Plant Mol Biol Rep (2013) 31:231–239
DOI 10.1007/s11105-012-0495-6

ORIGINAL PAPER

Development and Characterization of Genomic and Gene-Based Microsatellite Markers in North American Red Oak Species

Alexis R. Sullivan · Jennifer F. Lind · Tim S. McCleary · Jeanne Romero-Severson · Oliver Gailing

Published online: 31 July 2012

Abstract Oaks (*Quercus*: Fagaceae) are ecological and economic keystones of many forested ecosystems but effective genetic management strategies are hindered by high levels of phenotypic plasticity within species and frequent hybridization among them. These same features, however, make oak communities suited for the study of speciation, hybridization, and genetic adaptation. Efforts to develop new and to adapt existing genomic resources to less-studied members of this genus should not only improve oak conservation and management but also aid the study of fundamental evolutionary processes. Here, we present a suite of 27 highly polymorphic simple sequence repeat (SSR) markers tested in four North American red oak (*Quercus* section *Lobatae*) species: *Q. rubra*, *Q. ellipsoidalis*, *Q. coccinea*, and *Q. velutina*. Five markers are genomic SSRs (gSSRs) — four novel and one previously transferred from *Q. petraea* — and 22 are gene-based SSRs derived from *Q. robur* and *Q. petraea* expressed sequence tags (EST-SSRs). Overall, levels of polymorphism detected with these primer pairs were high, with gene diversity (H_e) averaging 0.66 across all loci in natural populations. In addition, we show that EST-SSR markers may have the potential to detect divergent selection at stress-resistance candidate genes among closely related oak species.

Keywords Molecular markers · Expressed sequence tags · Microsatellites · *Quercus* · Red oaks · SSRs · Transferability · Outlier loci · Divergent selection

Introduction

Oaks (*Quercus*: Fagaceae) comprise the most economically important hardwood species in North America, Europe, and the Russian Federation (UNECE/FAO 2011). Ecologically, oaks drive the population dynamics of other organisms (McShea et al. 2007) and can influence interactions between disjunct trophic levels (e.g., Ostfeld et al. 1996; Forkner and Hunter 2000; Clotfelter et al. 2007) through pulses in leaf, bud, and mast availability. Increased exploitation coupled with poorly understood environmental changes have led to the apparent decline of many oak species, but insufficient data exists to accurately assess the conservation status of the majority of oak taxa (Oldfield and Eastwood 2007). Efforts to manage oak gene pools have been hampered by their characteristic phenotypic plasticity (Blue and Jensen 1988; Bruschi et al. 2000) and complex patterns of genetic diversity (e.g., Kremer et al. 2002; Curtu et al. 2007a). Sympatric oaks retain distinct species identities despite relatively common intra-sectional hybridization (reviewed by Aldrich and Cavender-Bares 2011). Furthermore, oak taxa frequently exhibit weak interspecific differentiation at molecular markers (e.g., Bodénès et al. 1997; Mariette et al. 2002; Coart et al. 2002; Aldrich et al. 2003a). Although the often unresolved taxonomies within oak sections complicate their management, this attribute also makes sympatric *Quercus* communities well suited for the investigation of speciation, hybridization, and the molecular basis of adaptation (e.g., Scotti-Saintagne et al. 2004). Understanding patterns of gene flow and genetic diversity within and among species should not only improve oak conservation and management

Electronic supplementary material The online version of this article (doi:10.1007/s11105-012-0495-6) contains supplementary material, which is available to authorized users.

A. R. Sullivan · J. F. Lind · O. Gailing (✉)
School of Forest Resources and Environmental Science,
Michigan Technological University,
Houghton, MI 49931, USA
e-mail: ogailing@mtu.edu

T. S. McCleary · J. Romero-Severson
Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556, USA

but also help to elucidate fundamental evolutionary processes in natural populations.

Until recently, most studies have focused on the European white oaks (*Quercus* section *Quercus*). Now, a growing number of genomic resources for *Quercus* in general have rapidly advanced our understanding of the genetic structure in red oaks (*Quercus* section *Lobatae*), a group indigenous to the Americas (Aldrich and Cavender-Bares 2011). Isozyme and dominant markers can detect patterns of genetic diversity within and among the red oaks (e.g., Guttman and Weigt 1989; Hokanson et al. 1993; Sork et al. 1993; Tomlinson et al. 2000), but the higher information content of simple sequence repeats (SSRs; also microsatellites) have made them the markers of choice in out crossing forest trees. SSR markers are co-dominant, easily reproduced and scored, abundant throughout the genome, and highly polymorphic (Zane et al. 2002). Expressed sequence tags (ESTs) are an increasingly important source of SSRs (EST-SSRs), principally because they are easily developed from public databases (e.g., Zhu et al. 2012). Additionally, EST-SSRs show promise as functional markers (Bouck and Vision 2007). By virtue of their frequent occurrence in gene-rich regions of the genome, the functional identity of an EST-SSR locus can often be determined through comparative analysis of protein databases. Designing primers around SSRs in ESTs associated with a gene of interest may allow for the direct characterization of adaptive genetic variation (Vasemägi et al. 2005; Bouck and Vision 2007). Despite these advantages, genomic SSRs (gSSRs), which normally occur in non-coding regions, may be more appropriate for analyses that assume selective neutrality, such as migration rate and population size estimates, along with other demographic inferences.

Previously, several gSSR markers have been developed in *Q. rubra* (14 loci, Aldrich et al. 2002; 16 loci, Aldrich et al. 2003b) or transferred to this and other red oaks from section *Quercus* (Fernández et al. 2000; Steinkellner et al. 2003). Largely replacing the use of isozymes, these markers have been used, for example, to study: hybridization and post-zygotic selection (Moran et al. 2012); demographic and spatial genetic structure (Aldrich et al. 2005); loss of genetic diversity following historic deforestation (Gerwein and Kesseli 2006); and the management implications of morphologically determined species assignments (Aldrich et al. 2003a). While the number of available gSSR loci may be sufficient for many analyses, additional markers will help elucidate the genomic structure of section *Lobatae* taxa through the creation of dense genetic linkage maps. These maps can be applied to comparative studies of genomic structure, shedding light on the processes shaping genome evolution (e.g., Barreneche et al. 2004) and to the identification of candidate genes underlying important traits through quantitative trait locus (QTL) mapping.

