Transferability of Microsatellite Markers in the Betulaceae

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ABSTRACT. Microsatellite-containing sequences for the Betulaceae (*Betula*, *Corylus*, and *Alnus*) were retrieved from GenBank and used to develop twelve new microsatellite marker primer pairs that amplified and were polymorphic in european hazelnut (*Corylus avellana*). The primer pairs were characterized using 50 european hazelnut accessions. Nine of these microsatellites that segregated in a mapping population were assigned to linkage groups. The 12 new primer pairs will be useful in genetic studies in *Corylus* and *Betula*. To investigate transferability of microsatellite primer pairs in the family Betulaceae, we assessed the ability of 129 simple sequence repeat (SSR) primer pairs (75 from *Corylus*, 52 from *Betula*, and two from *Alnus*) to amplify DNA of 69 accessions representing diverse taxa. Microsatellite primer pairs from *Betula* amplified 92% of *Betula*, 51% of *Alnus*, 41% of *Corylus*, 37% of *Carpinus*, 35% of *Ostryopsis*, and 34% of *Ostrya* accessions. In the 69 accessions, microsatellite primer pairs from *Corylus* amplified 81% of *Corylus*, 55% of *Carpinus*, 53% of *Ostrya*, 51% of *Ostryopsis*, 41% of *Alnus*, and 39% of *Betula* accessions. An additional 147 SSR primer pairs developed from *Corylus*, used to amplify a subset of 32 accessions, gave similar values: 92% in *Corylus*, 33% in *Carpinus*, 33% in *Ostrya*, 44% in *Ostryopsis*, 35% in *Alnus*, and 54% in *Betula*. The high transferability (>39%) of microsatellite primer pairs between *Betula* and *Corylus* will allow comparative studies of the two genera with the greatest economic importance.

European hazelnut belongs to the family Betulaceae, which is one of eight families of the Order Fagales. Betulaceae, which includes six genera and ≈140 species, is subdivided into two clades, Betuloideae and Coryloideae. Betuloideae includes *Alnus* (35 species), and *Betula* (35–60 species), while Coryloideae includes *Carpinus* (35 species), *Corylus* (11–13 species), *Ostrya* (10 species), and *Ostryopsis* (two species) (Chen et al., 1999; Yoo and Wen, 2002). The basic chromosome number is 14 for *Alnus* and *Betula*, 11 for *Corylus*, and 8 for *Carpinus*, *Ostrya*, and *Ostryopsis* (Botta et al., 1986; Snow and Goldblatt, 1992). *Betula* species form a polyploid series, with chromosome numbers of 28, 56, 70, 84, and 112 (Järvinen et al., 2004), while *Corylus* species are diploid. Most species in the Betulaceae are wind-pollinated and monoecious. Most are distributed in temperate regions of the Northern Hemisphere, while *Ostryopsis* is endemic to eastern Asia. Despite the variation in chromosome number and genome size among species of the Betulaceae, some degree of microsatellite marker transferability is expected based on results in other plant families.

Microsatellites, also known as simple sequence repeats (SSR), are molecular markers that are highly polymorphic and abundant in plant genomes. Moreover, the transferability of SSR markers allows their use in related species for fingerprinting, mapping, and marker-assisted breeding. Examples include the high transferability of expressed sequence tag (EST)-SSRs from bread wheat (*Triticum aestivum*) to other cereals (Zhang et al., 2005), across the major pulses (Pandian et al., 2000), from apple (*Malus ×domestica*) to european pear (*Pyrus communis*) (Pierantoni et al., 2004), from apricot (*Prunus armeniaca*) and grape (*Vitis vinifera*) to related taxa (Decroocq et al., 2003), from peach (*Prunus persica*) to related *Prunus* species (Cipriani et al., 1999), and from olive (*Olea europaea*) to other

species in the genus *Olea* (Rallo et al., 2003). Recent reviews of SSR transferability include Ellis and Burke (2007) and Barbara et al. (2007).

In this study, we developed new SSR markers based on DNA sequences of *Alnus*, *Betula*, and *Corylus* retrieved from a public database. We used these new SSR primer pairs, published polymorphic marker primer pairs for *Betula*, and markers developed in our laboratory for *Corylus*, to investigate their transferability in the family Betulaceae. *Betula* primer pairs that amplified in *Corylus* were characterized, and new primer pairs that segregated in our *Corylus* mapping population were assigned to linkage groups of the european hazelnut.

Materials and Methods

PLANT MATERIAL. A total of 50 hazelnut accessions, including the parents of our mapping population, were used to characterize the SSR primer pairs (Gürcan et al., 2010). Of these, 24 were used in the initial screening of markers. These 50 accessions were chosen as a diverse subset of the 198 unique accessions fingerprinted by Gökirmak et al. (2009). Trees of all hazelnut accessions were growing in the field at the U.S. Department of Agriculture-Agriculture Research Service-National Clonal Germplasm Repository (USDA-ARS-NCGR) and the Oregon State University Department of Horticulture's Smith Horticulture Research Farm in Corvallis. A mapping population of 144 seedlings (Mehlenbacher et al., 2006) was used for segregation analysis.

Sixty-nine genotypes representing six genera (Table 1), including 9 species of *Alnus*, 15 species of *Betula*, 4 species of *Carpinus*, 11 species of *Corylus*, 3 species of *Ostrya*, and 2 species of *Ostryopsis*, were used in the transferability study. Leaf material was obtained from several sources, including four in Corvallis, OR (USDA-ARS-NCGR, Smith Horticultural Research Farm, Oregon State University campus, and Peavy Arboretum), the Morris Arboretum of the University of Pennsylvania in Philadelphia, and the U.S. National Arboretum in Washington, DC.

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Table 1. Betulaceae species and accessions used to study transferability of microsatellite markers.

Species	Cultivar or accession	Source ^z
<i>Alnus glutinosa</i>		OSU campus
<i>Alnus hirsuta</i> ^y		U.S. National Arboretum
<i>Alnus incana</i>		OSU campus
<i>A. incana</i> ^y		Peavy Arboretum
<i>Alnus japonica</i> ^y		Peavy Arboretum
<i>Alnus rhombifolia</i> ^y		Peavy Arboretum
<i>Alnus rubra</i> ^y		OSU campus
<i>Alnus serrulata</i>		U.S. National Arboretum
<i>Alnus sinuta</i> ^y		Peavy Arboretum
<i>Alnus tenuifolia</i>		Peavy Arboretum
<i>Betula albosinensis</i> ^y		U.S. National Arboretum
<i>Betula alleghaniensis</i> ^y		U.S. National Arboretum
<i>Betula chinensis</i>		U.S. National Arboretum
<i>Betula davurica</i> ^y		U.S. National Arboretum
<i>Betula fruiticosa</i> ^y		U.S. National Arboretum
<i>Betula grossa</i>		U.S. National Arboretum
<i>Betula jacquemontii</i>		OSU campus
<i>Betula lenta</i> ^y		U.S. National Arboretum
<i>Betula maximowicziana</i> ^y		U.S. National Arboretum
<i>Betula nigra</i> ^y	'Heritage'	U.S. National Arboretum
<i>B. nigra</i>		OSU campus
<i>Betula ovalifolia</i>		U.S. National Arboretum
<i>Betula papyrifera</i> ^y		U.S. National Arboretum
<i>B. papyrifera</i>		OSU campus
<i>Betula pendula</i>	<i>youngii</i>	OSU campus
<i>B. pendula</i>		OSU campus
<i>B. pendula</i> ^y	<i>laciniata</i>	OSU campus
<i>B. pendula</i>	<i>purpurea</i>	OSU campus
<i>Betula populifolia</i>		OSU campus
<i>Betula uber</i>		U.S. National Arboretum
<i>Carpinus betulus</i>	<i>fastigiata</i>	OSU campus
<i>C. betulus</i>	<i>pendula</i>	U.S. National Arboretum
<i>C. betulus</i> ^y		U.S. National Arboretum
<i>C. betulus</i>		OSU campus
<i>Carpinus caroliniana</i> ^y		OSU campus
<i>C. caroliniana</i>		U.S. National Arboretum
<i>Carpinus cordata</i>		U.S. National Arboretum
<i>Carpinus turczaninovi</i> ^y		U.S. National Arboretum
<i>Corylus americana</i>	CCOR 193	USDA-NCGR
<i>C. americana</i> ^y	CCOR 686 (N02.68)	USDA-NCGR
<i>Corylus avellana</i>	'Imperiale de Trebizonde'	USDA-NCGR
<i>C. avellana</i>	'Tombul' (V26a)	USDA-NCGR
<i>Corylus californica</i> ^y	#2 (N2.66)	USDA-NCGR
<i>C. californica</i>	CCOR239, SCS 59-1 (N05.57)	USDA-NCGR
<i>Corylus chinensis</i>	OSU 529.001	USDA-NCGR
<i>C. chinensis</i>	OSU 567.005	USDA-NCGR
<i>C. chinensis</i> ^y	W05	USDA-NCGR
<i>Corylus colurna</i>	CCOR453, N550 (N04.08)	USDA-NCGR
<i>C. colurna</i> ^y	X-11	USDA-NCGR
<i>Corylus cornuta</i> ^y	CC3.01 (N02.59)	USDA-NCGR
<i>C. cornuta</i>	CC3.113 (N07.04)	USDA-NCGR
<i>Corylus fargesii</i>	#83	USDA-NCGR
<i>C. fargesii</i>	96-574J	USDA-NCGR
<i>C. fargesii</i> ^y	Gas Pump	USDA-NCGR
<i>Corylus ferox</i>	OSU weather station	USDA-NCGR
<i>Corylus heterophylla</i> ^y	<i>sutchuensis</i> (N05.22)	USDA-NCGR
<i>C. heterophylla</i>	CCOR 691 (N07.22)	USDA-NCGR
<i>C. heterophylla</i>	D80-190 (V15)	USDA-NCGR

