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Isolation and characterization of microsatellite markers for red elm (*Ulmus rubra* Muhl.) and cross-species amplification with Siberian elm (*Ulmus pumila* L.)

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Abstract

***Ulmus pumila* is an elm species, non-native to the USA that hybridizes with *Ulmus rubra*. In order to study the genetic structure and hybridization patterns between these two elm species, we developed 15 primer pairs for microsatellite loci in *U. rubra* and tested their cross-amplification in *U. pumila*. All 15 primers amplified in both species, 11 of which possessed species-specific alleles. Eight loci were polymorphic in *U. pumila* and eight in *U. rubra*, each with two to eight alleles per locus. In addition, five primer pairs previously developed in *U. laevis* and *U. carpinifolia* (syn. *U. minor*) cross-amplified and showed polymorphic loci in *U. pumila* and/or *U. rubra*. These markers will facilitate the study of genetic structure and gene flow between *U. rubra* and exotic, invasive *U. pumila*.**

Keywords: cross-species amplification, SSR, Ulmus laevis, Ulmus minor

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Ulmus rubra Muhl (syn. *U. fulva* Michx.; red elm; 2n = 28) is a diploid elm species native to eastern North America from North Dakota east to southern Quebec and south to northern Florida and eastern Texas (Cooley & Sambeek 1990). This elm species is a broad-leaved, deciduous tree which is characteristic of moist, rich soils such as those of bottomland forests, but it can also be found in mesic upland forests. Although comparatively less susceptible to Dutch elm disease (DED) than other elms (e.g. *U. americana*), *U. rubra* populations have been depleted across North America. Surviving populations of red elm are usually composed of relatively small statured trees that seldom reach 20 cm in diameter before succumbing to DED (Lester & Smalley 1972). In order to study genetic diversity, population structure, and gene flow between *U. rubra* (DED susceptible) and exotic, invasive *U. pumila* (DED tolerant), microsatellites markers were isolated and characterized in *U. rubra*. Additionally, six polymorphic microsatellite markers previously developed in *U. laevis* (Whiteley *et al*. 2003) and five in *U. carpinifolia* (syn. *U. minor*; Collada *et al*. 2004) were tested in *U. rubra* and *U. pumila*.

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Young leaves from elm trees were collected and freeze-dried for 72 h using a BenchTop lyophilizer (Virtis Inc.). DNA was extracted using a DNeasy kit (QIAGEN), and concentrations were measured in a Turner Quantech Fluorometer (Barnstead). Microsatellite-enriched libraries were developed from a single *U. rubra* tree collected at the 2971-acre Coulee Experimental Forest (La Crosse County, Wisconsin). Microsatellite loci were isolated using a bead capture enrichment protocol (Glenn & Schable 2005). Briefly, total DNA was digested with *Rsa*I (New England BioLabs, Inc.), and fragments were ligated to double-stranded SuperSNX24 linkers (forward 5′-GTTTAAGGCCTAGCTA-GCAGAATC-3′, reverse 5′-GATTCTGCTAGCTAGGCCT-TAAAC-3′). Fragments were then hybridized to biotinylated oligonucleotides using magnetic streptavidin beads (Dynal). The three biotinylated oligo mixes used for double enrichment were the following: Mix 1 = $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$; Mix 2 = $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACAG)_6$ $(ACCT)_6$, $ACTC)_6$, $ACTG)_6$; and Mix 3 = $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ $(AGAT)_8$. Recovered enriched DNA fragments were polymerase chain reaction (PCR) amplified using the SuperSNX24 forward primer. The DNA products were ligated into PCR 2.1-TOPO vector and used to transform One Shot Top 10

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Table 1 Characteristics of 11 microsatellite loci developed in *Ulmus rubra* and cross-species amplification with *Ulmus pumila*