To our knowledge, no published set of EST-SSR markers for section *Lobatae* exists, despite the abundance of untested primer sequences (http://fagaceae.org/markers). As functional markers, they may allow characterization of variation at loci involved in stress responses and flowering time, traits of particular interest because of their potential role in speciation. These same traits are also of interest to managers establishing germplasm banks, implementing reforestation and restoration programs, and delineating seed orchard zones.

We present a panel of 27 genomic and EST-derived SSR markers for the red oaks. Of these, 22 are EST-SSRs adapted from *Q. robur* (Durand et al. 2010), four are novel gSSRs developed from *Q. rubra* (northern red oak), and one is a newly characterized, but previously adapted, gSSR from *Q. petraea* (Steinkellner et al. 2003). We report genetic diversity metrics for these markers in natural populations of *Q. rubra*, *Q. ellipsoidals*, *Q. velutina*, and *Q. coccinea* and evaluate the ability of EST-SSRs to detect divergent selection at loci with known or putative functions in the red oaks.

Material and Methods

In brief, we developed four novel gSSRs from an enriched genomic library and assessed the transferability of 90 EST-SSRs to section *Lobatae*. Markers were assessed for Mendelian segregation patterns using the parents and seven full-sibs from a *Q. rubra* mapping population. To assess the ability of these markers to detect differentiation between a notoriously taxonomically difficult species, *Q. ellipsoidalis*, and species with which it forms putative hybrids, *Q. rubra* (Hipp and Weber 2008), we characterized the four novel gSSRs, a previously adapted gSSR (QpZAG15; Steinkellner et al. 2003), and four of the EST-SSRs in a *Q. rubra* and *Q. ellipsoidalis* population pair. The remaining 18 EST-SSRs were characterized in population samples of *Q. rubra*, *Q. ellipsoidalis*, *Q. coccinea*, and *Q. velutina.* Among these four interfertile species, we considered markers with F_{ST} values significantly deviating from neutral expectations as potentially under selection (Beaumont and Nichols 1996; Beaumont 2005).

Plant Material

A *Q. rubra* mapping family comprising about 500 full-sibs was identified from the progeny of an open-pollinated tree (SM1) located on the Purdue University campus in West Lafayette, Indiana, USA. Paternity exclusion analysis was conducted in program CERVUS (Slate et al. 2000) using all 29 potential male parents located within a 200-m radius of the female parent. Potential male parents were genotyped at six previously published gSSR loci (Aldrich et al. 2002) to

identify probable full-sib progeny. Subsequent analysis using 13 to 18 additional gSSRs markers (Aldrich et al. 2002, 2003b) produced genotypes consistent with the initial determination and excluded all but one candidate male parent (Romero-Severson et al. unpublished results). The two crossing parents (SM1, female parent; SM2, male parent) and seven full-sibs were used for the initial screening of transferred SSRs and for an assessment of Mendelian segregation patterns in all presented markers.

In order to characterize the markers in natural populations, leaves were collected from a total of 210 individuals comprising six populations and four species: 26 from a pure *Q. rubra* stand in the Nicolet-Chequamegon National Forest, Wisconsin (45°20′N, 88° 23′W), 49 from a *Q. rubra* stand and 77 from a neighboring *Q. ellipsoidalis* stand both located in the Ford Research Forest, Michigan (46°39′N, 88°30′W), 18 from a *Q. ellipsoidalis* population in northern Wisconsin (44°40′N, 88°5′W), 20 from a *Q. velutina* population in the Manistee National Forest, Michigan (43°26′N, 85°37′W), and 20 from a *Q. coccinea* population in the Shawnee National Forest, Illinois (37°36′N, 88°16′W). Species identities were previously determined through genetic admixture analyses using either AFLP markers in the case of the *Q. ellipsoidalis* population located in Wisconsin and the *Q. velutina* and *Q. coccinea* populations (Hipp and Weber 2008) or seven EST-SSR and eight gSSR markers for the remaining populations (Lind and Gailing, submitted).

Development of gSSRs for *Q. rubra*

Total genomic DNA extracted from a single *Q. rubra* individual was sent to Genetic Identification Services (Chatsworth, CA, USA) for the construction of microsatellite libraries enriched in $(CA)_n$ repeats. Enriched genomic fragments of 350–700 bp were ligated into the pUC19 vector, electrotransformed into *Escherichia coli* strain DH5α cells, and cultured on selective medium. Positive clones enriched for $(CA)_n$ repeats were amplified using M13 universal primers and sequenced.[1] Primer pairs were designed in consensus sequences with the program PRIMER3 v. 0.4.0 (Rozen and Skaletsky 2000) and optimized following the methods of Aldrich et al. (2002).

DNA Analysis

Total genomic DNA was isolated from ~1 cm^2 of frozen leaf tissue using the DNeasy® 96 Plant Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Polymerase chain reactions (PCR) were carried out in a 10-µl solution comprising: 2 µl of 5× HOT FIREPol® Blend Master Mix Ready to Load containing 10 mM $MgCl_2$, 0.4 U of HOT FIREPol® *Taq* polymerase, and 2 mM dNTPs (Solis BioDyne, Estonia), 10 pmol of each primer (Sigma Aldrich, St. Louis, MO and Applied Biosystems, Foster City, CA), 2.5 µl deionized RNase-free water, and 1.5 µl genomic DNA (~1.8 ng/µl). The PCR profile consisted of an initial denaturation at 95 °C for 15 min followed by 35 cycles of 94 °C for 45 s, annealing at T_a (Table 1) for 45 s, and 72 °C for 45 s. Final extension of amplified DNA occurred at 72 °C for 20 min. All PCR reactions were carried out in a Peltier Thermal Cycler (GeneAmp® PCR system 2700, Applied Biosystems).