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DNA EXTRACTION. DNA from *Corylus* accessions and the mapping population was extracted from young leaves of field-planted trees, as described by Davis et al. (1998) and modified by Lunde et al. (2000). DNA was extracted from other genera using a modified Puregene extraction protocol (Gentra Systems, Minneapolis). Proteinase K and RNase A digestion steps were included in the extraction, and protein precipitation was repeated twice. All template DNA was diluted in TE buffer to 3.5 ng-μL⁻¹ before amplification.

MARKER SOURCES. Twenty new SSR markers were developed from sequences retrieved from GenBank (National Center for Biotechnology Information, 2010): 16 for *Betula*, 2 for *Alnus*, and 2 for *Corylus*. Thirty-six SSR primer pairs developed from genomic libraries in *Betula* (Kulju et al., 2004; Oygü et al., 2003; Truong et al., 2005; Wu et al., 2002) for which published observed heterozygosity was high ($H_o \geq 0.5$) were also used. Ten *Betula platyphylla* primer pairs were from Wu et al. (2002) (Bp01, Bp04, Bp07, BpTA, Bp10, Bp11, Bp13, Bp15, Bp16, and BpA), 5 *Betula maximowicziana* primer pairs were from Oygü et al. (2003) (Bmax097, Bmax544, Bmax624, Bmax630, and Bmax 671), 15 *Betula pendula* primer pairs were from Kulju et al. (2004) (L2.2, L2.7, L3.1, L3.4, L37.1, L7.4, L4.4, L5.1, L5.4, L5.5, L7.3, L7.8, L13.1, L63, and L022), and 6 *B. pendula* primer pairs were from Truong et al. (2005) (L1.10, L2.5, L021, Bo.F394, Bo.F330, and Bo.G182). Primer pairs developed from enriched libraries of *Corylus avellana* (Bassil et al., 2005a, 2005b; Boccacci et al., 2005; Gürcan et al., 2010), primer pairs developed from ISSR fragments (Gürcan, 2009), and new *Corylus* primer pairs from various sources (Table 2) were used in this study.

In 2006, all Betulaceae sequences were retrieved from the GenBank database: 3300 for *Betula*, 23 for *Alnus*, and 45 for *Corylus*. Unique sequences were identified using the sequence assembly program CAP3 (Huang and Madan, 1999) at the Oregon State University (OSU) Center for Genome Research and Biocomputing, and then

Table 1. Continued.

Species	Cultivar or accession	Source ^z
<i>C. heterophylla</i> ^v	<i>yunnanensis</i> 286 (N06.21)	USDA-NCGR
<i>C. heterophylla</i>	'Ogyoo' (T26)	USDA-NCGR
<i>Corylus jacquemontii</i> ^v	#1 (N01.64)	USDA-NCGR
<i>C. jacquemontii</i>	#2 (N07.65)	USDA-NCGR
<i>Corylus sieboldiana</i> ^v	#2 (N05.55)	USDA-NCGR
<i>C. sieboldiana</i>	CCOR 350 (N02.61)	USDA-NCGR
<i>Ostrya carpinifolia</i> ^v		Morris Arboretum
<i>Ostrya japonica</i> ^v		Morris Arboretum
<i>Ostrya virginiana</i>		OSU campus
<i>Ostryopsis davidiana</i> ^v		UC Botanical Garden
<i>Ostrya nobilis</i> ^v		UC Botanical Garden

^vOregon State University (OSU), Corvallis; Peavy Arboretum, OSU, Corvallis; U.S. National Arboretum, Washington, DC; U.S. Department of Agriculture-Agricultural Research Service-National Clonal Germplasm Repository (USDA-NCGR), Corvallis, OR; Morris Arboretum, University of Pennsylvania, Philadelphia; University of California (UC) Botanical Garden, Berkeley.

^zUsed to amplify 157 additional SSR loci from *Corylus*.

sequences were screened for the presence of SSRs with the program SSRIT (Temnykh et al., 2001). Primers that flanked the microsatellites were designed using the Primer3 program (Rozen and Skaletsky, 2000) with the following criteria: 22 to 24 bp in length, 30% to 55% guanine-cytosine (GC) content, and 60 °C annealing temperature. From these database sequences, we developed 20 new SSR markers: 16 from *Betula* sequences, 2 from *Alnus*, and 2 from *Corylus* (Table 2).

MARKER TRANSFERABILITY. Polymerase chain reactions (PCRs) were conducted in a 15-μL PCR mixture containing 0.3 μM each of forward and reverse primers, 1 × Biolase NH₄ reaction buffer, 3 mM MgCl₂, 200 μM of each dNTP, 3 to 5 ng of template DNA, and 0.25 units of Biolase DNA polymerase (Bioline, Randolph, MA). The thermocycler was programmed to denature DNA at 94 °C for 3 min, followed by 40 cycles of 94 °C for 40 s, annealing temperature for 60 s, 72 °C for 60 s, and a final 7-min extension step at 72 °C. PCR products were separated by electrophoresis through 3% agarose. The gels were stained with ethidium bromide, placed under ultraviolet light, and photographed using an imaging system (UVP, Upland, CA). Amplification was indicated by visible bands of the expected size (intense or faint) on the agarose gels. Amplifications that gave multiple bands with a large size range were scored as zero and were not considered transferable.

CHARACTERIZATION OF POLYMORPHIC MARKERS. The initial screening of the new markers in *C. avellana* used 24 accessions (Gürçan et al., 2010) and the PCR mixture and thermocycler program described above. PCR products were separated on 3% agarose gels, and polymorphic primer pairs were identified visually. For polymorphic primer pairs, forward primers were labeled fluorescently with 6-FAM, HEX, or NED and were used to amplify 48 accessions plus the parents of our mapping population (OSU 252.146 × OSU 414.062) (Mehlenbacher et al., 2006) using the same PCR cocktail and thermocycler program described above. PCR products, 1 μL from each of four primer pairs, were multiplexed by diluting in 80 μL of distilled water. A 1-μL aliquot of the mixture was sent to the Core Laboratories of the OSU Center for Genome Research and Biocomputing where fragment sizes were determined using a capillary electrophoresis instrument (ABI 3100; Applied Biosystems, Foster City, CA).

Expected heterozygosity (H_e), observed heterozygosity (H_o), and polymorphic information content (PIC) were calculated for the polymorphic primer pairs using PowerMarker (Liu and Muse, 2005). H_e was calculated according to the formula $H_e = 1 - \sum p_i^2$, where p_i is the frequency of the i^{th} allele (Nei, 1973). H_o was from direct counts, and the PIC value for each locus was estimated using PowerMarker as $PIC = 1 - \sum_i p_i^2 - \sum_{i=1} \sum_{j=i+1} 2p_i p_j^2$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, respectively. The frequency of null alleles (r) was estimated as $r = (H_e - H_o)/(1 + H_e)$ (Botstein et al., 1980) in a spreadsheet (Table 3).

SEGREGATION AND MAPPING OF POLYMORPHIC MARKERS. Observed segregation ratios in the mapping population were compared with expected Mendelian ratios using chi-square goodness-of-fit tests (Table 4). The new markers were placed on the hazelnut linkage map using JoinMap 3.0 (van Ooijen and Voorrips, 2001) as described by Mehlenbacher et al. (2006). The Kosambi mapping function was used for the calculation of map distances and a LOD score of 7.0 was used for the determination of linkage groups.

