Development of Microsatellites from Fothergilla ×intermedia (Hamamelidaceae) and Cross Transfer to Four other Genera within Hamamelidaceae

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PRIMER NOTE

DEVELOPMENT OF MICROSATELLITES FROM *FOTHERGILLA* ×*INTERMEDIA* (HAMAMELIDACEAE) AND CROSS TRANSFER TO FOUR OTHER GENERA WITHIN HAMAMELIDACEAE[1]

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- *Premise of the study:* We developed microsatellites from *Fothergilla* ×*intermedia* to establish loci capable of distinguishing species and cultivars, and to assess genetic diversity for use by ornamental breeders and to transfer within Hamamelidaceae.
- *Methods and Results:* We sequenced a small insert genomic library enriched for microsatellites to develop 12 polymorphic microsatellite loci. The number of alleles detected ranged from four to 15 across five genera within Hamamelidaceae. Shannon's information index ranged from 0.07 to 0.14.
- *Conclusions:* These microsatellite loci provide a set of markers to evaluate genetic diversity of natural and cultivated collections and assist ornamental plant breeders for genetic studies of five popular genera of woody ornamental plants.

Key words: *Corylopsis*; Hamamelidaceae; *Hamamelis*; *Loropetalum*; *Parrotia*; simple sequence repeats.

Hamamelidaceae comprises 31 genera and more than 140 species (Li et al., 1999) and includes several ornamental genera within *Corylopsis* Siebold & Zucc., *Fothergilla* L., *Hamamelis* L., *Loropetalum* R. Br., and *Parrotia* C. A. Mey. *Fothergilla* has been used in ornamental plantings for over two centuries and there are fewer than 15 cultivars, whereas *Loropetalum* has 19 cultivars and *Corylopsis* and *Parrotia* have five or fewer cultivars (Dirr, 1998). *Hamamelis* species are used as an astringent and are more widely recognized, with more than 75 cultivars (Marquard et al., 1997). Many cultivars from these genera are commercially available, but the pedigrees are not well known as they are often selected from spontaneous mutations, wild-grown seedlings, or open-pollinated crosses, as is the case with *Hamamelis* (Marquard et al., 1997).

The phylogeny of Hamamelidaceae has been examined and, based on nrDNA ITS sequences, a well-supported phylogeny with three clades was resolved (Li et al., 1999). *Fothergilla*, *Hamamelis*, and *Parrotia* were in one clade, whereas *Corylopsis* and *Loropetalum* were in a different clade. *Corylopsis*, *Loropetalum*, and *Parrotia* are native to Asia. *Loropetalum chinense* (R. Br.) Oliv. is found in Japan and southeastern China, whereas *L. lanceum* Hand.-Mazz. is more widely distributed throughout Japan, China, and northeastern India (Zhang et al., 2003). Only four populations of *L. subcordatum* (Benth.) Oliv. remain, making it one of the most endangered angiosperm species in China (Gong et al., 2010). *Parrotia persica* (DC.) C. A. Mey. is a deciduous tree endemic to northern Iran; it is the only extant species in the genus and could become a conservation concern if habitat destruction continues (Sefidi et al., 2011). Species from the genus *Hamamelis* are found on both the North American and Asian continents (Zhang et al., 2003). *Fothergilla* is the only genus exclusively limited to North America. The genus is found in the southeastern United States and includes only two species, *F. major* Lodd. and *F. gardenia* L., as well as the hybrid *Fothergilla* ×*intermedia*. Both species are of conservation concern, *F. major* in Tennessee and *F. gardenii* in both Florida and Georgia (USDA, 2012).

Molecular markers can be used to determine diversity in wild populations and assist in breeding and conservation studies. The purpose of this study was to develop a microsatellite-enriched library from *Fothergilla* ×*intermedia* to establish loci capable of distinguishing species and cultivars, to assess genetic diversity for use by ornamental breeders, and to test these loci for crossover to other members of Hamamelidaceae, such as those in the related genera *Corylopsis*, *Hamamelis*, *Loropetalum*, and *Parrotia*.