					U. rubra					*U. pumila*	
Locus name (GenBank no.)	Primer sequence 5′–3′	T_a (°C)	Repeat motif	Clone size (bp)	A (*n*)	Size range (bp)	H_E	H_O	HWE	A (*n*)	Size range (bp)
UR101 (EF123150)	F-gggaagtcaaattcccatga *R-ctccaatggcatcttcacaa	60	$(TA)_5(CA)_9$	128	3 (20)	126–130	0.68	0.20	$P < 0.001$	3 (15)	131–139
UR123 (EF123154)	*F-agcaataaaccttgtgtcgtg R-gagcttgctatgcttcgtctc	60	$(CA)_4CG(CA)_5(TTA)_5$	250	7 (20)	246–262	0.77	0.85	NS	7 (15)	239–263
UR138 (EF123155)	*F-ctagaaccccttcgaaacc R-acaaaaagcccacacacctc	60	$(GA)_8$	232	5 (20)	226–244	0.72	0.75	NS	4 (15)	232–242
UR141 (EF123156)	*F-ttgtgtttgcgtgaaaagga R-gttccatgggttttcattgg	60	$(GA)_{10}$	156	1 (20)	154	—	—	—	1 (15)	150
UR153 (EF123157)	*F-agatttcatgcctccagtcg R-cctttcgaaatgcagaggtag	55	$(CTT)_7$	191	3 (20)	189–196	0.41	0.45	NS	5 (15)	178–200
UR158 (EF123158)	*F-ttcttcataggcgctgaggt R-tgcaccctgtcaaagctaaa	55	$(TGTA)_5$	199	3 (20)	196–205	0.19	0.20	NS	2 (15)	180–187
UR159 (EF123159)	*F-tgcatgaacatggacttcatt R-tgatgttaagataagaagtcattagga	55	$(TCA)_5$	245	1 (20)	245	—	—	—	3 (15)	249–279
UR173a (EF123160)	*F-ataaaggacgctaaggcagtca R-agacaaactcttcgccatcaat	55	$(CT)_{19}$	175	8 (20)	156–178	0.75	0.95	NS	1 (15)	146
UR173b (EF123160)	*F-ccgtgcaactttcctgctac R-tgactgccttagcgtcctttat	55	$(TATTT)_3$	160	1 (20)	160	—	—	—	2 (15)	158–168
UR175 (EF123161)	*F-tgccaatttgttgaaatttacg R-ttgttggttgtggtttgtga	55	$(TG)_8$	222	4 (20)	220–227	0.69	0.50	$P = 0.041$	7 (15)	220–236
UR188a (EF123162)	*F-aaaactaacgcgtcccttcc R-atttcgcttcaattgcgagt	55	$(AC)_{15}$	124	8 (20)	114–132	0.76	0.80	NS	1 (15)	108

*Primer label with 6-FAM fluorophore.
T_a (°C), initial annealing temperature.
A, number of alleles; *n*, number of individuals genotyped.
H_E, expected heterozygosity; H_O, observed heterozygosity; HWE, probability value for Hardy–Weinberg equilibrium test.

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Table 2 Cross-species amplification of five microsatellite loci developed in *Ulmus leavis* (Whiteley *et al*. 2003) and *Ulmus carpinifolia* (Collada *et al*. 2004) in *U. rubra* and *U. pumila*

		U. rubra					*U. pumila*	
Locus name	Primer source	*A* (*n*)	Size range (bp)	H_E	H_O	HWE	*A* (*n*)	Size range (bp)
Ulm2	Whiteley *et al*. 2003	2 (20)	103–106	0.48	0.45	NS	1 (15)	100
Ulm3	Whiteley *et al*. 2003	4 (20)	144–173	0.62	0.60	NS	5 (15)	159–185
Ulmi1–21	Collada *et al*. 2004	5 (20)	206–220	0.66	0.60	NS	2 (15)	194–196
Ulmi1–98	Collada *et al*. 2004	4 (20)	143–165	0.47	0.45	NS	6 (15)	122–136
Ulmi1–165	Collada *et al*. 2004	10 (20)	147–188	0.84	0.90	NS	8 (15)	152–180

A, number of alleles; *n*, number of individuals genotyped.
H_E, expected heterozygosity; H_O, observed heterozygosity; HWE, probability value for Hardy–Weinberg equilibrium test.