Marker Transferability Tests

Ninety EST-SSRs (Durand et al. 2010) developed in *Q. robur* were selected based on linkage group position (Durand et al. 2010), associated gene function, and annealing temperature for screening in two *Q. rubra* mapping parents. Putative functions associated with EST-SSR markers were obtained by performing a homology search of the translational products of reassembled ESTs (Durand et al. 2010) against gene family members in the nonredundant (nr) NCBI database using the BLASTx algorithm (Altschul et al. 1997). Results with expect-values $\leq 10^{-4}$ and with similarity over >55 % of the sequence were considered significant.

Markers were first screened in the two *Q. rubra* mapping parents to test for the presence of amplicons. PCR products were separated on 2.5 % agarose gels and stained with ethidium bromide. Primer pairs producing visible amplicons within the expected size range were amplified in seven full-sibs and resolved electrophoretically on the QIAxcel System using the QIAxcel DNA High Resolution Kit (Qiagen) to assess polymorphisms and Mendelian segregation patterns. Primer pairs were considered transferable and polymorphic if: (1) at least two different alleles were detected in the mapping parents, (2) only parental fragments were observed in the offspring, and (3) if allelic fragments were easily reproduced and interpreted. Of the successfully transferable pairs, 22 EST-SSRs were selected for characterization in population samples on the basis of linkage group position and putative function. Primer sequences, repeat motifs, linkage group position in *Q. robur* (Durand et al. 2010), annealing temperatures, observed size ranges, and putative protein products are presented in Table 1.

Marker Characterization

All five gSSR markers and four EST-SSRs (PIE040, GOT004, FIR004 and GOT021) were characterized in the *Q. rubra*–*Q.*

[1] Sequence data have been deposited in the GenBank, DDBJ Nucleotide Sequence Database and EMBL databases under the accession numbers JQ929659 (locus 1P10), JQ929660 (2P24), JQ929661 (3A05), and JQ929662 (3D15).

Table 1 Characteristics of 26 simple sequence repeat (SSR) markers

Locus	Repeat motif	L.G.	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	T_a (°C)	Range (bp)	BLASTx sequence description
1P10	$(TG)_{12}GCC(TG)_3$	–	atttctgatgcagggtgtcg	taggccaaggaccagagacc	62	237-265	–
2P24	CA_{14}	–	gcaagagatcacacacaaactagc	ctttgggttcaccaaacagc	62	136–164	–
3A05	$CA_{11}CT_2$	–	aacgtgacctctctcacagc	agtgctggagtgctcatgg	62	138–160	–
3D15	CA_{15}	–	ggtggtggcagatacactgg	gactcagacaaccaacttcagg	62	208–236	–
FIR004	CT_{18}	3	tctctctcagggcagcttct	aaccaaactcagatccagattca	59	128–180	No hit
FIR024	CCT_6	5	cgcttctcctcatcctcaag	ctcaaaaggcacgattctcc	56	214–229	DNA gyrase subunit a
FIR030	AG_7	7	ggacatatttatctaggagacgaggt	atgtcccatagcacagagca	56	161–179	NADH dehydrogenase
FIR031	TC_7	11	acgagtccaacggaagttgt	cacaacttcacaaggcaagg	56	139–174	5-Adenylylsulfate reductase
FIR039	CT_7	1	gagcctctttcatcgctcac	tcaacaccccaaaactccat	56	109–123	Histone deacetylase
FIR048	CT_9	2	tgcaccaaaattggaggatg	ttgatgcaaggtgcagtttc	56	187–219	Cell division protein
FIR053	GTG_7	5	agtttccccacatttgttgc	taccatgcaccaagcaattc	56	126–150	Glutaredoxin c9
FIR104	GGT_7	11	ttaactcggtttgcgactca	agcacgtgactcgacctgta	56	206–221	r2r3-myb transcription factor
FIR110†	AG_{12}	6	acttgctcgcttcaaccttc	attcctcctcatcaggctca	56	191–239	Protein transport protein sec23
GOT004	TG_{12}	7	gggcatattgatcgcttagg	tgacgattcatacattccatgat	59	266–292	Tonoplast intrinsic protein
GOT009	TC_7	12	cacctcactaagcaacctgtca	ttttggaggcggagataatg	56	228–248	Uncharacterized protein
GOT021	AT_{13}	3	agaaagttccagggaaagca	cttcgtccccagttgaatgt	59	95–101	Histidine kinase 4-like
GOT037	CT_{11}	5	ccatccttttcattctttcca	tgttgttgttgctgttgtcg	56	239–265	5-Nucleotidase sure-like
GOT040	GA_{11}	6	aaggcactcgtcgctttcta	accgatttgaagctcgagaa	56	234–254	40s ribosomal protein s16
PIE039	CTT_8	12	cctcaccctctgcggtct	cagaaagggctgcaaagc	56	157–178	Uncharacterized protein
PIE040	TTC_8	–	gtgagagagagagagacaaa gaagaaaaa	aaattctccgccacattgag	59	155-174	Basic leucine zipper transcription factor-like protein
PIE099	TC_9	8	gtaaaacgacggccagtgtggc taccgactactaccacttc	cggtggacccaatatgtaac	56	178-202	Uncharacterized protein
PIE125	$GGAAGC_3$	6	aatacaaatcgcaggaggtg	ctaacccatcgttcatggag	56	147-162	dnaj-like protein
POR003	CT_8	3	ctcgctctcctcccctaatc	agctttgatcgagtccgaaa	56	91-113	Uncharacterized protein
POR016	GGT_6	6	agcaacagcagagccaaaat	cagcggctttgaggtaattc	56	115-119	Tetratricopeptide repeat-like
VIT057	$AACTC_3$	9	tcagcaaaatcccaactttgt	acacttcgctgttcctcgat	56	133-147	Ethylene-responsive transcription factor-like
VIT107	TA_{13}	3	tgatcacagattggagcttaaca	cccccacttaggaaagaagc	56	127-139	lhca2 protein

The first five markers are novel genomic SSRs while the remainder are gene-based EST-SSR markers developed in *Q. robur* (Durand et al. 2010) and transferred to section *Lobatae*. L.G. denotes the linkage group on which the EST-SSR is located in *Q. robur* (Durand et al. 2010). BLASTx descriptions are reported only for sequences with expect values $\leq 10^{-4}$ and with similarity over >55 % of the sequence

ellipsoidalis population pair originating from the Ford Research Forest, Michigan. The remaining 18 EST-SSR markers were characterized in the *Q. rubra* and *Q. ellipsoidalis* populations originating in Wisconsin and the *Q. velutina* and *Q. coccinea* populations. Population samples were amplified using fluorescently labeled primers and resolved on an ABI Prism® Genetic Analyzer 3730 using the GeneScan™ LIZ-500 internal size standard (Applied Biosystems). All fragments were scored using GeneMapper® v. 4.0 and PeakScanner™ Software v. 1.0 (Applied Biosystems).