Results and Discussion

DEVELOPMENT OF NEW MARKERS FROM DATABASE SEQUENCES. We downloaded 3368 sequences from GenBank: 3300 for *Betula*, 23 for *Alnus*, and 45 for *Corylus*. Alignment of the *Betula* sequences resulted in 527 contigs and 605 singlet fragments. In these 1132 unique fragments, 254 fragments contained SSRs with repeats ≥ 10 bp, of which 35 contained repeats ≥ 20 bp. Comparison of these 35 with published SSR-containing sequences revealed that 19 had already been used for SSR marker development, while 16 had not yet been used. All 23 *Alnus* sequences were unique; four SSR-containing regions were ≥ 10 bp, of which two in a single sequence had SSRs ≥ 20 bp. All 45 *Corylus* sequences were unique; 10 regions in these sequences had SSRs ≥ 10 bp, of which three in a single sequence had repeats ≥ 20 bp. Unique sequences with SSRs ≥ 20 bp (16 from *Betula*, 2 from *Alnus*, and 3 from *Corylus*) were used to develop SSR markers. Of the 16 *Betula* primer pairs, 6 failed to amplify in *Corylus*, and only 4 of the 10 that amplified *Corylus* were polymorphic. Of the two *Alnus* primer pairs, one failed to amplify in *Corylus*, and the other generated monomorphic products. Three *Corylus* primer pairs were designed from a single sequence. Two (AJ417975b and AJ417975c) produced polymorphic bands, but amplification with the third primer pair (AJ417975a) was poor and the locus was not investigated further. Only AJ417975c segregated in the mapping population.

CHARACTERIZATION AND MAPPING OF POLYMORPHIC PRIMER PAIRS. Four articles describe the development of 52 markers for *Betula* (Kulju et al., 2004; Oygü et al., 2003; Truong et al., 2005; Wu et al., 2002). For 36 of these markers, observed heterozygosity was ≥ 0.5 . DNA from at least 18 of 24 *Corylus* accessions was amplified using the published primer sequences

Table 2. New microsatellite markers developed from sequences of *Betula*, *Alnus*, and *Corylus*.

GenBank accession no.	Locus	Synonym	Sequence source ^z	Primers		Repeat motif	Allele length (bp)	Annealing temp (°C)
				Forward	Reverse			
Markers from <i>Betula</i>								
AF310860 ^y	—	—	Genomic library	CCTGCAGTCGACCTAGAGGA	CCAAAAGTTTGACAGCATCAGA	(TC) ₁₅ TA(TG) ₁₀	161	60
AJ490266 ^y	—	—	Mads2 gene	ACTGCAGCCCTTCAACTACG	AACGCCTACCTCATGTTTGG	(CT) ₁₃ Ns(CT) ₁₃	210–224	60
AY423613 ^x	—	—	Genomic library	CTTACCGTCTTGCCAAAGGT	ACCACACAGCCACAACC	(AG) ₁₇	235	60
AY423614 ^x	—	—	Genomic library	TCGGAATTGGTGGGTCTATT	CGAAACCCCTTTGATGAGTT	(AG) ₁₈	190	60
AY423621 ^x	—	—	Genomic library	TGGCAGTTCAAATCATGTGGT	CCATGTACCCCATCAAAACTTCA	(AT) ₁₄ (AG) ₂₃	205	60
CD276825 ^y	—	—	cDNA	GGGGATCGTTTTCCTCTCTG	GCTGCTCAAAGCCAAAGATT	(CTT) ₁₀	283	60
CD276757 ^y	—	—	cDNA	GGTCGCCTATAGGTACGTAG AAAA	CCAGAAACTACCACTGAACC	(GAC) ₆ Ns(TTA) ₇	219	60
CD276827 ^y	—	—	cDNA	GAATAGAGCGTTGCGCAGAT	CAGGTTCTCTCTCCACTG	(AG) ₁₁	190	60
CD277232 ^y	—	—	cDNA	GCAATTTCTACCAACAGAAACC	CTCTGGATCCAACGGTCAAAG	(CT) ₁₄	185	60
CD277302 ^y	—	—	cDNA	AAAAACCAACCAAGCCAAAGTG	CTGCACCTCCCAACAGTTCT	(CT) ₁₁	232	60
CD277382 ^y	—	—	cDNA	GGGACTCTCAAAACCTCCTT	TTCCAACGACCATTCGATT	(TC) ₁₁	170	60
CD278084 ^y	—	—	cDNA	GCGACAGGAAATTCACCCAC	CACCTTCCATTTTCCCGAGA	(TC) ₁₁	175	60
CD278264 ^y	—	—	cDNA	TTTTTGATGCACGGGATGAGA	ACACGTGTCACACGAAAGC	(ATG) ₇	174	60
CD278994 ^y	—	—	cDNA	CTTACCAGATTGCGGCAC	AAGAGGAGCACCACATGAC	(CT) ₁₀	248	60
CD278733 ^y	—	—	cDNA	CGGTGCTCCTCTACTCCT	AACGTTCCCATTACTCAAAGTCA	(AT) ₉ Ns(TC) ₁₀	300	60
Z72433 ^w	—	—	Bet v 1 gene	GCCTGGAACCATCAAGAAGA	TCCACCTCATCAACCCTCTC	(CA) ₁₇ T(AT) ₁₆	169–187	60
Markers from <i>Alnus glutinosa</i>								
Y08436	—	—	AG164 gene	ATGGGAATGGACATGGACAC	GCCACATAAGACGACTTAGACACA	(TA) ₁₇	215	60
Y08436	—	—	AG164 gene	TTGTCATGTGTAATTCTGC TTTTT	TCTTATTCACGTACAACCTTTCAA	(TA) ₂₉	168	60
Polymorphic loci from <i>Corylus</i>								
AJ417975	AJ417975b	—	Lox gene	Hex-GGTTTCCCTGCAGCTCATTA	CTTCAACCACTAGGCCAAACC	(TA) ₁₇	190–230	60
AJ417975	AJ417975c	—	Lox gene	Ned-AAAAAGAAACCAAGGTGT GGAA	CCAAACTTTCAATTGCGACA	(T) ₃₃ (TTAGT) ₇	203–250	60
GU338869	B639 ^z	—	Genomic library	Fam-CTGCAATTGATGGATTGGTTG	TTAAGAAAAGGTACAAAGGGCTCTC	(GA) ₂₇	348	60
FJ98543	B768 ^z	—	Genomic library	Fam-GTGGCAACGATGCCTTGATA	TCGCA TGGTAGGTTCAAGTTG	(TC) ₁₄ Ns(TGA) ₅ Ns(TGA) ₇	288–308	60
CC875206	W07-SSR ^y	—	RAPD marker	CCCAACATTTTGAATCTACTGCA ACCTC	Hex-TGCAAGACACCAAAAC CACAATTAAGAAA	(TC) ₉ Ns(AC) ₄	238	67
Monomorphic loci from <i>Corylus</i>								
GU338868	B620	—	Genomic library	GGACTGGGAGGCAAGAA	TTCAGCATCCACACCTATTGTT	(GAA) ₅ Ns(CAA) ₅ Ns(TC) ₁₄	360	60
FJ986517	B702b	—	Genomic library	AAAGCGGACAGCGAATAATG	TTCGAACAAAGTCCGGATAGC	(CT) ₅ Ns(TC) ₅	244	60
GU181800	B747	—	Genomic library	GTTACAGGCTGTTGGGTTT	CGTGTGCTCATGTGTTGTG	(GT) ₅ (GA) ₁₂	187	60
GU181815	KG901	SSR 064	ISSR fragment	AAGCGATTCTGTAAGTACGGA AATTGT	AGGTAGCTTGAACATTACAA CCATCTG	T ₁₄ CCGT(TG) ₇	186	67
GU181812	KG902	SSR 022	ISSR fragment	TAAAGCATAGGGGCCTCTAC ATACACG	GAAAAATGAGTCAAAATTCAGCA AAACAA	(AO) ₁₁	329	67
GU181824	KG903	K18–28–24	ISSR fragment	TGTTTGGCAGAGAAATAGAGT	GGGTAGGCAGGAAATAGAA	(AAAAG) ₄	272	54

continued next page

Table 2. Continued.

