quercus pedunculiflora	3	215-224	2/2		*Quercus pedunculiflora*	2	293-305	2/2
	Quercus petraea	1	218	1/2		*Quercus petraea*	2	290-293	2/2
	Fagus sylvatica	1	203	1/2		*Fagus sylvatica*	2	272-290	2/2
	Quercus ellipsoidalis	2	193-196	5/5		*Quercus ellipsoidalis*	3	210-216	5/5
	Quercus georgiana	2	193-196	5/5		*Quercus georgiana*	2	210-213	5/5
	Quercus robur	3	196-202	5/5		*Quercus robur*	3	216-222	4/5
FS_C2361	*Quercus alba*	2	196-199	2/2	Qr2927	*Quercus alba*	1	216	2/2
	Quercus pedunculiflora	2	196-202	2/2		*Quercus pedunculiflora*	3	216-222	2/2
	Quercus petraea	2	196-199	2/2		*Quercus petraea*	2	216-225	2/2
	Fagus sylvatica	3	196-205	2/2		*Fagus sylvatica*	-	-	0/2
	Quercus ellipsoidalis	3	217-223	4/5		*Quercus ellipsoidalis*	4	462-479	5/5
	Quercus georgiana	4	217-226	5/5		*Quercus georgiana*	3	462-476	5/5
	Quercus robur	4	217-226	5/5		*Quercus robur*	4	491-500	5/5
FS_C2660	*Quercus alba*	3	217-232	2/2	Qr3173	*Quercus alba*	2	497-500	2/2
	Quercus pedunculiflora	3	217-223	2/2		*Quercus pedunculiflora*	3	491-500	2/2
	Quercus petraea	2	217-223	2/2		*Quercus petraea*	3	494-500	2/2
	Fagus sylvatica	1	138	2/2		*Fagus sylvatica*	-	-	0/2
	Quercus ellipsoidalis	1	289	5/5		*Quercus ellipsoidalis*	2	322-384	5/5
	Quercus georgiana	1	289	5/5		*Quercus georgiana*	2	322-384	5/5
	Quercus robur	1	293	5/5		*Quercus robur*	-	-	0/5
FS_C2791	*Quercus alba*	2	271-291	2/2	Qr3552	*Quercus alba*	2	322-384	2/2
	Quercus pedunculiflora	1	291	2/2		*Quercus pedunculiflora*	1	384	1/2
	Quercus petraea	1	293	1/2		*Quercus petraea*	1	322	1/2
	Fagus sylvatica	1	293	2/2		*Fagus sylvatica*	-	-	0/2

Table 3 (continuation)

Marker	Species	Number of alleles	Size (bp)	Amplification success	Marker	Species	Number of alleles	Size (bp)	Amplification success
	Quercus ellipsoidalis	3	202-211	5/5		*Quercus ellipsoidalis*	3	244-250	5/5
	Quercus georgiana	2	208-211	3/5		*Quercus georgiana*	2	247-250	4/5
	Quercus robur	5	199-214	5/5		*Quercus robur*	4	226-244	5/5
FS_C8183	*Quercus alba*	3	202-214	2/2	Qr5117	*Quercus alba*	2	238-241	2/2
	Quercus pedunculiflora	2	199-205	2/2		*Quercus pedunculiflora*	2	238-244	2/2
	Quercus petraea	2	205-214	2/2		*Quercus petraea*	1	238	2/2
	Fagus sylvatica	1	196	2/2		*Fagus sylvatica*	1	241	2/2
	Quercus ellipsoidalis	5	128-152	5/5		*Quercus ellipsoidalis*	4	200-212	5/5
	Quercus georgiana	3	131-140	5/5		*Quercus georgiana*	4	203-212	5/5
	Quercus robur	6	134-152	5/5		*Quercus robur*	3	203-212	5/5
Qr0057	*Quercus alba*	1	134	2/2	Qr5439	*Quercus alba*	3	203-212	2/2
	Quercus pedunculiflora	2	134-137	2/2		*Quercus pedunculiflora*	3	203-212	2/2
	Quercus petraea	1	131	1/2		*Quercus petraea*	3	206-212	2/2
	Fagus sylvatica	2	134-140	2/2		*Fagus sylvatica*	1	224	2/2
	Quercus ellipsoidalis	4	119-140	5/5		*Quercus ellipsoidalis*	4	252-273	5/5
	Quercus georgiana	4	128-143	5/5		*Quercus georgiana*	3	267-279	5/5
	Quercus robur	2	131-161	5/5		*Quercus robur*	6	258-285	5/5
Qr0237	*Quercus alba*	2	131-137	2/2	Qr5623	*Quercus alba*	4	273-285	2/2
	Quercus pedunculiflora	1	131	2/2		*Quercus pedunculiflora*	4	270-294	2/2
	Quercus petraea	2	131-137	2/2		*Quercus petraea*	4	279-285	2/2
	Fagus sylvatica	-	-	0/2		*Fagus sylvatica*	3	267-279	2/2
	Quercus ellipsoidalis	4	154-172	5/5		*Quercus ellipsoidalis*	5	116-130	5/5
	Quercus georgiana	2	160-163	5/5		*Quercus georgiana*	4	116-128	5/5
	Quercus robur	3	160-169	5/5		*Quercus robur*	3	112-122	5/5
Qr0332	*Quercus alba*	2	163-166	2/2	Qr6333b	*Quercus alba*	2	122-124	2/2
	Quercus pedunculiflora	2	160-166	2/2		*Quercus pedunculiflora*	2	122-124	2/2
	Quercus petraea	1	160	2/2		*Quercus petraea*	2	122-124	2/2
	Fagus sylvatica	-	-	0/2		*Fagus sylvatica*	1	120	2/2