METHODS AND RESULTS

Leaf tissue or unopened flower buds were obtained from the J. C. Raulston Arboretum at North Carolina State University (Raleigh, North Carolina, USA),

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TABLE 1. Characteristics of 12 microsatellite loci isolated from *Fothergilla* ×*intermedia* for three *Corylopsis*, 15 *Fothergilla*, 14 *Hamamelis*, two *Loropetalum*, and two *Parrotia* accessions.[a]

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	*A*	Shannon's information index	GenBank accession no.
Foth001	F: ATCCTAAAGAGCGGTCAGATTG	$(AC)_{10}$	152–198	12	0.14	KJ461123
	R: ATGATTCGAAACTGACAATCCA					
Foth002	F: GCAGCAATAGCCAAAATTATCC	$T(AC)_4AT(AC)_8(AT)_5$	180–207	11	0.11	KJ461124
	R: GGTTTCGTTGAGTTTTGAATGA					
Foth004	F: TCTTCAATTTTCTCAGCAATCAA	$(AC)_6(AT)_5$	143–177	14	0.11	KJ461125
	R: AACTCAAGGGAAAAACCCTAAGA					
Foth009	F: CGGATTAGAAGTTGTAAAATTTTGGT	$(TC)_5(TCTTTTTCTC)_2$	159–226	9	0.13	KJ461126
	R: GTCGACGTAGACATACCTGCAA					
Foth016	F: ACAGAAAGAAGAAAACCCCACA	$(AC)_{15}$	158–207	15	0.11	KJ461127
	R: GTGACTCTGGATTTGCCCATA					
Foth018	F: TCTTCTTCAGGAGTCCATAGCC	$(GT)_{17}(GA)_{15}$	163–208	14	0.11	KJ461128
	R: ACTCTTTCCCATCTCTCCGATT					
Foth021	F: AACTTATTTGGATTTTGGTTTTTGA	$(TG)_9CGA(GT)_7$	114–163	8	0.11	KJ461129
	R: CAAACTCAAAAATAGATGGGTTTTC					
Foth027	F: TTTGAAGTCTTTATAGGGAAGAGC	$(TG)_{12}$	111–138	9	0.12	KJ461130
	R: CAAAAATTTTATCAAATGAAATGCAC					
Foth029	F: AAGGGTTTTGTAAAATGGTCTCA	$(CA)_6CC(CA)_5$	156–159	4	0.07	KJ461131
	R: TAACAGATGAATCCACCTTAGCC					
Foth032	F: CAACCAGGCTACTACCAAATTCA	$(CA)_7CG(CA)_6$	128–209	14	0.11	KJ461132
	R: CGGTGGACATTACATGATGATAG					
Foth040	F: TCAAAATACTATCGGCTGTGTGA	$(TG)_{13}$	147–177	9	0.10	KJ461133
	R: ATGCGAGGTATTAGAATTGGACA					
Foth045	F: TCTTCTTCTGTGGCTAAGTGGAG	$(TG)_{13}$	175–211	9	0.09	KJ461134
	R: TATTTGAATGCCCATTATCCATT					

Note: *A* = number of alleles.
[a]Optimum annealing temperature was 55°C.

Spring Grove Cemetery and Arboretum (Cincinnati, Ohio, USA), Arnold Arboretum of Harvard University (Boston, Massachusetts, USA), and University of Tennessee Gardens (Knoxville, Tennessee, USA). Approximately 100 mg of tissue was homogenized in 2.0-mL microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) containing silica beads (2.3 mm; BioSpec Products, Bartlesville, Oklahoma, USA) and frozen in liquid nitrogen for 5 min followed by agitation in a Bio101 FastPrep Homogenization System FP120 (Thermo Savant, Waltham, Massachusetts, USA) for 30 s at the 5.0 speed setting and freezing and agitation were repeated once. DNA was isolated using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) with the following modifications: 2% (w/v) insoluble polyvinylpyrrolidone (PVP) and 6 µL of RNase were added to 600 µL of AP1 buffer and cell lysis incubation was 20 min. DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). An enriched microsatellite library was created from accession number AA#182-96*A (Appendix 1) following the procedures of Wang et al. (2007). Microsatellite-containing sequences were identified using Imperfect SSR Finder (Stieneke and Eujayl, 2007). Primer3 (Rozen and Skaletsky, 1999) was used to design 50 primer pairs, which were screened for amplification against a subset of four *Fothergilla* samples. A single 10-µL PCR reaction contained 10 ng DNA, 2.5 mM $MgCl_2$, 1× GeneAmp PCR Buffer II (Applied Biosystems, Carlsbad, California, USA), 0.2 mM dNTPs, 0.25 µM primers (forward and reverse), 5% dimethyl sulfide (DMSO; Fisher Scientific), 0.4 unit AmpliTaq Gold DNA Polymerase (Applied Biosystems), and sterile water. The reactions were amplified using the following conditions: 94°C for 3 min; 35 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 30 s; and a final extension at 72°C for 4 min. The amplicons were separated by electrophoresis through a 2% agarose gel stained with ethidium bromide. Twelve loci were polymorphic, whereas the remaining loci did not amplify, produced a smear pattern, or were monomorphic. The polymorphic loci were used to characterize a larger sample size.