GenBank accession no.	Locus	Synonym	Sequence source ²	Primers		Repeat motif	Allele length (bp)	Annealing temp (°C)
				Forward	Reverse			
GU181811	KG904	SSR 014	ISSR fragment	TGCGTTTATATTTTGAGCTGGA TGGTTT	TTGACACCTCAGATTGCAGGA ATGATA	(CA) ₁₆	341	67
GU181818	KG906	SSR 110F	ISSR fragment	TTTTATGGATCGAGTCAACTC CTACCG	GCTATTTTGTGGCTTTCAA TTGCT	(GT) ₁₁	357	67
GU181809	KG907	SSR 011	ISSR fragment	CCCCCTCTGGCCTTTGGTATATC CTTA	TGCTAACTACCCTCAGAAGG GCAATG	(TG) ₅ CA(GT) ₈	373	67
GU181825	KG908	K56-28-33	ISSR fragment	TTCCGAGTAGTGACAATCAAC	ACAATAGAGCAATGAAACTGG	(AAATT) ₅ AAAT	399	54
GU181816	KG909	SSR 075	ISSR fragment	AATAAAGGTAGCACAAATTTGGT GGGAAA	CGTTAGAACGGCTCTTCTGTCTG AACT	(GA) ₈	176	57
GU181817	KG910	SSR 104	ISSR fragment	ATCCCCAAATGGAGGATTGGTT AGAC	AATTGCCCTTATCTTCTTGAGTG GGATT	(CCCT) ₅	154	67
GU181826	KG911	K57-1-32	ISSR fragment	AGGGAAGCCATCGTCATT	TGCTGTGTAGAGTGTGTGAGA	(TTTA) ₅	171	56
GU181828	KG912	K76-2-10	ISSR fragment	GCTTGACACCATTTCTTTCTTT	CTATTTTCAGATTTTCGGTAGTTT	(CAAAAT) ₄	274	54
GU181829	KG913	K76-28-26	ISSR fragment	CTATTTTCAGATTTTCGGTAGTTT	GCTTGACACCATTTCTTTCTTT	(TGATT) ₄	274	54
GU181813	KG914	SSR 025	ISSR fragment	TGCGGTTTGTATTTCTTCTTTTC ACTCT	CAGTTCAATATCACAATGCTGTC TCCAG	(AC) ₁₃	330	57
GU181827	KG925	K62-15-12	ISSR fragment	GCAAAAGAGATAAGGAAAGAGG	TGGAGAAAGAGGATGATGAG	(CAT) ₇	399	55
Primer pairs from <i>Corylus</i> that amplify two or more loci								
GU338877	A003a	—	Genomic library	CCATCATATCTTTCACCTTCACG	CCGAAACCATGTAAATCTGTGT	(CTT) ₉	293	60
GU338878	A017	—	Genomic library	TGCGTCATCCATACATAGACAT	TGCTTCATACAGAGTTCACGTG	(GT) ₁₂	188	60
GU181833	A038	—	Genomic library	TTACCAAAAACCAGAGACCT	CCTCCCTTGCTCATCACATG	(CA) ₁₃	261	60
GU338872	A607	—	Genomic library	CTTTGGAAGCGAGCATTTGTT	TTGTGAACATGTTGTTGTAGTGC	(CA) ₁₃	176	60
GU338873	A609	—	Genomic library	CACATCCACCATCAAAACCAGT	CGGTCGATAGTGGATCAGGT	(CA) ₇ (AC) ₂₀	230	60
GU338874	A617	—	Genomic library	GCTTCATACAGAGTTCCAGTGC	TTGTGTGTGCGTCATCCATA	(AC) ₁₃	196	60
GU338875	A618	—	Genomic library	CAGGACTTGTGAGAGCGAGA	TTGCATGCTCATCTTCTATTG	(AC) ₁₇ (AT) ₇	245	60
GU181823	A620	—	Genomic library	AACGCTTGAATGGATTGACC	AAGACCTACAAATAAAAGGCACA	(CA) ₁₂ (AT) ₅	166	60
GU338876	A637	—	Genomic library	CTCCACTCGAGGCAACATTC	ACAAACGCCAACCCATTCTA	(CT) ₁₁ (CA) ₁₇	283	60
GU181835	B013	—	Genomic library	CCGCTATTGAAGAAAGTGGTAG	GCAACGAGAAAAGAGAGAGAGA	(TC) ₁₂	257	58
GU338870	B661	—	Genomic library	AGCTTGGGGTGTAGATGTGC	CCAAGACCTTTGTGCCTACC	(GT) ₉ (GA) ₁₁	172	60
GU338871	B666	—	Genomic library	TTTTCAGACCTCCCCATCAG	CCCAAAGCAGTTTAGGAACC	(AG) ₈ Ns(GA) ₅ Ns(GA) ₅ Ns(GA) ₁₉	383	60
GU181803	B677	—	Genomic library	GGGACTGCTTAACGGTTTCA	GCTCGATCACAGTCACATGG	(TC) ₁₇ A(CT) ₁₀	233	60
GU181801	B682	—	Genomic library	CAGCCTGCCCTCTAGTGGTTC	AGGTTTCCACCTCATCACCA	(AG) ₁₆	237	60
GU181802	B683	—	Genomic library	TGAGTTAGTGTGCGCATGGATT	GAAGAAGAAATTTGCTCCACCA	(TC) ₁₇	297	60
GU181804	B690	—	Genomic library	AACCCTAACCCCTGTTTTGTTC	CTCTGGCGAGTTAGGTCGAG	(TC) ₇ Ns(CT) ₇	210	60
GU181805	B691	—	Genomic library	CGGATTGTTAAAGTGGTTTGTTC	CCCCACTTGAAGAGCAAAAA	(TC) ₂₀ Ns(CT) ₉	383	60
GU181806	B713	—	Genomic library	GGGGATTGGAAGAAATGGT	GGCGGGTTGTGGTTATAAAT	(GA) ₈ Ns(AG) ₅ Ns(AG) ₁₅	383	60
GU181807	B742	—	Genomic library	CAAAAGCATTCACTTTGTCTCA	TCTTCAATGGCTGAATGTGC	(AG) ₁₅	294	60
GU181808	B781	—	Genomic library	TCTCCATTGGTGAAGTAGCTTG	AAGCTTATCACACGCACACA	(CT) ₁₅	221	60
GU181831	KG916	K79-1-21	ISSR fragment	AGATACAAGTGAAGGAAGATGG	ATTTGGGTTGGGTTTGAC	(TC) ₁₆	381	55

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Table 2. Continued.

GenBank accession no.	Locus	Synonym	Sequence source ^a	Primers		Repeat motif	Allele length (bp)	Annealing temp (°C)
				Forward	Reverse			
GU181820	KG917	SSR 020	ISSR fragment	GCAGATTCCCTAAATGAACCCCTA AACATT	TTACTTCCCTATGTCAAAATA CACCCCAAT	(CT) ₄ T(CT) ₃ T(CT) ₂ C(CT) ₃ C(CT) ₂	259	67
GU181830	KG918	K51-1-20	ISSR fragment	CGCAAGTTTAAACGCAAAATCTC	AGGAGGAAGATGGCAGACT	(ATT) ₁₀	323	58
GU181832	KG919	K88-28-3	ISSR fragment	GTGGTGTTCCTTGTGTAG	AGCATTCACCTTGTCTCATC	(TC) ₁₅	367	55
GU181822	KG920	SSR 077	ISSR fragment	AATCAAAAACCTAAATGTCAAA CCCAACA	ATACATGATTTTGGCCACGTC ACCAAT	(GA) ₂ G(GA) ₂ G(GA) ₇	343	67
GU181821	KG921	SSR 053	ISSR fragment	ATTGACTGATAAGAGGCGA TCGACTGAG	ACTCACCATCAAAATGCACACA TAGGATT	(GT) ₁₃	293	67

^aMarkers developed from internal and end repeats of ISSR fragments, and from end repeat of RAPD marker.^bDeveloped from *Betula pendula* sequence.^cDeveloped from *Betula pubescens* sequence.^dDeveloped from *Betula verrucosa* sequence.

for 18 of the 36 primer pairs, but eight of these were monomorphic and four produced several bands over a large size range. The remaining six were polymorphic and single locus, and were characterized using 50 *C. avellana* accessions. Five of the six were polymorphic in our mapping population and were assigned to linkage groups (Table 4). For the 12 loci that were single-locus and polymorphic in *Corylus* (six published *Betula* loci, four new loci developed from *Betula* sequences, and two newly developed from a *Corylus* sequence), one primer of each pair was fluorescently labeled and used to amplify 50 hazelnut accessions. For the 10 *Betula* loci, mean values for H_e , H_o , and PIC were 0.66, 0.53, and 0.62, respectively (Table 3). For the two *Corylus* loci, mean values for H_e , H_o , and PIC were 0.88, 0.48, and 0.47, respectively (Table 3). A total of 78 alleles was produced at the 10 *Betula* loci (Table 3). The number of alleles ranged from 2 to 12, with a mean of 8.58. A total of 29 alleles was produced at the two *Corylus* loci (Table 3). A high frequency of null alleles was observed at *Betula* locus CD277232 and *Corylus* locus AJ417975c.

Eight of the 10 *Betula* and one of the two *Corylus* markers segregated in the mapping population and were assigned to a linkage group (LG) based on cosegregation with existing markers (Mehlenbacher et al., 2006) (Table 4). AJ490266 showed a deficiency of 224/224 homozygotes, but the other seven loci showed good fit to Mendelian expectation.

The *Betula* genetic map of Pekkinen et al. (2005) includes 16 microsatellite markers of which we were able to map four (L5.5, L13.1, L7.8, and L1.10) in hazelnut. Three (BL55, BL131, and BL78) that were placed on LG2 in *B. pendula* were assigned to LG10 in hazelnut. BLK110 on LG5 in *Betula* was assigned to LG2 in *Corylus*. Of the remaining loci mapped in *Betula* by Pekkinen et al. (2005), three (L7.1, L5.4, and L3.1) amplified in hazelnut but were not polymorphic, five (L7.4, L7.3, L63, L3.4, and L2.2) failed to amplify in *Corylus*, and three (L2.3, L3.3, and L52) were not included in this study due to their low PIC values (≤ 0.5). We were not able to map AG164 from *Alnus* because it was not polymorphic in our mapping population. SSR locus AJ417975b from the *Corylus* Lox gene was assigned to hazelnut LG7. Lipoxigenases (LOXs) are a class of dioxygenases that have diverse functions in several physiological processes such as growth, development, and response to biotic and abiotic stresses (Santino et al., 2003).

