Transferability of Microsatellite Markers across Eleven Species of *Magnolia* L.

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Abstract. The genus *Magnolia* (Magnoliaceae) comprises more than 130 species distributed predominantly in temperate and tropical regions in Southeast Asia and is valued worldwide for its ornamental traits as well as for timber and medicinal products, and in trade. Despite their favored status, many species of *Magnolia* are faced with threats from logging, agricultural land use, development, and collection, and are at risk of extinction. Conservation of these species through habitat preservation and in ex situ collections is needed to prevent extinction. To provide a tool for conservation of *Magnolia* species, microsatellite markers developed previously for *Magnolia ashei* were tested in 10 other species of *Magnolia* to determine their transferability across species. Of the 64 primer pairs tested, 21 amplified alleles in the expected size range in all samples; 11 primer pairs amplified clean products in most, but not all, species; 18 primer pairs consistently amplified a polymerase chain reaction (PCR) product in most species, but had either low peak height or other amplification issues; and 14 primers showed excessive stutter, nonspecific amplification, or no amplification. Cluster analysis using the 129 alleles amplified by these 21 simple sequence repeat (SSR) primer pairs generated groups that corresponded to the known taxonomic relationships in this genus.

Magnolia L. (Magnoliaceae) is a popular genus comprising more than 130 species distributed predominantly in temperate and tropical regions in Southeast Asia (Azuma et al., 1999; Figlar and Nootboom, 2004; Kim et al., 2001). They are valued worldwide for their ornamental traits as well as for timber and medicinal products, and in trade, and are well recognized botanically as one of the earliest flowering plants (Raven et al., 1986). Despite their favored status, many species of *Magnolia* are faced with threats from logging, agricultural land use, development, and collection, and are at risk of extinction. According to a recent study by Botanic Gardens Conservation International (BGCI), 48% of the species studied are

threatened in the wild (critically endangered, endangered, or vulnerable), including 75% of the species from neotropical regions (Rivers et al., 2016). Conservation of these species in situ through habitat preservation and in ex situ collections is needed to prevent extinction.

Microsatellite markers, or SSRs, are an efficient method to assess the genetic diversity and population structure of plant populations [Powell et al. (1996); reviewed in Varshney et al. (2005); Wang et al. (2009)], and have proved useful for guiding conservation efforts in several *Magnolia* species, including *M. ashei* Weath. (von Kohn et al., 2018), *M. obovata* Thunb. (Isagi et al., 1999), *M. stellata* (Seibold & Zucc.) Maxim. (Ueno et al., 2005), *M. sieboldii* K. Koch (Kikuchi and Isagi, 2002), *M. tripetala* (L.) L. (Gilkison, 2013), *M. sharpii* Miranda, and *M. schiedeana* Schltdl. (Newton et al., 2008), among others. The availability of additional SSR markers that are useful in multiple *Magnolia* species could facilitate the characterization of threatened species for ex situ conservation. The objective of this study was to determine the transferability of genomic SSR primers developed for *M. ashei* (von Kohn et al., 2018) to 10 additional *Magnolia* species to provide a tool for population and conservation studies.

Materials and Methods

Plant material. Shoot tips from 22 samples representing 11 *Magnolia* species (Table 1) were collected or sent from co-operators and refrigerated before DNA extraction. Genomic DNA was extracted from 0.040 to 0.100 g vegetative buds using a PowerPlant Pro DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions with the following modifications: the addition of a small amount of garnet matrix (BIO 101, Inc. Vista, CA) to aid in homogenization, inclusion of the optional phenolic separation solution, and an additional wash step before elution. DNA was quantified using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

SSR primer evaluation and PCR. Genomic SSR primers were developed and selected from *Magnolia ashei* as described

Table 1. List of 11 *Magnolia* species used in this study, including section, ploidy, number of samples per species tested, and source of samples.

Species ^z	Section ^z	Ploidy ^y	No. of samples tested	Sources ^x (accession no.)
<i>M. acuminata</i> (L.) L.	<i>Yulania</i>	4x	3	UCBG (94.0923); MA (35-91*3); ABG (var. <i>subcordata</i>)
<i>M. ashei</i> Weath.	<i>Macrophylla</i>	2x	3	USNA (NA81565, 81555, 81560)
<i>M. dealbata</i> Zucc.	<i>Macrophylla</i>	2x	2	UCBG (86.0507)
<i>M. fraseri</i> Walter	<i>Auriculata</i>	2x	2	ABG; UCBG (82.2174)
<i>M. guatemalensis</i> Donn.Sm.	<i>Magnolia</i>	2x	2	UCBG (72.0658)
<i>M. macrophylla</i> Michx.	<i>Macrophylla</i>	2x	2	USNA (leaf material from Alabama)
<i>M. pyramidata</i> W. Bartram	<i>Auriculata</i>	2x	2	USNA (leaf material from Florida)
<i>M. sharpii</i> Miranda ^x	<i>Magnolia</i>	2x	1	UCBG (81.0939)
<i>M. tamulipana</i> A. Vázquez ^x	<i>Magnolia</i>	6x	2	UCBG (91.0683; 91.1423)
<i>M. tripetala</i> (L.) L.	<i>Rytidospermum</i>	2x	2	ABG; MA (164-2001*1)
<i>M. virginiana</i> L.	<i>Magnolia</i>	2x	1	MA (1-98*2)