The 36 accessions of Hamamelidaceae were genotyped and analyzed in the same manner as primer screening (Appendix 1). PCR products were separated using the QIAxcel Capillary Electrophoresis System (QIAGEN) and sized with

TABLE 2. Unique alleles detected at 12 microsatellite loci for five genera within Hamamelidaceae.[a]

Locus	*Corylopsis* spp. (*n* = 3)	*Fothergilla* spp. (*n* = 15)	*Hamamelis* spp. (*n* = 14)	*Loropetalum chinense* (*n* = 2)	*Parrotia persica* (*n* = 2)
Foth001	1	4	0	2	1
Foth002	1	6	2	1	0
Foth004	1	4	1	3	1
Foth009	0	5	1	—	—
Foth016	3	8	0	—	0
Foth018	0	7	2	—	0
Foth021	1	4	—	—	0
Foth027	0	2	2	—	0
Foth029	0	1	0	1	1
Foth032	0	7	1	1	0
Foth040	—	6	0	—	—
Foth045	—	9	—	—	—

Note: — = no amplification.
[a]Analyzed species and cultivars are listed in Appendix 1.

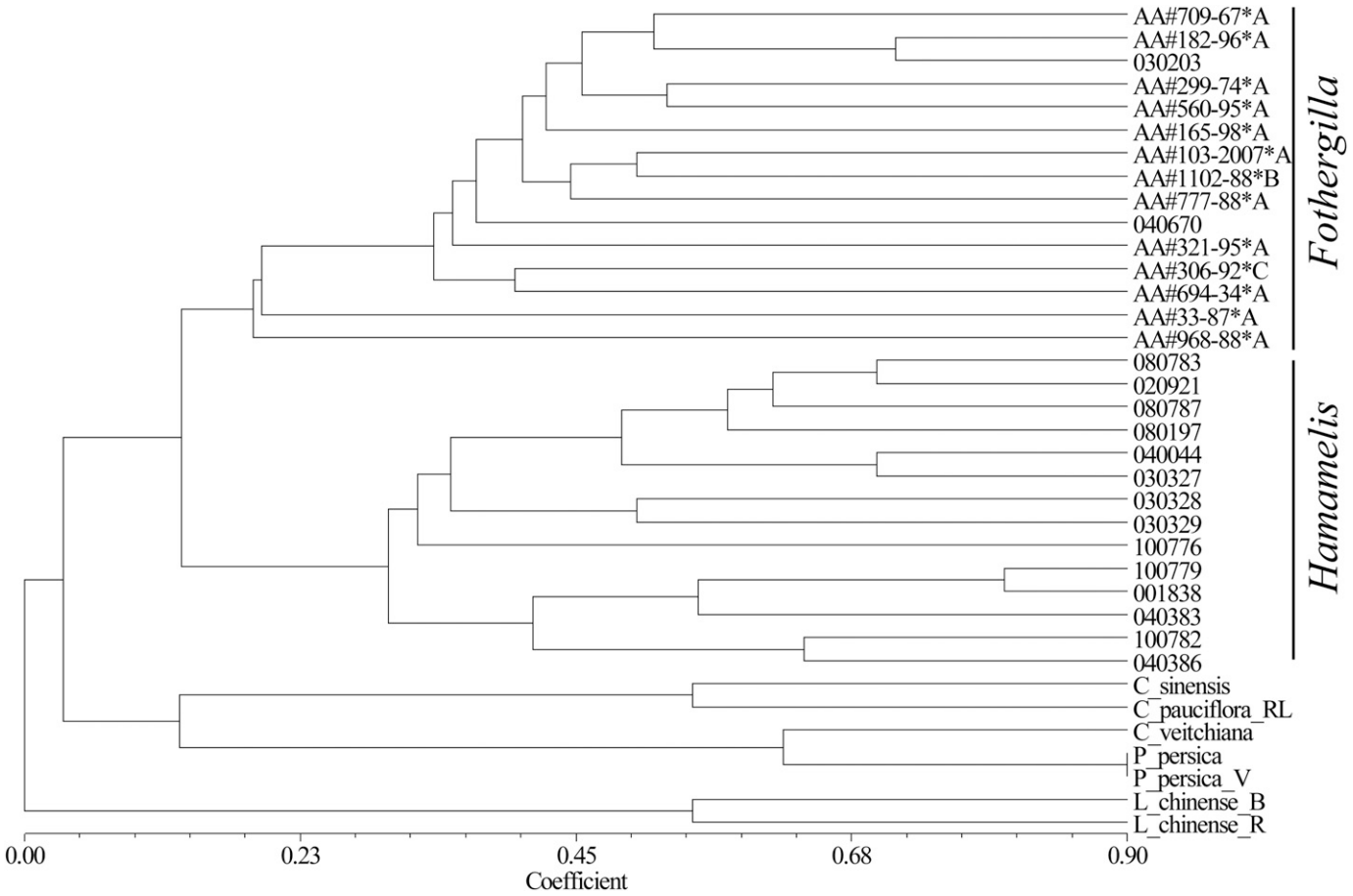

Fig. 1. Cluster analysis of three *Corylopsis*, 15 *Fothergilla*, 14 *Hamamelis*, two *Loropetalum*, and two *Parrotia* accessions (see Appendix 1) after unweighted pair group method with arithmetic mean (UPGMA) clustering using 12 microsatellite loci coded as dominant (presence/absence) markers. The similarity of the accessions was calculated using the Dice coefficient. The cophenetic correlation coefficient value ($r = 0.91$), suggested a strong fit between the Dice similarity matrix and the UPGMA dendrogram using the parameters of Sneath and Sokal (1973).

a 25–300-bp marker. Raw allele data for each individual were binned into allelic classes using FLEXIBIN (Amos et al., 2007). We used a conservative 2-bp allelic category size determination standard error range for reproducibility and the 2-bp resolution of the QIAxcel Capillary Electrophoresis System. Ploidy level varies from 4*x* to 6*x* in *Fothergilla* (Ranney et al., 2007) and 2*x* to 6*x* in *Corylopsis* as compared to the diploid *Parrotia* and *Hamamelis* species (Zhang et al., 2003). Due to ploidy variation, each allele was scored as either 0 (absent) or 1 (present), and nonamplified loci were scored as missing data. The data were analyzed using GenAlEx version 6.5 (Peakall and Smouse, 2012). For cluster analysis, genetic similarity indices were calculated for all pairwise comparisons using Dice's similarity coefficient and then clustered using the unweighted pair group method with arithmetic mean (UPGMA) using NTSYS-pc 2.20q (Rohlf, 2008). The cophenetic coefficient between the Dice similarity matrix and the UPGMA dendrogram was calculated (Rohlf, 2008).

Twelve primer pairs were polymorphic and used to genotype 36 accessions of Hamamelidaceae (Table 1). The number of alleles ranged from four (Foth029) to 15 (Foth16), and Shannon's information index ranged from 0.07 to 0.14. Five loci amplified across all genera: Foth001, Foth002, Foth004, Foth029, and Foth032. In total, 128 alleles were identified and 90 were unique to individual genera (Table 2). Foth045 amplified only *Fothergilla* accessions and 63 unique alleles were detected. Ten loci amplified in *Corylopsis*, with five loci detecting a total of seven unique alleles. Ten loci amplified in *Hamamelis*, with six loci detecting nine unique alleles. Nine loci amplified the *Parrotia* accessions and detected three unique alleles. For *Loropetalum*, five loci amplified and detected eight unique alleles. Cluster analysis grouped the accessions into five groups, which were separated by genus (Fig. 1). The cophenetic correlation coefficient value ($r = 0.91$) suggested a strong fit between the Dice similarity matrix and the UPGMA dendrogram using the parameters of Sneath and Sokal (1973).