The locus AJ490266, developed from the *Betula* gene BpMADS2, was assigned to hazelnut LG7. BpMADS2 is similar to PISTILLATA (PI) from *Arabidopsis thaliana* and GLOBOSA (GLO) from *Antirrhinum majus*. They are homeotic B-function genes for the specification of the identity of petals and stamens (Järvinen et al., 2003). Locus Z72433, developed from the major *Betula* pollen allergen BetV 1 (Hoffmann-Sommergruber et al., 1997), was placed on hazelnut LG1.

Hazelnut pollen and food allergies are common in humans. In Europe, 0.1% to 0.5% of the population shows an allergic reaction to hazelnut pollen. Allergens from *Corylus* and *Betula* have been identified and characterized at the molecular and biochemical level (Lüttkopf et al., 2001; Schocker et al., 2002). Several forms of the hazelnut allergen have been sequenced, including Cor a 1.04, Cor a 1.01, Cor a 2, Cor a 8, Cor a 9, and Cor a 11. The hazelnut pollen allergens are similar to the major *Betula* pollen allergen Bet v 1 (Swoboda et al., 1995), which is a homolog of Cor a 1.04.

TRANSFERABILITY OF *BETULA* AND *ALNUS* SSR PRIMER PAIRS. The amplification ability of 52 SSR primer pairs from *Betula*

Table 3. Characteristics of new polymorphic SSR loci from *Betula* and *Corylus* sequences based on amplification of 50 *Corylus avellana* accessions.

Marker locus	GenBank accession no.	Allele length (bp)		Characteristics ^z					
		<i>Betula</i>	<i>Corylus</i>	T _a	n	He	Ho	PIC	r
<i>Betula</i> loci from publications									
Bp16	AB084483	166–172	181–207	56	11	0.76	0.72	0.72	0.02
L7.8	AF310866	295–307	260–274	57	9	0.78	0.86	0.75	−0.05
L13.1	AF310871	93–108	96–100	57	3	0.5	0.38	0.4	0.08
L5.5	AF310863	121–146	108–116	57	2	0.13	0.1	0.12	0.03
L1.10	AF310856	168–209	168–190	57	9	0.84	0.88	0.82	−0.02
L021	AF310877	168–236	226–254	60	11	0.59	0.34	0.57	0.16
<i>Betula</i> loci from GenBank sequences									
CD277232	CD277232	185	360–408	60	12	0.8	0.54	0.78	0.15
Z72433	Z72433	231	169–187	60	9	0.7	0.68	0.66	0.01
CD278264	CD278264	174	182–198	60	7	0.61	0.6	0.54	0
AJ490266	AJ490266	221	210–224	60	12	0.88	0.16	0.86	0.38
Total for <i>Betula</i>					85	6.58	5.26	6.23	0.76
Avg for <i>Betula</i>					8.5	0.66	0.53	0.62	0.08
<i>Corylus</i> loci from GenBank sequence									
AJ417975b	AJ417975	—	190–230	60	16	0.85	0.8	0.84	0.03
AJ417975c	AJ417975	—	203–250	60	13	0.9	0.16	0.9	0.39
Total for <i>Corylus</i>					29	1.75	0.96	1.73	0.42
Avg for <i>Corylus</i>					14.5	0.88	0.48	0.87	0.21

^zT_a = annealing temperature (°C), n = number of alleles, He = expected heterozygosity, Ho = observed heterozygosity, PIC = polymorphism information content, r = null allele frequency.

Table 4. Segregation at nine SSR loci developed from database sequences and linkage group assignments.

Locus	Species source	Allele sizes [♀ × ♂ (bp)]	Observed ratio	Expected ratio	Chi-square	P	Linkage group ^z
AJ490266	<i>Betula pendula</i>	210/224 × 224/224	86:57	1:1	5.88	0.015 ^y	7
BL55	<i>B. pendula</i>	108/108 × 108/115	71:73	1:1	0.03	0.87	10
BL131	<i>B. pendula</i>	99/99 × 99/101	61:83	1:1	3.36	0.07	10
BP16	<i>Betula platyphylla</i>	203/205 × 197/197	70:74	1:1	0.11	0.74	1
CD277232	<i>B. pendula</i>	390/405 × 396/396	72:68	1:1	0.11	0.74	3
BLK110	<i>B. pendula</i>	175/190 × 177/183	38:39:29:37	1:1:1:1	1.76	0.62	2
BL78	<i>B. pendula</i>	260/268 × 264/272	39:44:29:30	1:1:1:1	4.42	0.22	10
Z72433	<i>Betula verrucosa</i>	168/180 × 168/170	36:24:45:35	1:1:1:1	6.34	0.10	1
AJ417975c	<i>Corylus avellana</i>	215/247 × 203/null	41:31:36:30	1:1:1:1	2.23	0.53	2

^zLinkage group assignments based on cosegregation with the markers of Mehlenbacher et al., (2006).

^yObserved segregation deviates from expected Mendelian ratios.

and two primer pairs from *Alnus* was investigated using 69 accessions of the Betulaceae (Table 5). Amplification of *Betula* accessions with individual SSR primer pairs ranged from 40% to 100%, and exceeded 80% for 44 of the 52 primer pairs. Amplification using *Betula* primer pairs averaged 92.3% in *Betula*, 50.6% in *Alnus*, 41.2% in *Corylus*, 36.5% in *Carpinus*, 34.0% in *Ostrya*, and 34.6% in *Ostryopsis*. Average amplification in the five other genera was 41.6%, and the overall average in the six genera was 56.3%. Amplification of *Corylus* accessions exceeded 80% at 14 of the 52 *Betula* SSR primer pairs. These values represent a useful level of transferability of *Betula* SSR primer pairs across the Betulaceae. Of the two primer pairs from *Alnus*, amplification with AGAG164a was excellent overall, while AGAG164b was successful only in nine of the 10 *Alnus* accessions.

TRANSFERABILITY OF *BETULA* SSR PRIMER PAIRS COMPARED BY ORIGIN. The 52 *Betula* SSR primer pairs studied include 2 from gene sequences, 10 from expressed sequence tags (ESTs), and 40 from genomic sequences. Z72433 developed from the *Bet v*

1 gene sequence amplified all accessions in all genera. AJ490266 developed from the *MADS2* gene amplified 100% of the *Betula* accessions and 94% of the accessions in other genera. These values indicate that the sequences of *Bet v* 1 and *MADS2* are highly conserved in the Betulaceae. The 10 primer pairs developed from EST sequences amplified 70% to 100% of the *Betula* accessions and 0% to 86% of the accessions in other genera. The 40 primer pairs from genomic sequences amplified 40% to 100% of the *Betula* accessions and 0% to 100% of the accessions in other genera.

TRANSFERABILITY OF *CORYLUS* SSR PRIMER PAIRS. The transferability of *Corylus* primer pairs was investigated with two sets of templates. The full set of 69 Betulaceae accessions was used to study transferability of 75 *Corylus* primer pairs (Table 5), and a subset of 32 accessions was used to study an additional 147 *Corylus* primer pairs (Table 6). For the 75 primer pairs used to amplify 69 accessions, average amplification was 81.4% in *Corylus*, 54.5% in *Carpinus*, 52.9% in *Ostrya*, 51.3% in *Ostryopsis*, 40.5% in *Alnus*, and 38.9% in *Betula*. Average

Table 5. Amplification of 69 accessions of the Betulaceae using microsatellite markers developed from *Betula*, *Alnus*, and *Corylus* sequences.