Competent *Escherichia coli* cells (TOPO TA cloning kit; Invitrogen). Colonies were screened for inserts using the β-galactosidase gene, from which 96 positive colonies were isolated and purified. The resulting plasmids were PCR amplified using M13 forward and reverse primers. Sequencing of PCR products of between 500 and 1000 bp was conducted on an automated ABI377-96 sequencer (Applied Biosystems) using BigDye Terminator (version 3.1). Sequences were edited with SEQUENCHER version 4.1 (Genecodes). TROLL (Castelo *et al*. 2002) was used to search for microsatellites, and 38 primers were designed using PRIMER 3 (Rozen & Skaletsky 2000).

DNA was extracted from 20 *U. rubra* trees from a population collected at the 2971-acre Coulee Experimental Forest. Additionally, 15 samples from *U. pumila* (original accessions from Henan Province, China), housed at the University of Wisconsin-Madison elm arboretum (Arlington, Columbia County, Wisconsin), were collected and used for genotypic analysis of microsatellite loci. Each PCR was performed using a 15-μL volume and contained 1.5 μL 10× PCR buffer, 1.8 μL 25 mM $MgCl_2$, 2.4 μL dNTPs (1.25 mM of each dATP, dGTP, dTTP, and dCTP), 1.0 μL 5 μM of primer, 2 μL 10 ng/μL genomic DNA, 1 U *Taq* DNA polymerase, and 6.2 μL H_2O. Thermocycling conditions were as follows: an initial melting step (94 °C for 3 min), then 30 cycles (94 °C for 15 s, 55 °C/60 °C for 90 s, and 72 °C for 2 min), a final elongation step (72 °C for 20 min), and then an indefinite soak at 4 °C. Marker analysis was initially performed by gel electrophoresis (4.0% agarose gels run for 5 h at 80 v) and by primer labelling using ChromaTide dUTP Alexa Fluor 546–14-dUTP (Invitrogen). After such initial analysis, 15 primers were labelled at the 5′ end using 6-FAM fluorophore (IDT Inc.), and the microsatellite allele sequences were submitted to GenBank. Genotyping using fluorescent-labelled primers was performed at the University of Wisconsin Biotechnology Center DNA Sequence Facility using an Applied Biosystems 3730 fluorescent sequencer (POP-6 and a 50-cm array) and the GENEMARKER software version 1.5 (SoftGenetics).

All 15 primers pairs amplified some type of allele pattern (i.e. monomorphic, polymorphic, or unclear) in both *U. rubra* and *U. pumila*. While four primers (UR103, UR107, UR120, and UR189) revealed unclear allele patterns (data not presented, details in GenBank), 11 primers amplified clear, consistent alleles (Table 1). Eight primer pairs amplified polymorphic loci in *U. rubra*, and eight amplified polymorphic loci in *U. pumila* with the number of alleles per locus ranging from two to eight. All loci, monomorphic and/or polymorphic, possessed species-specific alleles rendering them useful for hybridization studies. Expected heterozygosity for the eight *U. rubra* polymorphic loci ranged from 0.19 to 0.77 (Table 1). Only two loci (UR101 and UR175) showed significant deviation from Hardy–Weinberg equilibrium (GENEPOP, http://wbiomed.curtin.edu.au/genepop). One pair of loci (UR138 and UR188) yielded significant linkage disequilibrium in *U. rubra*. A total of five of the microsatellite loci previously developed in *U. laevis* (ULM2 and ULM3; Whiteley *et al*. 2003) and *U. carpinifolia* (Ulmi1–21, Ulmi1–98, and Ulmi1–165; Collada *et al*. 2004) showed clear, polymorphic amplification in *U. rubra* and/or *U. pumila* (Table 2).

The microsatellite markers developed in the present study were considerably more polymorphic than allozymes used in *Ulmus* (Cogolludo *et al*. 2000), and the polymorphism levels were comparable to microsatellite loci described previously in other elm species (Tables 1 and 2). Thus, the microsatellite loci presented herein will likely be useful for investigating population-level processes in both *U. rubra* and *U. pumila* and to study hybridization patterns between the two elm species.

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