^zSpecies authority and classification are according to the U.S. Department of Agriculture, Agricultural Research Service (2018) and Kim and Suh (2013). All species are in subgenus *Magnolia* except *M. acuminata*, which is in subgenus *Yulania* (Spach) Rchb.

^yPloidy based on Parris et al. (2010).

^x*M. sharpii* and *M. tamulipana* are classified as endangered (Rivers et al., 2016).

ABG = Atlanta Botanical Garden-Gainesville; MA = Morton Arboretum; UCBG = University of California Botanical Garden; USNA = U.S. National Arboretum.

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Table 2. Characteristics of the 50 polymorphic simple sequence repeat loci derived from *M. ashei* including sequence, repeat unit, annealing temperature, allelic statistics, and their transferability to 10 other *Magnolia* species. The 18 primers pairs from loci below the dashed horizontal line consistently amplified a polymerase chain reaction product in the indicated species, but had either low peak height, ambiguous alleles, unexpected number of repeats, or did not amplify a product in at least one sample in a species that otherwise had that locus.

Locus name ^z	Forward/reverse primer sequences (5'-3')	Repeat unit	T _a (°C)	H _o ^y	H _e	PIC	K	No. of alleles amplified in each species (no. samples tested) ^x										
								ac (3)	as (3)	de (2)	fr (2)	gu (2)	ma (2)	py (2)	sh (1)	ta (2)	tr (2)	vi (1)
**MA3-22	TCC AAG ATT TCC TCG TCT GG CGA GGG AGG AGT TCA CGT AG	GCT	57	1.00	0.83	0.78	6	2	2	2	2	2	2	2	2	2	2	2
**MA3-28	TCG TTT TTC CAT CAA TAT GCA G TGC TGT TTT TCC ACT GTG ATT C	TTC	57	0.35	0.87	0.83	9	3	3	2	3	1	1	3	1	5	1	1
**MA4-16	TGC GTG TGT GTT TGA GTG AG TTA GAA AAG CGT GGC TGG TT	AGTG	56	0.29	0.60	0.55	6	1	3	1	2	1	2	2	1	1	1	2
**MA6-17	ATG CTG GAG GGA TGA AAC AT TCT TCA GTG TTA GCC GCT CAT	CCATCA	57	0.12	0.76	0.69	4	2	1	1	1	2	1	1	1	2	1	1
**MA3-23	TCT TGC CGA AGT CAA AAT GA TAC CGA ATG CCA TGA AAA CA	AGC	55	0.06	0.79	0.72	5	4	2	1	1	1	1	1	1	3	1	1
**MA5-12	TCA TTT TCG ATA GGG GAC CA AGT CGG ACT TGG GTT GAG AA	CCCAA	57	0.18	0.63	0.57	5	2	2	2	1	1	1	1	1	2	1	1
**MA6-19	GAG AAA CCC TGC GAA AGA GA ATG GTT AGC ACC GAG CAT TT	TGGTGC	57	0.35	0.81	0.75	6	1	3	1	2	1	3	1	1	1	2	2
MA3-30	TAC AGG CCA AGC AGA CTT CC TTG GAC CCC AAC CCT TAT TT	GGT	55	0.47	0.74	0.66	4	1	2	1	1	2	2	2	1	1	1	1
MA3-31	CAC AGC TGC AGT TTT GGG TA ATC CAT AGC GAG GTC AAT GC	TGG	56	0.65	0.7	0.62	5	2	2	2	1	2	2	1	2	3	1	2