Locus	GenBank accession no.	Accessions amplified by genus (no.)						Amplification (%)		
		<i>Alnus</i> (n = 10)	<i>Betula</i> (n = 20)	<i>Corylus</i> (n = 26)	<i>Carpinus</i> (n = 8)	<i>Ostrya</i> (n = 3)	<i>Ostryopsis</i> (n = 2)	in <i>Betula</i>	in other genera	Total
Markers from <i>Betula</i> database sequences (52)										
Bp01	AB084473	9	20	0	1	0	0	100	20.41	43.48
Bp04	AB084474	10	20	13	7	3	0	100	67.35	76.81
Bp07	AB084475	10	17	20	8	2	1	85	83.67	84.06
BpTA	AB084476	0	20	2	3	0	0	100	10.20	36.23
Bp10	AB084477	0	20	3	0	1	0	100	8.2	34.8
Bp11	AB084478	10	20	0	0	0	0	100	20.41	43.48
Bp13	AB084480	0	18	0	0	0	0	90	0.00	26.09
Bp15	AB084482	0	19	0	0	0	0	95	0.00	27.54
Bp16	AB084483	10	20	24	7	3	2	100	93.88	95.65
BpA	AB084484	2	19	7	0	0	0	95	18.37	40.58
Bmax544	AB094098	5	20	26	7	2	2	100	85.71	89.86
Bmax624	AB094100	9	20	26	4	0	0	100	79.59	85.51
Bmax630	AB094101	8	20	25	3	1	0	100	75.51	82.61
Bmax671	AB094102	0	13	1	0	0	0	65	2.04	20.29
Bmax097	AB094104	9	20	26	8	3	2	100	97.96	98.55
L2.2	AF310846	0	20	0	0	1	0	100	2.04	30.43
L2.5	AF310848	9	20	0	1	0	2	100	24.49	46.38
L2.7	AF310850	7	20	18	5	3	2	100	71.43	79.71
L3.1	AF310851	8	20	20	6	2	2	100	77.55	84.06
L3.4	AF310852	7	20	6	4	0	1	100	36.73	55.07
L7.1	AF310854	10	20	25	6	3	2	100	93.88	95.65
L7.4	AF310855	0	17	0	0	0	0	85	0.00	24.64
L1.10	AF310856	10	20	23	8	3	2	100	93.88	95.65
L4.4	AF310858	0	12	1	0	0	0	60	2.04	18.84
—	AF310860	5	8	15	1	0	0	40	42.86	42.03
L5.1	AF310861	0	20	0	0	0	0	100	0.00	28.99
L5.4	AF310862	9	20	20	5	2	2	100	77.55	84.06
L5.5	AF310863	10	20	26	8	3	2	100	100.00	100.00
L7.3	AF310864	1	14	0	0	0	0	70	2.04	21.74
L7.8	AF310866	9	20	26	4	1	2	100	85.71	89.86
L13.1	AF310871	9	20	25	7	3	2	100	93.88	95.65
L63	AF310873	1	20	0	0	0	0	100	2.04	30.43
L022	AF310874	9	20	0	1	0	0	100	20.41	43.48
L021	AF310877	0	19	21	2	0	0	95	46.94	60.87
—	AJ490266	10	20	26	8	3	2	100	100.00	100.00
Bo.F394	AY423608	1	19	5	1	0	0	95	14.29	37.68
Bo.F330	AY423611	10	20	26	8	3	2	100	100.00	100.00
—	AY423613	1	20	0	0	0	0	100	2.04	30.43
—	AY423614	0	15	0	0	0	0	75	0.00	21.74
Bo.G182	AY423617	8	20	26	7	3	2	100	93.88	95.65
—	AY423621	1	18	0	0	0	0	90	2.04	27.54
—	CD276757	0	16	0	0	0	0	80	0.00	23.19
—	CD276825	0	17	0	0	0	0	85	0.00	24.64
—	CD276827	3	15	0	0	0	0	75	6.12	26.09
—	CD277232	4	20	7	3	1	0	100	30.61	50.72
—	CD277302	5	20	22	5	1	0	100	67.35	76.81
—	CD277382	6	14	0	0	0	0	70	12.2	29.0
—	CD278084	0	19	0	0	0	0	95	0.00	27.54
—	CD278264	9	18	22	6	3	2	90	85.71	86.96
—	CD278733	9	18	0	1	0	0	90	20.41	40.58
—	CD278994	0	15	0	0	0	0	75	0.00	21.74
—	Z72433	10	20	24	7	3	2	100	93.88	95.65
Mean of 52 loci (no.)		5.06	18.46	10.71	2.92	1.02	0.69	18.46	20.40	38.87
Mean of 52 loci (%)		50.6	92.3	41.2	36.5	34.0	34.6	92.3	41.6	56.3

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Table 5. Continued.

Locus	GenBank accession no.	Accessions amplified by genus (no.)						Amplification (%)		
		<i>Alnus</i> (n = 10)	<i>Betula</i> (n = 20)	<i>Corylus</i> (n = 26)	<i>Carpinus</i> (n = 8)	<i>Ostrya</i> (n = 3)	<i>Ostryopsis</i> (n = 2)	in <i>Alnus</i>	in other genera	Total
Markers from <i>Alnus</i> database sequences										
AGAG164a	Y08436	10	19	26	8	3	2	100	98.3	98.6
AGAG164b	Y08436	9	0	0	0	0	0	90	0.0	13.0
Mean of two loci (no.)		9.5	9.5	13	4	1.5	1			
Mean of two loci (%)		95.0	47.5	50.0	50.0	50.0	50.0	95.0	49.2	55.8
Markers from <i>Corylus</i> database sequences										
AJ417975b	AJ417975	1	0	20	0	0	0	76.9	2.3	30.4
AJ417975c	AJ417975	0	0	12	2	1	1	46.2	9.3	23.2
Mean of two loci (no.)		0.50	0.00	16.00	1.00	0.50	0.50			
Mean of two loci (%)		5.0	0	61.5	12.5	16.7	25.0	61.5	5.8	26.8
Markers from <i>Corylus</i> enriched genomic libraries										
A014a	—	1	0	23	6	3	2	88.5	27.9	50.7
A040	—	0	0	15	1	2	2	57.7	4.7	24.6
B005	—	10	19	26	8	3	2	100.0	97.7	98.6
B010	—	5	11	25	5	1	2	96.2	55.8	71.0
B028	—	4	0	26	2	0	0	100.0	14.0	46.4
B029b	—	4	13	26	5	1	0	100.0	53.5	71.0
B105	—	6	0	20	1	0	0	76.9	20.9	42.0
B111	—	1	0	15	0	0	0	57.7	2.3	23.2
B502	—	1	4	25	6	1	0	96.2	27.9	53.6
B505	—	3	11	26	7	1	0	100.0	51.2	69.6
B507	—	10	13	26	8	3	2	100.0	83.7	89.9
B508	—	2	13	21	8	3	2	80.8	65.1	71.0
B602	—	7	20	26	8	2	2	100.0	90.7	94.2
B606	—	0	0	20	5	3	2	76.9	23.3	43.5
B613	—	2	2	25	2	0	0	96.2	14.0	44.9
B617	—	10	9	26	5	3	1	100.0	72.1	82.6
B619	—	10	9	26	6	3	2	100.0	74.4	84.1
B634	—	10	20	26	7	3	2	100.0	97.7	98.6
B641a	—	0	20	25	1	3	0	92.3	55.8	69.6
B641b	—	0	2	20	6	3	2	76.9	30.2	47.8
B648	—	6	13	26	6	0	0	100.0	58.1	73.9
B656	—	1	0	24	1	0	0	92.3	4.7	37.7
B657	—	0	16	26	3	0	2	100.0	48.8	68.1
B664	—	10	19	26	8	3	2	100.0	97.7	98.6
B665	—	6	9	25	2	1	2	96.2	46.5	65.2
B702a	—	10	19	23	8	3	2	92.3	97.7	95.7
B709	—	10	15	26	8	3	2	100.0	88.4	92.8
B719	—	5	9	24	5	0	0	92.3	46.5	63.8
B720	—	9	1	26	6	3	1	100.0	46.5	66.7
B732	—	0	2	26	1	0	0	100.0	7.0	42.0
B733	—	3	5	24	3	3	0	88.5	27.9	50.7
B741	—	3	0	26	7	3	1	100.0	32.6	58.0
B749	—	0	19	26	1	0	1	100.0	48.8	68.1
B751	—	6	14	25	3	2	1	96.2	65.1	76.8
B753	—	0	2	22	1	1	0	84.6	9.3	37.7
B762	—	6	12	25	6	1	2	96.2	62.8	75.4
B773	—	3	5	24	3	3	0	92.3	32.6	55.1
B774	—	1	0	26	7	0	0	96.2	18.6	49.3
B776	—	4	11	26	6	2	2	100.0	60.5	75.4
B779	—	3	14	26	8	3	2	100.0	67.4	79.7
B789	—	4	11	26	8	2	2	100.0	62.8	76.8
B791	—	5	1	24	3	2	2	92.3	30.2	53.6
B793	—	9	20	26	7	3	0	100.0	93.0	95.7
C028	—	7	13	26	8	3	2	100.0	76.7	85.5

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Table 5. Continued.