Data Analysis and Interpretation

Allelic richness was calculated using program HP-RARE v. 1.0 and rarefacted to the smallest population size (n=18, Wisconsin *Q. ellipsoidalis* population) to allow for comparisons among unequal sample sizes (Kalinowski 2004, 2005). Observed and expected heterozygosities (H_o, H_e) were calculated by locus for each population separately. The inbreeding coefficient F_{IS} was computed following standard ANOVA (Weir and Cockerham 1984) and tested for significant deviation from Hardy–Weinberg proportions

using the Markov chain (MC) algorithm with the default parameters as implemented in program GENEPOP v. 4.0.10 (Raymond and Rousset 1995). Population differentiation was estimated by F_{ST} (Weir and Cockerham 1984) and assessed for significant deviation from zero using the exact G test in GENEPOP v. 4.0.10 (Raymond and Rousset 1995). MICROCHECKER v. 2.2.3 was employed to check for potential genotyping errors arising from null alleles, large allele drop-out, and stuttering (van Oosterhout et al. 2004). Bonferroni adjustments were applied to p values to correct for multiple comparisons (Rice 1989).

Divergent selection among the four red oak species was inferred though pairwise analysis of variance in F_{ST} estimates following the approach of Beaumont and Nichols (1996) as implemented in LOSITAN (Antao et al. 2008). This method of outlier loci detection simulates an expected distribution of F_{ST} as a function of expected heterozygosity under an island model of migration. Loci more strongly differentiated than expected under neutral conditions were considered potentially under divergent selection (Beaumont and Nichols 1996; Beaumont 2005). First, we conducted 50,000 coalescent simulations assuming a stepwise mutational model to detect loci potentially under selection. Loci falling outside the 95 % confidence envelope were removed and F_{ST} was recomputed in order to obtain the best approximation of mean neutral F_{ST} (Beaumont and Nichols 1996). Then, we reran the simulations using all loci and the approximated mean neutral F_{ST} to identify the final panel of candidate loci potentially under divergent selection.

Results and Discussion

Transferability of Markers

Of the 90 EST-SSR primer pairs tested, 81 % [73] amplified a single product of expected size and 42 % [38] showed polymorphism in at least one *Q. rubra* mapping parent (see online supplementary material). While the percentage of *Q. robur*-derived EST-SSRs producing allelic amplicons was high and comparable to values reported for other intrageneric transfers (reviewed by Ellis and Burke 2007), the percentage of polymorphic, and therefore useful, markers was closer to the transferability rates observed in gSSRs (Steinkellner et al. 2003; Fernández et al. 2000; Aldrich et al. 2003b; Barreneche et al. 2004). While using more than two individuals for the initial marker screening may have detected more polymorphic primer pairs, our method yielded a panel of EST-SSRs (N_a=7, rarefacted to 18 individuals), on average, as multiallelic as the novel gSSRs (N_a=7).

Assessment of Mendelian segregation of EST-SSR products in the seven *Q. rubra* full-sibs revealed a null allele at locus GOT037, as inferred from the homozygous genotypes of two progeny that can only be explained by the presence of a null allele in a crossing parent. However, null alleles are apparently rare in natural populations for this marker: F_{IS} values showed no significant deviation from Hardy–Weinberg equilibrium, suggesting that the number of genotyping errors made due to null alleles were low. Furthermore, the MICROCHECKER analysis did not find evidence for the presence of null alleles at locus GOT037 in any of the four characterized populations. The FIR110 primer pair amplified two regularly segregating loci. For these two loci, an overlap in allele sizes of 1–6 (mean=3, SD=2) bp was observed in the six characterized populations, which could lead to genotyping errors due to erroneous assignment of fragments to loci. Indeed, the MICROCHECKER analysis indicated possible genotyping errors leading to an apparent excess of homozygotes at this marker in the *Q. velutina* and Wisconsin *Q. ellipsoidalis* populations but not in the *Q. coccinea* or Wisconsin *Q. rubra* populations.

Marker Characterization

Mean observed heterozygosity across all loci and populations was moderate (H_o=0.65; Tables 2 and 3) and within the range reported for gSSRs characterized in other oak communities (e.g., Curtu et al. 2007b). Genomic SSRs detected significantly higher levels of genetic diversity than EST-SSRs (H_e=0.79, 0.65, respectively; two-tailed *t*-test; p <0.05), a result commonly reported in other studies (e.g., Liewlaksaneeyanawin et al. 2004). Expected levels of heterozygosity were not as consistently high at EST-SSR loci, with H_e ranging from 0.04 to 0.92 versus 0.64 to 0.86 for gSSRs (Tables 2 and 3). Mean expected levels of heterozygosity at EST-SSR loci for all six red oak populations (mean H_e=0.65) were lower than reported for *Q. petraea* (Durand et al. 2010), a species closely related to the source species, *Q. robur* (section *Quercus*). Reduced polymorphism in transferred SSRs is expected because portable markers tend to be located in genomic regions less tolerant of mutation (e.g., Castillo et al. 2010).