MA6-10	CTG TGT TGG ACC CGA TTT CT GAG ATG GTG TGC TCG TCA CA	TGGAGA	58	0.53	0.71	0.65	5	1	2	2	1	2	1	1	2	2	2	2
MA3-25	AGC ACA TTG ATG ACT TGT GGA GGC CAT AGG GAC ATG AT	GAT	58	0.47	0.86	0.82	8	6	3	2	1	1	3	1	1	2	1	2
**MA6-18	CAT CAC CAT CAC CAT CAT CC TAA GGC TCT CGC CAA TAG GA	CATCCT	55	0.35	0.8	0.75	6	1	2	2	1	2	1	1	2	1	1	1
**MA7-6	GGG AAT GCC TCT AAT GAT GC GGG TGA GTG AAA CCT CTT GC	AAAAGAA	56	0.12	0.6	0.53	4	2	2	1	1	1	2	1	1	2	1	1
MA3-14	CCC TCA CCC ATC CAT ACA AG GCG AGG ACC CAT CAT TTC T	TGG	56	0.12	0.65	0.59	6	3	1	1	1	1	1	2	1	1	2	2
MA4-22	TTT GAT GGC GTG TGA AGT AAA GGG CCT TCA ACA TGT CCT C	TGTC	57	0.06	0.66	0.57	4	1	1	1	1	1	1	2	1	2	1	1
**MA4-13	TCA TTC CCA CCT TCT CAT CC GGC CCA TCA TAT CAG CAG TT	GAGT	55	0.18	0.8	0.74	6	1	2	1	2	1	1	2	1	2	1	1
MA4-9	GTG AAT GTG TGG ATG GAT GC CAA TTC ACC TTT CGG TGT CA	ATGC	55	0.18	0.17	0.16	3	4	1	1	1	1	1	1	1	1	2	2
MA5-19	TAA CTA GGG GTG CAG CTT GG AGG ATT GGG CTG GGT AGA TT	TGGGT	57	0.65	0.85	0.81	7	2	2	1	4	2	1	3	2	3	2	1
MA9-1	GGC ACT GAA ATC CAA ATT CC TTG GGT TTG GGC TAA TGA AG	AGAAGACA	55	0.71	0.77	0.7	5	2	2	2	2	2	1	2	2	2	2	1
MA7-4	CGT TTA ACC CTA AGC GAG TGA GAA ACG GTG AGG TCC AAG AA	AGAAGAA	56	0.71	0.61	0.54	4	3	2	2	1	2	2	1	1	2	2	2
MA5-24	TTG ATT AAA TGG GTC ACA GTG G AAT CCC ATC ATC GCA GTA GC	TGGGT	55	0.29	0.59	0.54	6	2	1	1	1	1	1	2	2	1	2	1
**MA3-7	CAT GCT AAC CCA TCT AGT CAC G TCC CAA TAC CCA TCC CAG TA	GAA	57				4	4	1	3	—	3	3	3	—	—	1	2
**MA3-12	AGC CCA AGG AGA CAA CAG AA GGG TTT CTT CGC ATG TTG TT	GGA	55				3	1	1	2	—	1	2	1	2	1	2	1
**MA3-27	ATC ACC GAT TTT AGC CTC CA GAC TGG CCC GTA TGT TTG TC	TTC	57				4	2	1	2	1	2	1	3	3	1	3	—
**MA4-19	TGG AAA GTG CAC ACT GGA AG	CCAT	56				1	2	1	—	1	1	1	1	1	2	—	1

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Table 2. (Continued) Characteristics of the 50 polymorphic simple sequence repeat loci derived from *M. ashet* including sequence, repeat unit, annealing temperature, allelic statistics, and their transferability to 10 other *Magnolia* species. The 18 primers pairs from loci below the dashed horizontal line consistently amplified a polymerase chain reaction product in the indicated species, but had either low peak height, ambiguous alleles, unexpected number of repeats, or did not amplify a product in at least one sample in a species that otherwise had that locus.