Locus	GenBank accession no.	Accessions amplified by genus (no.)						Amplification (%)		
		<i>Alnus</i> (n = 10)	<i>Betula</i> (n = 20)	<i>Corylus</i> (n = 26)	<i>Carpinus</i> (n = 8)	<i>Ostrya</i> (n = 3)	<i>Ostryopsis</i> (n = 2)	in <i>Alnus</i>	in other genera	Total
C040	—	4	14	25	6	3	2	96.2	62.8	75.4
C115	—	1	5	24	7	2	2	92.3	39.5	59.4
C118	—	10	19	22	5	3	2	84.6	90.7	88.4
C119	—	9	19	26	8	3	2	100.0	95.3	97.1
C504	—	10	19	26	8	3	2	100.0	97.7	98.6
Mean of 59 loci (no.)		3.92	8.00	20.25	4.24	1.58	1.00			
Mean of 59 loci (%)		39.2	40.0	77.9	53.0	52.5	50.0	77.8	43.7	56.6
Markers from <i>Corylus</i> internal repeats in ISSR fragments										
KG802	—	3	11	23	5	1	0	88.5	46.5	62.3
KG803	—	10	19	26	8	2	2	100.0	95.3	97.1
KG806	—	2	2	26	8	2	0	100.0	32.6	58.0
KG807	—	9	18	26	8	3	2	100.0	93.0	95.7
KG808	—	10	20	26	8	3	2	100.0	100.0	100.0
KG809	—	5	6	26	3	2	2	100.0	41.9	63.8
KG810	—	2	7	26	3	1	2	100.0	32.6	58.0
KG813	—	4	9	26	4	2	1	100.0	44.2	65.2
KG815	—	10	6	25	7	2	2	96.2	62.8	75.4
KG816	—	2	7	26	6	3	1	100.0	44.2	65.2
KG817	—	8	6	26	3	0	0	100.0	39.5	62.3
KG819	—	0	1	26	5	2	1	100.0	20.9	50.7
KG821	—	7	0	26	7	2	2	100.0	41.9	63.8
KG822	—	0	0	26	0	0	0	100.0	0.0	37.7
Mean of 14 loci (no.)		5.14	8.00	25.71	5.36	1.79	1.21			
Mean of 14 loci (%)		51.4	40.0	98.9	67.0	59.5	60.7	98.9	50.0	68.4
Mean of 75 <i>Corylus</i> loci (%)		40.5	38.9	81.4	54.5	52.9	51.3	81.4	43.8	57.9

amplification of other genera was 43.8%, and the overall average was 57.9%. Amplification of *Corylus* accessions at individual SSR primer pairs ranged from 46.2% to 100%, and was lower in the two primer pairs developed from the *Lox* gene sequence than from enriched libraries or ISSR fragments. Amplification of *Corylus* exceeded 80% at 57 of the 75 primer pairs. Over all 69 accessions, amplification exceeded 80% at 17 of the 75 primer pairs. For the two SSR primer pairs developed from the *Corylus* *lox* gene (AJ417975b and AJ417975c), amplification was 76.9% and 46.2% in *Corylus* accessions, and 2.3% and 4.6% in accessions of other genera, respectively.

For the 147 *Corylus* primer pairs used to amplify the subset of 32 accessions (Table 6), average amplification was 91.9% in *Corylus*, 32.9% in *Carpinus*, 32.7% in *Ostrya*, 43.9% in *Ostryopsis*, 34.9% in *Alnus*, and 53.9% in *Betula*. Average amplification of other genera was 43.0%, and the overall average was 58.3%. In genera other than *Corylus*, average amplification at polymorphic primer pairs developed from enriched libraries (33.2%) was lower than for polymorphic primer pairs developed from ISSR sequences, monomorphic primer pairs, and primer pairs that amplified more than one locus. Amplification exceeded 80% at 127 of the 147 primer pairs in *Corylus*, and at 34 of the primer pairs in other genera. As with the *Betula* SSR primer pairs, the values for these two sets of *Corylus* primer pairs represent a useful level of transferability across the Betulaceae.

As expected, amplification was higher in species of the genus from which the SSR locus was developed than in the other genera. The *Betula* primer pairs amplified 92.3% of the *Betula* accessions and 41.2% of the *Corylus* accessions. The first set

of *Corylus* primer pairs amplified 80.8% of the *Corylus* accessions and 38.4% of the *Betula* accessions. The second set of *Corylus* primer pairs amplified 91.9% of the *Corylus* accessions and 53.9% of the *Betula* accessions. Recent comparative genomic research has shown that despite vast diversity in angiosperms, plants have retained homologous genes with the same or very similar function during evolution. Homoeologous chromosomes in allopolyploid species and the chromosomes of closely related diploid species are extensively conserved (Bennetzen, 2000; Paterson et al., 2007). Comparisons of related species have shown gene synteny and marker colinearity with some chromosome rearrangements through translocation or inversions. Varshney et al. (2005) found that of 165 barley (*Hordeum vulgare*) EST-SSR loci studied, 78.2% showed amplification in wheat, followed by 75.2% in rye (*Secale cereale*), and 42.4% in rice (*Oryza sativa*). Heesacker et al. (2008) also reported that 88.6% of the 466 sunflower (*Helianthus annuus*) EST-SSR or INDEL markers amplified alleles from one or more wild species, whereas 14.8% amplified alleles from distantly related safflower (*Carthamus tinctorius*), and 14.4% amplified alleles from lettuce (*Lactuca sativa*), a distantly related genus in the Asteraceae. Dirlewanger et al. (2004) compared maps for *Prunus* and *Malus* using 30 homologous SSR markers. Barbara et al. (2007) reviewed 64 microsatellite marker transferability studies and found an average cross-species transferability success rate of about 60% in eudicots and about 40% in monocots, whereas between genera, it was only $\approx 10\%$ for eudicots. Our results show high transferability (38%–54%) in the Betulaceae that is unaffected by the polyploidy of some *Betula* accessions.

Table 6. Amplification of 32 Betulaceae accessions at 147 microsatellite loci from *Corylus*.

Locus	Accessions amplified by genus (no.)						Amplification (%) in		
	<i>Alnus</i> (n = 6)	<i>Betula</i> (n = 9)	<i>Corylus</i> (n = 10)	<i>Carpinus</i> (n = 3)	<i>Ostrya</i> (n = 2)	<i>Ostryopsis</i> (n = 2)	<i>Corylus</i>	Other Genera	Total
Polymorphic loci from enriched libraries (n = 54)									
A024b	0	6	7	0	0	0	70	27.3	40.6
A036	6	9	8	3	2	2	80	100.0	93.8
A601	0	2	10	0	0	1	100	13.6	40.6
A602	2	0	10	3	0	0	100	22.7	46.9
A604	6	8	10	3	2	2	100	95.5	96.9
A605	1	9	10	0	0	0	100	45.5	62.5
A606	1	0	8	0	2	0	80	13.6	34.4
A611	1	4	10	0	0	1	100	27.3	50.0
A613	4	9	10	3	2	2	100	90.9	93.8
A614	4	9	10	0	0	0	100	59.1	71.9
A616	6	7	10	3	2	2	100	90.9	93.8
A635	0	0	10	0	0	0	100	0.0	31.3
A640	0	3	10	0	0	0	100	13.6	40.6
B001	6	0	10	0	0	1	100	31.8	53.1
B501	0	1	10	0	1	1	100	13.6	40.6
B603	0	9	9	0	0	0	90	40.9	56.3
B604	6	0	9	3	1	2	90	54.5	65.6
B608	0	0	8	0	1	0	80	4.5	28.1
B628	0	7	10	0	0	1	100	36.4	56.3
B629	0	0	10	0	0	0	100	0.0	31.3
B631	6	9	10	3	2	2	100	100.0	100.0
B635	0	6	10	3	1	0	100	45.5	62.5
B639	1	9	9	2	1	1	90	63.6	71.9
B640	1	7	9	0	0	0	90	36.4	53.1
B643	0	8	9	0	0	0	90	36.4	53.1
B651	0	0	10	0	1	1	100	9.1	37.5
B652	0	1	10	0	0	0	100	4.5	34.4
B654	1	0	10	0	0	1	100	9.1	37.5
B655	0	0	10	0	0	0	100	0.0	31.3
B660	6	9	10	0	0	1	100	72.7	81.3
B662	1	9	10	0	0	0	100	45.5	62.5
B667	0	0	7	0	0	0	70	0.0	21.9
B670	0	1	10	0	0	0	100	4.5	34.4
B671	1	3	10	0	0	1	100	22.7	46.9
B686	4	9	5	1	2	1	50	77.3	68.8
B701	6	9	9	0	2	2	90	86.4	87.5
B706	0	9	8	2	2	2	80	68.2	71.9
B716	0	0	10	0	0	0	100	0.0	31.3
B726	0	5	8	0	0	0	80	22.7	40.6
B728	0	0	9	0	0	1	90	4.5	31.3
B734	2	3	10	0	1	0	100	27.3	50.0
B735	6	9	7	3	2	2	70	100.0	90.6
B737	0	0	9	0	0	0	90	0.0	28.1
B738	0	0	10	0	0	0	100	0.0	31.3
B760	0	0	10	0	0	0	100	0.0	31.3
B761	0	0	10	0	0	0	100	0.0	31.3
B767	0	8	9	0	0	0	90	36.4	53.1
B768	1	1	10	0	0	1	100	13.6	40.6
B769	3	3	10	0	0	0	100	27.3	50.0
B770	0	0	10	0	0	0	100	0.0	31.3
B777	0	9	10	0	0	1	100	45.5	62.5
B788	2	4	9	0	0	0	90	27.3	46.9
B790	1	4	10	0	0	0	100	22.7	46.9
B795	0	0	10	0	0	0	100	0.0	31.3

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Table 6. Continued.