After applying Bonferroni corrections, two gSSR loci (2P24 and 3A05) and two EST-SSR loci (FIR004 and GOT004) showed significant deviations from Hardy–Weinberg proportions in the adjacent *Q. rubra–Q. ellipsoidalis* population pair (Table 2; p<0.05). Apparent homozygote excess may result from technical problems with primer pairs, such as point mutations in the annealing sites leading to null alleles and allelic dropout (Pompanon et al. 2005). Consistent with the presence of such technical artifacts, analysis with MICROCHECKER indicated the possibility of null

Table 2 Genetic variation at nine microsatellite loci as characterized in a *Q. rubra* and *Q. ellipsoidalis* population pair located in the Ford Forestry Center, Michigan

		Q. rubra				*Q. ellipsoidalis*			
Locus	F_{ST}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}
Parameter estimates by locus and population									
3D15	**0.11**	9	0.71	0.84	0.16	5	0.80	0.69	−0.15
1P10	0.02	9	0.82	0.83	0.03	9	0.75	0.80	0.07
2P24	**0.05**	7	0.61	0.83	**0.27**	8	0.61	0.81	**0.26**
3A05	**0.21**	7	0.50	0.75	**0.34**	5	0.44	0.64	0.32
QpZAG15	**0.06**	10	0.76	0.86	0.13	11	0.82	0.84	0.03
PIE040	0.01	7	0.57	0.66	0.14	5	0.70	0.67	0.04
GOT004	**0.04**	8	0.63	0.78	**0.21**	8	0.59	0.84	**0.31**
FIR004	**0.02**	12	0.52	0.89	**0.42**	15	0.68	0.92	**0.27**
GOT021	**0.29**	2	0.55	0.44	−0.25	2	0.04	0.04	−0.01
Parameter estimates by population									
	0.08	8	0.63	0.76	**0.16**	8	0.60	0.69	**0.13**

For each locus, the following parameters were calculated: pairwise genetic differentiation (F_{ST}), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficents (F_{IS}), and number of alleles rarefacted to 18 individuals (N_a). Mean values of H_e, H_o, N_a and F_{IS} were calculated across all loci for each species. Mutlilocus F_{ST} values are given as population-level estimates of population differentiation. Values in bold indicate significant (α=0.05) deviations from zero after applying Bonferroni corrections

alleles at these loci. However, further characterization of these two populations using these and an additional three EST-SSRs and three gSSRs indicated a significant deviation from Hardy–Weinberg proportions in both these populations due to homozygote excess (Lind and Gailing, submitted; HW exact test $p<0.05$). Because the null allele estimation algorithms employed by MICROCHECKER are not applicable to populations deviating from panmixia (van Oosterhout et al. 2006), we conclude that homozygote excess at these markers predominately reflects the population structure rather than null alleles or allelic dropout.

Conversely, locus VIT107 significantly deviated from Hardy–Weinberg proportions due to heterozygote excess (F_{IS}=−0.50; Table 3) in the *Q. coccinea* population. Additionally, F_{IS} estimates for this locus were relatively low, although nonsignificant, in the *Q. rubra*, *Q. ellipsoidalis*, and *Q. velutina* populations. F_{IS} estimates for locus POR016 (F_{IS}=−0.62 to −0.11; Table 3) were also low in all populations, although the null hypothesis of random union of gametes could not be rejected. Markers exhibiting such F_{IS} patterns may be located in or linked to a gene subject to some form of balancing selection (Charlesworth 2006). We suggest these preliminary results provide justification for further investigation of the mechanisms shaping polymorphism at these loci.

The suite of 18 EST-SSR markers characterized in *Q. rubra*, *Q. ellipsoidalis*, *Q. velutina*, and *Q. coccinea* revealed similar levels of genetic diversity among all four species (Table 3; average H_e=0.65, SD=0.03). The panel of four EST-SSRs and five gSSRs applied to the *Q. rubra–Q. ellipsoidalis* population pair suggested higher levels of genetic diversity (average H_e=0.73, SD=0.05), consistent with the higher levels of polymorphism detected by gSSRs (Table 2). Mean N_a was consistent among all six populations, ranging from an average of six to eight alleles per locus (Tables 2 and 3).

Genetic differentiation between the neighboring *Q. rubra* and *Q. ellipsoidalis* populations was low but significant (F_{ST}=0.08, $p<0.05$). Seven of the nine loci were significantly differentiated (Table 2), but pairwise differentiation varied by locus from low (PIE040, F_{ST}=0.01) to relatively high differentiation (GOT021, F_{ST}=0.29). EST-SSRs were not more strongly differentiated than gSSRs (two-tailed *t*-test, $p>0.05$). In the *Q. rubra, Q. ellipsoidalis*, *Q. coccinea*, and *Q. velutina* populations characterized with the panel of 18 EST-SSRs (Table 3), significant differentiation was observed at 89 % [16] of loci and overall differentiation was relatively low but significant (F_{ST}=0.01, $p<0.05$).

Some heterogeneity in F_{ST} estimates across loci is expected due to the random effects of genetic drift, but this pattern could also result from selection on some loci. When accompanied by differential reduction in genetic variation — as observed at loci GOT021 (Table 2), FIR039, and FIR053 (Table 3) — such a pattern may indicate a selective sweep, that is, a reduction in diversity in the flanking regions of beneficial mutations due to strong and recent selection towards different optima (Maynard Smith and Haigh 1974). Selective sweeps have been reported in the European white oak species *Q. robur* and *Q. petreaea* (e.g., Scotti-Saintagne et al. 2004), including at a locus mapping to leaf QTLs

Table 3 Characterization of 18 EST-SSR loci in *Q. rubra*, *Q. ellipsoidalis*, *Q. coccinea*, and *Q. veultina* populations