Table 3 (continuation)

Marker	Species	Number of alleles	Size (bp)	Amplification success
Qr1266	*Quercus ellipsoidalis*	2	258-264	5/5
	Quercus georgiana	4	234-249	5/5
	Quercus robur	4	234-249	5/5
	Quercus alba	3	252-264	2/2
	Quercus pedunculiflora	2	240-246	2/2
	Quercus petraea	3	240-249	2/2
	Fagus sylvatica	1	249	2/2
Qr1423	*Quercus ellipsoidalis*	3	276-282	5/5
	Quercus georgiana	2	276-279	5/5
	Quercus robur	1	279	5/5
	Quercus alba	1	279	2/2
	Quercus pedunculiflora	1	279	2/2
	Quercus petraea	1	279	2/2
	Fagus sylvatica	-	-	0/2
Qr6783	*Quercus ellipsoidalis*	3	260-266	4/5
	Quercus georgiana	6	260-278	5/5
	Quercus robur	5	239-266	5/5
	Quercus alba	3	257-266	2/2
	Quercus pedunculiflora	3	251-257	2/2
	Quercus petraea	2	242-257	2/2
	Fagus sylvatica	-	-	0/2
Qr6859	*Quercus ellipsoidalis*	1	255	5/5
	Quercus georgiana	2	255-262	5/5
	Quercus robur	2	255-258	5/5
	Quercus alba	1	255	2/2
	Quercus pedunculiflora	2	255-258	2/2
	Quercus petraea	1	255	2/2
	Fagus sylvatica	1	255	2/2

(e.g., Lind & Gailing 2013, Sullivan et al. 2013, Lalagüe et al. 2014, Müller & Finkeldey 2016). The STRUCTURE analysis and, at a lower level, the PCoA, showed a separation of population FC-A from the other two populations, HMR-IH and MTU-1. The reason for this differentiation remains open.

The transferability of the reported EST-SSR markers in *Q. rubra* to six other oak species and European beech was successfully tested. In general, a high transferability of EST-SSRs is expected among taxa (Ellis & Burke 2007). In agreement with this, all loci were successfully transferred to all tested oak species (except marker Qr3552 to *Q. robur*). Thus, the amplification success was generally not lower in oaks belonging to section *Quercus* (*Q. alba*, *Q. robur*, and *Q. pedunculiflora*), despite the markers were transferred from *Q. rubra*, which belongs to section *Lobatae*. The transferability to *F. sylvatica*, however, was lower. In total, 13 of 20 markers (65 %) were transferable to this species. The transferability rates are in agreement with other studies. For instance, Steinkellner et al. (1997) found transferability rates for SSRs developed in *Q. petraea* of 100 % for *Q. robur* and *Q. pubescens* Willd., of 64 % for *Quercus cerris* L., of 47 % for *Quercus palustris* Münchh., *Q. rubra* and *Castanea sativa* Mill., and of 24 % for *F. sylvatica*. Barreneche et al. (2004) found that SSRs developed for *Q. rubra*, *Q. petraea*, and *Quercus macrocarpa* Michx, showed 100 % transferability to *Q. robur*, and a transferability of 70 % to *C. sativa*.

The reported set of 20 polymorphic EST-SSRs can be used in future population genetic studies. Since all EST-SSRs were successfully annotated (Table 1), they might be especially useful for the investigation of adaptive processes in *Q. rubra*.

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Supporting Information

The online version of the article includes Supporting Information:

Table S1 Primers that did not amplify in *Q. rubra*

Figure S1 Plots of (a) ΔK and (b) log likelihood for each K