CONCLUSIONS

We have developed the first set of microsatellites from *Fothergilla* and have demonstrated cross transfer to other species within Hamamelidaceae. These loci provide a set of markers to evaluate genetic diversity of natural and cultivated collections and assist ornamental plant breeders for genetic studies of five popular genera of woody ornamental plants.

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Appendix 1. Hamamelidaceae accessions and cultivars that were analyzed using 12 microsatellite loci.

Accession no.[a]	Species or cultivar	Source
N/A	*Corylopsis pauciflora* ‘Red Leaf’	University of Tennessee Gardens
N/A	*C. spicata*	University of Tennessee Gardens
N/A	*C. veitchiana*	University of Tennessee Gardens
AA#306-92*C	*Fothergilla gardenii* ‘Blue Mist’	Arnold Arboretum
AA#103-2007*A	*F. major*	Arnold Arboretum
AA#1102-88*B	*F. major*	Arnold Arboretum
AA#165-98*A	*F. major*	Arnold Arboretum
AA#777-88*A	*F. major*	Arnold Arboretum
AA#321-95*A	*F. major*	Arnold Arboretum
AA#299-74*A	*F. major*	Arnold Arboretum
AA#560-95*A	*F. major*	Arnold Arboretum
AA#33-87*A	*F. major*	Arnold Arboretum
AA#968-88*A	*F. major*	Arnold Arboretum
AA#694-34*A	*F. major*	Arnold Arboretum
040670	*F. ×intermedia* ‘KLMfifteen’ Red Monarch	J. C. Raulston Arboretum
AA#182-96*A	*F. ×intermedia* ‘Mount Airy’	Arnold Arboretum
AA#709-67*A	*Fothergilla* (undetermined hybrid)	Arnold Arboretum
030203	*F. ×intermedia* ‘Sea Spray’	J. C. Raulston Arboretum
080783	*Hamamelis ovalis*	J. C. Raulston Arboretum
030328	*H. mollis* ‘Wisely Supreme’	J. C. Raulston Arboretum
080197	*H. vernalis* ‘KLMT’ Orange Sunrise	J. C. Raulston Arboretum
080787	*H. vernalis* ‘Quasimodo’	J. C. Raulston Arboretum
040044	*H. virginiana* ‘Green Thumb’	J. C. Raulston Arboretum
100776	*H. virginiana* ‘Harvest Moon’	J. C. Raulston Arboretum
030327	*H. virginiana* ‘Little Suzie’	J. C. Raulston Arboretum
020921	*H. virginiana* var. *mexicana*	J. C. Raulston Arboretum
030329	*H. ×intermedia* ‘Aurora’	J. C. Raulston Arboretum
100779	*H. ×intermedia* ‘Barmstedt Gold’	J. C. Raulston Arboretum
040383	*H. ×intermedia* ‘Diane’	J. C. Raulston Arboretum
001838	*H. ×intermedia* ‘Feuerzauber’	J. C. Raulston Arboretum
100782	*H. ×intermedia* ‘Westerstede’	J. C. Raulston Arboretum
040386	*H. ×intermedia* ‘Wiero’	J. C. Raulston Arboretum
N/A	*Loropetalum chinense* ‘Burgundy’	University of Tennessee Gardens
N/A	*L. chinense* ‘Ruby’	University of Tennessee Gardens
86-T24	*Parrotia persica*	Spring Grove Cemetery and Arboretum
N/A	*P. persica* ‘Vanessa’	University of Tennessee Gardens

[a] Specimens for the samples collected at the University of Tennessee Gardens have been deposited at the University of Tennessee herbarium (TENN), but accession numbers are not available.