		Q. rubra				*Q. ellipsoidalis*				*Q. coccinea*				*Q. velutina*			
Locus	F_{ST}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}
parameter estimates by locus and population																	
FIR024	**0.14**	5	0.52	0.55	0.08	3	0.67	0.53	−0.24	5	0.42	0.47	0.13	4	0.47	0.53	0.13
FIR030	**0.05**	7	0.46	0.50	0.10	4	0.61	0.57	−0.04	3	0.50	0.64	0.24	5	0.60	0.54	−0.08
FIR031	**0.08**	13	0.88	0.85	−0.02	13	0.78	0.84	0.10	7	0.68	0.79	0.16	11	0.80	0.84	0.08
FIR039	**0.25**	5	0.54	0.73	0.28	5	0.39	0.45	0.16	3	0.70	0.52	−0.31	3	0.20	0.19	−0.06
FIR048	**0.03**	12	0.77	0.84	0.10	14	0.89	0.89	0.03	11	0.95	0.87	−0.07	10	0.90	0.83	−0.05
FIR053	**0.15**	5	0.69	0.71	0.05	4	0.44	0.37	−0.17	3	0.50	0.56	0.13	5	0.75	0.69	−0.06
FIR104	**0.15**	3	0.38	0.38	−0.01	3	0.72	0.57	−0.23	3	0.55	0.64	0.16	5	0.60	0.67	0.13
FIR110	**0.05**	10	0.88	0.84	−0.03	11	0.61	0.88	0.33	8	0.85	0.78	−0.07	11	0.70	0.89	0.23
FIR110†	**0.14**	7	0.81	0.76	−0.04	11	0.67	0.74	0.12	15	0.90	0.91	0.04	7	0.45	0.46	0.05
GOT009	**0.08**	10	0.77	0.84	0.11	7	0.72	0.66	−0.07	6	0.65	0.65	0.02	8	0.60	0.77	0.25
GOT037	**0.03**	12	0.96	0.88	−0.07	9	0.72	0.84	0.17	12	0.95	0.90	−0.03	9	0.85	0.80	−0.03
GOT040	**0.17**	7	0.73	0.73	0.02	3	0.17	0.16	−0.04	2	0.05	0.05	−0.03	6	0.45	0.48	0.08
PIE039	**0.06**	6	0.69	0.69	0.02	4	0.67	0.68	0.04	6	0.50	0.51	0.05	7	0.60	0.58	−0.01
PIE099	**0.03**	12	0.85	0.89	0.07	9	0.67	0.81	0.21	14	0.90	0.89	0.02	11	0.65	0.80	0.21
PIE125	**0.18**	5	0.60	0.68	0.14	3	0.44	0.50	0.13	4	0.60	0.52	−0.13	3	0.50	0.49	−0.01
POR003	**0.04**	11	0.85	0.83	−0.01	10	0.89	0.84	−0.03	11	1.00	0.88	−0.11	11	0.95	0.82	−0.13
POR016	0.04	2	0.35	0.29	−0.19	2	0.78	0.48	−0.62	3	0.60	0.43	−0.36	5	0.55	0.45	−0.21
VIT057	0.03	3	0.27	0.30	0.13	4	0.50	0.54	0.11	4	0.45	0.50	0.13	4	0.55	0.56	0.04
VIT107	**0.08**	7	0.88	0.78	−0.11	6	0.83	0.73	−0.11	5	0.95	0.63	**−0.50**	5	0.95	0.66	−0.42
parameter estimates by population																	
	0.01	7	0.70	0.70	0.03	7	0.60	0.60	−0.01	6	0.67	0.60	−0.03	7	0.60	0.60	0.01

Genetic differentiation (F_{ST}), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficents (F_{IS}), and number of alleles rarefacted to 18 individuals (N_a) were calculated for each locus. Population-level diversity parameters were calculated means across all loci for H_e, H_o, N_a, and F_{IS} and as a mutlilocus estimate for F_{ST}. Values in bold indicate significant (α=0.05) deviations from zero after applying Bonferroni corrections for multiple comparisions

differentiating between the two species (Muir and Schlötterer 2005). The significance of this relationship between F_{ST} and heterozygosity can be determined through simulation-based outlier tests, such as that of Beaumont and Nichols (1996). Using this method of selection detection, our screen of all 27 markers indicated that GOT021, FIR039 and FIR053 were significantly more differentiated than expected under neutral expectations in at least one pairwise assessment. All three of these potential outlier loci are associated with genes involved in abiotic stress resistance, a result consistent with the distribution of these four *Lobatae* species along water and nutrient gradients.

Locus FIR039 is putatively associated with a gene encoding a HD2-class histone deacetylase (Table 1), a class of enzymes involved in drought stress responses (Sridha and Wu 2006) through the post-translational modification of histone tails. Allele frequencies at this locus were potentially differentially favored between *Q. rubra* and *Q. velutina* (F_{ST}=0.25, neutral F_{ST}=0.09, p<0.05), which are among the least and most drought-tolerant, respectively, of the eastern North American red oaks (Abrams 1990). Similarly, locus GOT021, putatively encoding a protein similar to the stress-responsive histidine kinase-4 (Tran et al. 2007), appeared to be under divergent selection between *Q. ellipsoidalis*, the most tolerant of drought and nutrient stress of all the red oaks (Abrams 1988), and the more mesic *Q. rubra* (F_{ST}=0.27, neutral F_{ST}=0.08, p<0.05). Locus FIR053, associated with a gene encoding a glutaredoxin-like protein (Table 1), was an outlier locus between *Q. ellipsoidalis* and *Q. coccinea* (F_{ST}=0.35, neutral F_{ST}=0.08, p<0.05). Gluteradoxins are important in drought and salinity responses through their role in antioxidative response systems (Guo et al. 2010). Both *Q. ellisoidalis* and *Q. coccinea* are found on relatively xeric sites (Abrams 1990), but to our knowledge no comparison of the antioxidative systems of these species exists.

Simultaneous detection of outlier loci using multiple selection detection strategies (e.g., Vasemägi et al. 2005) and incorporation of additional populations from each species would reduce the risk of false positives. Nevertheless, we demonstrate that outlier screens of EST-SSRs may provide

one way to assign putative functions to genomic regions under divergent selection in the red oaks. Further investigation of sequence variation among oak species at these three drought response candidate genes is warranted.

Our initial evaluation of a panel of EST-SSR and gSSR markers developed from heterologous sources within the *Quercus* genus demonstrates that both marker types perform well within section *Lobatae*. These highly polymorphic, multiallelic loci will be useful in the study of population dynamics and adaptive variation in the red oaks of North America.