Locus	Accessions amplified by genus (no.)						Amplification (%) in		
	<i>Alnus</i> (n = 6)	<i>Betula</i> (n = 9)	<i>Corylus</i> (n = 10)	<i>Carpinus</i> (n = 3)	<i>Ostrya</i> (n = 2)	<i>Ostryopsis</i> (n = 2)	<i>Corylus</i>	Other Genera	Total
Mean (no.)	1.6	4.0	9.4	0.6	0.5	0.6			
Mean (%)	26.2	44.9	93.7	19.8	25.0	29.6	93.7	33.2	52.1
Polymorphic loci from ISSR fragments (n = 50)									
KG800	1	9	10	3	2	0	100	68.2	78.1
KG801	0	1	10	0	0	1	100	9.1	37.5
KG803	0	5	10	3	0	0	100	36.4	56.3
KG804	0	0	10	0	0	0	100	0.0	31.3
KG805	0	0	8	0	0	0	80	0.0	25.0
KG811	0	0	10	0	0	0	100	0.0	31.3
KG812	5	0	10	1	0	0	100	27.3	50.0
KG814	6	9	10	3	2	2	100	100.0	100.0
KG820	1	0	9	0	0	1	90	9.1	34.4
KG823	6	9	9	3	2	2	90	100.0	96.9
KG824	6	3	9	3	2	2	90	72.7	78.1
KG825	0	9	8	0	1	2	80	54.5	62.5
KG826	6	9	8	3	0	2	80	90.9	87.5
KG827	0	5	10	2	0	2	100	40.9	59.4
KG828	6	9	9	3	1	2	90	95.5	93.8
KG829	2	9	10	0	1	1	100	59.1	71.9
KG830	6	9	10	3	2	2	100	100.0	100.0
KG831	0	3	7	1	0	0	70	18.2	34.4
KG832	3	9	10	3	2	0	100	77.3	84.4
KG833	1	1	9	0	0	0	90	9.1	34.4
KG834	0	0	10	0	2	2	100	18.2	43.8
KG836	3	3	8	0	1	0	80	31.8	46.9
KG838	6	8	7	2	1	2	70	86.4	81.3
KG840	5	9	10	0	0	0	100	63.6	75.0
KG841	4	7	10	2	0	0	100	59.1	71.9
KG842	4	9	10	0	0	2	100	68.2	78.1
KG843	2	9	10	3	2	2	100	81.8	87.5
KG844	4	3	10	0	0	1	100	36.4	56.3
KG845	0	1	10	0	0	1	100	9.1	37.5
KG846	3	0	10	2	0	1	100	27.3	50.0
KG847	0	5	10	0	0	2	100	31.8	53.1
KG848	0	2	10	0	0	0	100	9.1	37.5
KG851	3	6	10	1	1	2	100	59.1	71.9
KG852	4	6	10	0	0	2	100	54.5	68.8
KG854	6	9	7	3	2	2	70	100.0	90.6
KG855	6	9	5	3	2	2	50	100.0	84.4
KG857	0	0	10	0	0	0	100	0.0	31.3
KG858	6	9	10	1	0	2	100	81.8	87.5
KG859	6	9	10	1	2	1	100	86.4	90.6
KG860	5	7	10	0	1	2	100	68.2	78.1
KG861	0	1	10	0	0	0	100	4.5	34.4
KG864	2	9	6	0	0	1	60	54.5	56.3
KG865	6	9	5	3	2	2	50	100.0	84.4
KG867	1	7	10	0	1	1	100	45.5	62.5
KG868	6	9	8	3	2	2	80	100.0	93.8
KG869	0	1	10	0	0	0	100	4.5	34.4
KG870	1	9	9	1	2	2	90	68.2	75.0
KG873	4	8	9	2	1	2	90	77.3	81.3
KG874	4	8	10	2	1	1	100	72.7	81.3
W07-SSR	6	9	6	3	2	2	60	100.0	87.5
Mean (no.)	2.9	5.6	9.1	1.3	0.8	1.2			
Mean (%)	48.7	62.2	91.2	42.0	40.0	58.0	91.2	53.4	65.2

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Table 6. Continued.

Locus	Accessions amplified by genus (no.)						Amplification (%) in		
	<i>Alnus</i> (n = 6)	<i>Betula</i> (n = 9)	<i>Corylus</i> (n = 10)	<i>Carpinus</i> (n = 3)	<i>Ostrya</i> (n = 2)	<i>Ostryopsis</i> (n = 2)	<i>Corylus</i>	Other Genera	Total
Loci monomorphic in <i>Corylus avellana</i> (n = 17)									
B620	0	0	10	3	0	2	100	22.7	46.9
B702b	0	7	10	0	0	0	100	31.8	53.1
B747	6	9	6	0	1	0	60	72.7	68.8
SSR 011	0	2	10	0	1	1	100	18.2	43.8
SSR 014	0	9	10	0	0	0	100	40.9	59.4
SSR 022	3	9	10	1	2	2	100	77.3	84.4
SSR 025	2	3	8	1	0	1	80	31.8	46.9
SSR 064	3	9	10	3	2	2	100	86.4	90.6
SSR 075	0	0	10	0	0	2	100	9.1	37.5
SSR 104	0	1	10	0	0	0	100	4.5	34.4
SSR 110F	1	4	10	0	1	1	100	31.8	53.1
K18–28–24	0	9	10	0	0	1	100	45.5	62.5
K56–28–33	0	2	10	0	0	0	100	9.1	37.5
K57–1–32	0	0	10	0	0	0	100	0.0	31.3
K62–15–12	0	9	7	0	0	1	70	45.5	53.1
K76–2–10	6	9	9	3	2	2	90	100.0	96.9
K76–28–26	6	9	8	3	2	2	80	100.0	93.8
Mean (no.)	1.6	5.4	9.3	0.8	0.6	1.0			
Mean (%)	26.5	59.5	92.9	27.5	32.4	50.0	92.9	42.8	58.5
Primer pairs that amplify two or more loci (n = 26)									
A003a	1	9	9	1	0	0	90	50.0	62.5
A017	0	1	7	0	0	0	70	4.5	25.0
A038	6	8	10	0	0	0	100	63.6	75.0
A607	0	1	10	0	0	0	100	4.5	34.4
A609	4	6	9	3	2	2	90	77.3	81.3
A617	0	0	10	0	0	0	100	0.0	31.3
A618	1	9	10	1	0	0	100	50.0	65.6
A620	0	2	6	2	0	0	60	18.2	31.3
A637	0	3	10	0	0	1	100	18.2	43.8
B013	0	0	10	0	1	0	100	4.5	34.4
B661	0	7	10	2	2	2	100	59.1	71.9
B666	6	9	5	3	2	2	50	100.0	84.4
B677	0	2	7	0	0	0	70	9.1	28.1
B682	5	4	9	3	2	2	90	72.7	78.1
B683	0	1	10	3	0	1	100	22.7	46.9
B690	0	2	10	2	0	1	100	22.7	46.9
B691	6	9	10	0	0	0	100	68.2	78.1
B713	0	1	10	1	0	1	100	13.6	40.6
B742	1	4	9	0	1	1	90	31.8	50.0
B781	0	9	6	3	2	2	60	72.7	68.8
SSR 020	5	9	10	3	2	2	100	95.5	96.9
SSR 053	2	3	7	1	0	0	70	27.3	40.6
SSR 077	1	8	7	3	0	2	70	63.6	65.6
K51–1–20	6	8	10	2	2	1	100	86.4	90.6
K79–1–21	6	9	10	3	2	2	100	100.0	100.0
K88–28–3	0	0	10	0	0	0	100	0.0	31.3
Mean (no.)	1.9	4.8	8.9	1.4	0.7	0.8			
Mean (%)	32.1	53.0	88.8	46.2	34.6	42.3	88.8	43.7	57.8
Overall mean (no.)	2.1	4.9	9.2	1.0	0.7	0.9			
Overall mean (%)	34.9	53.9	91.9	32.9	32.7	43.9	91.9	43.0	58.3

RELATIONSHIP OF TRANSFERABILITY AND TAXONOMY. Results of SSR transferability are in agreement with the generally accepted taxonomy of the Betulaceae. Traditionally, Betula-

ceae is divided into two subfamilies, Betuloideae (*Alnus* and *Betula*) and Coryloideae (*Corylus*, *Carpinus*, *Ostrya*, and *Ostryopsis*) (Chen et al., 1999). The organelle sequences of

Chen et al. (1999) support this classification. *Betula* species were studied by Järvinen et al. (2004) and Schenk et al. (2008), *Alnus* species by Chen and Li (2004) and Navarro et al. (2003), *Carpinus* by Yoo and Wen (2002), and *Corylus* by Erdoğan and Mehlenbacher (2000) and Forest and Bruneau (2000). Our results indicate better amplification within the subfamily of origin, and lower amplification in the other subfamily.

We developed 20 new SSR primer pairs from GenBank sequences of the Betulaceae. Of these, 12 amplified and were polymorphic in hazelnut, and nine were assigned to linkage groups. We studied the transferability of 276 SSR primer pairs in the Betulaceae. Our study shows that SSR markers developed for *Betula* and *Corylus* are highly transferable across species within the genus of origin, and many are also transferable across genera in the Betulaceae. Transferable and polymorphic loci are well suited for genome studies in the Betulaceae and will allow alignment of the linkage maps for silver birch (*B. pendula*) and european hazelnut.

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