Acknowledgements We thank James Schmierer for his help in sample collection in the Ford Forestry Center and Dr. Andrew Hipp for providing *Q. ellipsoidalis*, *Q. coccinea* and *Q. velutina* samples. Additionally, we thank John Lampereur and Linda Parker for kindly guiding us to *Q. rubra* stands in the Nicolet-Chequamegon National Forest. We are also grateful to Erin Hickey who assisted in sample collection and marker analyses. Two anonymous referees provided helpful comments on an earlier draft. Funding for the study came from Michigan Technological University start-up funds to Oliver Gailing, the Michigan Technological University Research Excellence fund, the USDA McIntire Stennis fund, the Huron Mountain Wildlife Foundation, the Hanes Trust and the NSF Plant Genome Research program (NSF 1025974). We are also grateful to the Northern Institute of Applied Climate Science for their support.

References

Abrams MD (1988) Comparative water relationships of three successional hardwood species in central Wisconsin. Tree Physiol 4:263–273. doi:10.1093/treephys/4.3.263

Abrams MD (1990) Adaptations and responses to drought in *Quercus* species of North America. Tree Physiol 7:227–238. doi:10.1093/treephys/7.1-2-3-4.227

Aldrich PR, Cavender-Bares J (2011) *Quercus*. In: Kole C (ed) Wild crop relatives: Genomic and breeding resources, forest trees. Springer, Berlin, pp 89–129, doi: 10.1007/978-3-642-21250-5

Aldrich PR, Glaubitz JC, Parker GR, Rhodes OE, Michler CH (2005) Genetic structure inside a declining red oak community in old-growth forest. J Hered 96:627–634. doi:10.1093/jhered/esi115

Aldrich PR, Parker GR, Michler CH, Romero-Severson J (2003a) Whole-tree silvic identifications and the microsatellite genetic structure of a red oak species complex in an Indiana old-growth forest. Can J For Res 33:2228–2237. doi:10.1139/x03-160

Aldrich PR, Jagtap M, Michler CH, Romero-Severson J (2003b) Amplification of North American red oak microsatellite markers in European white oaks and Chinese chestnut. Silvae Genet 52:176–179

Aldrich PR, Michler CH, Sun WL, Romero-Severson J (2002) Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*). Mol Ecol Notes 2:472–474. doi:10.1046/j.1471-8278.2002.00282.x

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. BMC Bioinforma 8:323. doi:10.1186/1471-2105-9-323

Barreneche T, Casasoli M, Russell K, Akkak A, Meddour H, Plomion C, Villani F, Kremer A (2004) Comparative mapping between Quercus and Castanea using simple-sequence repeats (SSRs). Theor Appl Genet 108:558–566

Beaumont MA (2005) Adaptation and speciation: what can Fst tell us? Trends Ecol Evol 20:435–440

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proc Roc Soc B 263:1619–1626

Blue MP, Jensen RJ (1988) Positional and season variaion in oak (*Quercus*: Fagaceae) leaf morphology. Am J Bot 75:939–947. doi:10.2307/2443759

Bodénès C, Joandet S, Laigret F, Kremer A (1997) Detection of genomic regions differentiating two closely related oak species *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Heredity 78:433–444. doi:10.1038/hdy.1997.67

Bouck A, Vision T (2007) The molecular ecologist's guide to expressed sequence tags. Mol Ecol 16:907–924. doi:10.1111/j.1365-294X.2006.03195.x

Bruschi P, Vendramin GG, Bussotti F, Grossoni P (2000) Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (Fagaceae) in Northern and Central Italy. Ann Bot 85:325–333. doi:10.1006/anbo.1999.1046

Castillo A, Dorado G, Feuillet C, Sourdille P, Hernandez P (2010) Genetic structure and ecogeographical adaptation in wild barley (*Hordeum chilense* Roemer et Schultes) as revealed by microsatellite markers. BMC Plant Biol 10. doi:10.1186/1471-2229-10-266

Charlesworth D (2006) Balancing selection and its effects on sequences in nearby genome regions. PLoS Genet 2(4): e64. doi:10.1371/journal.pgen.0020064

Clotfelter ED, Pedersen AB, Cranford JA, Ram N, Snajdr EA, Nolan V, Ketterson ED (2007) Acorn mast drives long-term dynamics of rodent and songbird populations. Oecologia 154:493–503. doi:10.1007/s00442-007-0859-z

Coart E, Lamote V, De Loose M, Van Bockstaele E, Lootens P, Roldan-Ruiz I (2002) AFLP markers demonstrate local genetic differentiation between two indigenous oak species *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl in Flemish populations. Theor Appl Genet 105:431–439. doi:10.1007/s00122-002-0920-6

Curtu AL, Gailing O, Finkeldey R (2007a) Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. BMC Evol Biol 7:218. doi:10.1186/1471-2148-7-218

Curtu AL, Gailing O, Leinemann L, Finkeldey R (2007b) Genetic variation and differentiation within a natural community of five oak species (*Quercus* spp.). Plant Biol 9:116–126. doi:10.1055/s-2006-924542

Durand J, Bodénès C, Chancerel E, Frigero J-M, Vendramin GG, Sebastiani F, Buonamici A, Gailing O, Koelewijn H-P, Villani F, Mattioni C, Cherubini M, Goicoechea PG, Herran A, Ikaran Z, Cabane C, Ueno S, de Daruvar A, Kremer A, Plomion C (2010) A fast and cost-effective approach to develop and map EST-SSR markers: oak as a case study. BMC Genomics 11:570. doi:10.1186/1471-2164-11-570

Ellis JR, Burke JM (2007) EST-SSRs as a resource for population genetic analyses. Heredity 99:125–132. doi:10.1038/sj.hdy.6801001

Fernández J, Sork V, Gallego G, López J, Bohorques A, Tohme J (2000) Cross-amplification of microsatellite loci in a neotropical *Quercus* species and standardization of DNA extraction from mature leaves dried in silica gel. Plant Mol Biol Rep 18:397–397. doi:10.1007/bf02825070

Forkner RE, Hunter MD (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. Ecology 81:1588–1600. doi:10.2307/177309

Gerwein JB, Kesseli RV (2006) Genetic diversity and population structure of *Quercus rubra* (Fagaceae) in old-growth and

secondary forests in southern New England. Rhodora 108:1–18. doi:10.3119/05-9.1

Guo Y, Huang C, Xie Y, Song F, Zhou X (2010) A tomato glutaredoxin gene *SIGRX1* regulates plant responses to oxidative, drought and salt stresses. Planta 232:1499–1509. doi:10.1007/s00425-010-1271-1

Guttman SI, Weigt LA (1989) Electrophoretic evidence of relationships among *Quercus* (oaks) of eastern North America. Can J Bot 67:339–351

Hipp AL, Weber JA (2008) Taxonomy of Hill's oak (*Quercus ellipsoidalis*: Fagaceae): evidence from AFLP data. Syst Bot 33:148–158. doi:10.1600/036364408783887320

Hokanson SC, Isebrands JG, Jensen RJ, Hancock JF (1993) Isozyme variation in oaks of the Apostle Islands in Wisconsin — genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). Am J Bot 80:1349–1357. doi:10.2307/2445720

Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conserv Genet 5:539–543. doi:10.1023/B:COGE.0000041021.91777.1a

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189. doi:10.1111/j.1471-8286.2004.00845.x

Kremer A, Kleinschmit J, Cottrell J, Cundall EP, Deans JD, Ducousso A, Konig AO, Lowe AJ, Munro RC, Petit RJ, Stephan BR (2002) Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection on genetic diversity in European oaks? For Ecol Manage 156:75–87. doi:10.1016/s0378-1127(01)00635-1

Liewlaksaneeyanawin C, Ritland CE, El-Kassaby YA, Ritland K (2004) Single-copy, species-transferable microsatellite markers developed from loblolly pine ESTs. Theor Appl Genet 109:361–369. doi:10.1007/s00122-004-1635-7

Mariette S, Cottrell J, Csaikl UM, Goikoechea P, Konig A, Lowe AJ, Van Dam BC, Barreneche T, Bodénès C, Streiff R, Burg K, Groppe K, Munro RC, Tabbener H, Kremer A (2002) Comparison of levels of genetic diversity detected with AFLP and microsatellite markers within and among mixed *Q. petraea* (MATT.) LIEBL. and *Q. robur* L. stands. Silvae Genet 51:72–79

Maynard Smith J, Haigh J (1974) The hitch-hiking effect of a favourable gene. Genet Rese 23:23–35. doi:10.1017/S0016672300014634

McShea WJ, Healy WM, Devers P, Fearer T, Koch FH, Stauffer D, Waldon J (2007) Forestry matters: decline of oaks will impact wildlife in hardwood forests. J Wildl Manage 71:1717–1728. doi:10.219

Moran EV, Willis J, Clark JS (2012) Genetic evidence for hybridization in red oaks (*Quercus* sect. *Lobatae*, Fagaceae). Am J Bot 99:92–100. doi:10.3732/ajb.11000233/2006-169

Muir G, Schlötterer C (2005) Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiating two hybridising oaks (*Quercus* spp.). Mol Ecol 14:549–561. doi:10.1111/j.1365-294X.2004.02418.x

Oldfield S, Eastwood A (2007) The red list of oaks. Fauna & Flora International Cambridge, UK

Ostfeld RS, Jones CG, Wolff JO (1996) Of mice and mast. Bioscience 46:323–330. doi:10.2307/1312946

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Genet 6:847–859. doi:10.1038/nrg1707

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225. doi:10.2307/2409177

Rozen S, Skaletsky HJ (2000) PRIMER3 on the www for general users and for biologist programmers. In: Krawertz S, Misener S (eds) Bioinformatic methods and protocols: Methods in molecular biology. Humana Press, Totowa, pp 365–386

Scotti-Saintagne C, Mariette S, Porth I, Goicoechea PG, Barreneche T, Bodénès C, Burg K, Kremer A (2004) Genome scanning of interspecific differentiation between two closely related oak species (*Quercus robur* L. and *Q. petraea* (MATT.) LIEBL.). Genetics 168:1615–1626. doi:10.1534/genetics.104.026849

Slate J, Marshall TC, Pemberton JM (2000) A retrospective assessment of the accuracy of the paternity inference program CERVUS. Mol Ecol 9:801–808. doi:10.1046/j.1365-294x.2000.00930.x

Sork VL, Huang S, Wiener E (1993) Macrogeographic and fine-scale genetic structure in a North American oak species, *Quercus rubra* L. Ann Sci For 50(suppl 1):261s–270s

Sridha S, Wu K (2006) Identification of *AtHD2C* as a novel regulator of abscisic acid responses in *Arabidopsis*. Plant J 46:124–133. doi:10.1111/j.1365-313X.2006.02678.x

Steinkellner H, Lexer C, Turetschek E, Glössl J (2003) Conservation of (GA)n microsatellite loci between *Quercus* species. Mol Ecol 6:1189–1194. doi:10.1046/j.1365-294X.1997.00288.x

Tomlinson PT, Jensen RJ, Hancock JF (2000) Do whole tree silvic characters indicate hybridization in red oak (*Quercus* section *Lobatae*)? Am Midl Nat 143:154–168. doi:10.1674/0003-0031(2000)143[0154:dwtsci]2.0.co;2

Tran L-SP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci U S A 104:20623–20628. doi:10.1073/pnas.0706547105

United Nations Economic Commission for Europe/Food and Agriculture Organization of the United Nations (UNECE/FAO) (2011) Forest products annual market review 2010–2011. Geneva timber and forest study paper 27. United Nations Publications, Geneva

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Microchecker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x

van Oosterhout C, Weetman D, Hutchinson WF (2006) Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. Mol Ecol Notes 6:255–256. doi:10.1111/j.1471-8286.2005.01082.x

Vasemägi A, Nilsson J, Primmer CR (2005) Expressed sequence tag-linked microsatellites as a source of gene-associated polymorphisms for detecting signatures of divergent selection in Atlantic salmon (*Salmo salar* L.). Mol Biol Evol 22:1067–1076. doi:10.1093/molbev/msi09

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370. doi:10.2307/2408641

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. doi:10.1046/j.0962-1083.2001.01418.x

Zhu X, Raman H, Wu H, Lemerle D, Burrows G, Stanton R (2012) Development of SSR markers for menetic analysis of silverleaf nightshade (*Solanum elaeagnifolium*) and related species. Plant Mol Biol Report: 1–7. doi:10.1007/s11105-012-0473-z