Locus name ^z	Forward/reverse primer sequences (5'-3')	Repeat unit	T _a (°C)	H _o ^y	H _e	P _{IC}	K	No. of alleles amplified in each species (no. samples tested) ^x										
								ac (3)	as (3)	de (2)	fr (2)	gu (2)	ma (2)	py (2)	sh (1)	ta (2)	tr (2)	vi (1)
*MA5-5	TTT CCA TTA ATC CGG GTC TG	GAAAG	57					—	2	1	2	—	2	2	—	—	1	1
	AAC GTT CAG CTT CTT GTT GGA																	
	AGT CCA AGA CCG AGC GAG T																	
*MA5-13	GCT TGC ATG CAT ATC ACG TT	ACCTT	56					—	2	1	1	1	1	2	1	2	1	1
	ACT CAG ACC GGA CGT AAT GG																	
	GCT CGT GCA CAA AAG AAG GT																	
*MA6-8	CAG GCT GAG GTC TTT CCA AG	TGGGCT	56					—	1	2	1	—	1	1	1	1	1	1
	GTG GAT ATC ATG GGC CTC AC																	
	GGA GAA GTC CCT GCA TGT GT																	
MA5-25	TGA ACT GGG GTT GAG TTG AGT	GGTTG	56					3	1	1	3	2	2	—	1	5	2	1
	CCA TGG ACT ACC CAT GGT CT																	
	CCC CAC ACA GCT TCA ATG TA																	
MA6-15	CTT GAC AAG GCC CAG TTT TT	TGGGTT	55					1	3	1	1	1	1	1	1	3	—	2
	TCC TGT TTT CGT TGC CAT AA																	
	GTG GCT GAT GGG ATC GTA GT																	
MA6-6		ACCATC	58					4	3	1	1	—	3	2	—	1	2	2
MA3-9	GGC AGC AGG TTT GTG TTT TT	CCA	55					1	1	—	1	1	1	1	1	1	1	1
	TGG ATG AAG GAT TTC GTC TTT T																	
	GCT CTG AGC CAA AAC ACC A																	
MA4-21	TGT GGA GAA GGC TTT GGA TT	GGAG	56					2	1	1	1	1	1	1	3	2	1	
	GGT TGT TGT TGA TGG TGG ATT																	
	TTC TCC TTC ACC CTC TTG GA																	
MA6-9	GGA ATT CAA GAT GGG CAG AC	AGAGGG	56					2	2	1	1	1	3	1	1	1	1	1
	CTC GAA CCA GTT TCC TTC GT																	
	GCT CGG GTA GTG GAA AGT TG																	
MA4-20	AAT GGT GCA TGT TTG CAT GT	GATG	55					1	1	1	—	—	1	—	—	—	—	
	GAA CCA TCC CCA AAT AAG CA																	
	CAA CAA TTC TGT GCG TCT GG																	
MA5-23	CAT CAT CGT TGT CGT CGT CT	GTGG	56					2	1	1	1	1	1	1	2	1	1	1
	GTG CAA AGG AGG CCA AGT AG																	
	AGA TCG ACC CAC ATT CCA AC																	
MA6-5	AAC ATA TTG GGG TGC AGG AG	GAGAG	57					1	1	1	1	1	2	1	1	1	1	—
	GGA ATT GCA AAA AGC AAC AAA																	
	TAG ACC CAA CAG CCC AAA AC																	
MA5-15	AGG TTA CCC AAT TTC AAA GCA A	CCTTGC	55					5	2	2	2	2	1	2	4	1	2	
	ATG GTA GTG GTG GTG GT																	
	GAA TTG CCT GGA AAA CGA AA																	
MA3-4	GGT GAA AAT TCT GTG GGG ATT	ACTCCC	57					1	1	2	1	1	2	1	1	2	—	1
	ACG CTC GAT CAT GGT GTA TG																	
	GGT TTG GTC GGG TCT TGT TA																	
MA6-3	CGT AAA TGA TTC CGG TCC AT	ACCAC	58					6	2	2	2	2	2	2	3	2	2	3
	TCC AAT CCA CAA CAG AAC CA																	
	GAT GGT CCG ACA TCA AAA CC																	
MA5-10	GCA CCG AAG TTT CAG GAA GA	GCT	55					1	2	1	2	1	2	2	1	1	1	1
	CAA ATA CTG CAT GGG AGG AAA																	
	TGC CAG AGC TTG TCC TTA AAA																	
MA8-2	GTG GGT TTG GAC TGC TGA TT	GAAGCA	56					1	2	1	1	—	2	2	1	—	1	—
	CAT CCT CCA CTT CCA AGT CC																	
	CAT GCA CAC CTA TGC ACA TAT AGA																	
MA5-21		ACCCA	55					2	2	1	1	—	2	1	—	1	1	1
MA3-3		ACTTTTTT	56					—	1	1	2	1	2	1	1	1	1	1
MA4-4		AGGGC	55					3	2	1	—	1	2	—	1	2	1	2

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Table 2. (Continued) Characteristics of the 50 polymorphic simple sequence repeat loci derived from *M. ashei* including sequence, repeat unit, annealing temperature, allelic statistics, and their transferability to 10 other *Magnolia* species. The 18 primers pairs from loci below the dashed horizontal line consistently amplified a polymerase chain reaction product in the indicated species, but had either low peak height, ambiguous alleles, unexpected number of repeats, or did not amplify a product in at least one sample in a species that otherwise had that locus.

Locus name ^a	Forward/reverse primer sequences (5'-3')	Repeat unit	T _a (°C)	H _o ^b	H _e	PIC	K	No. of alleles amplified in each species (no. samples tested) ^c									
								ac (3)	as (3)	de (2)	fr (2)	gu (2)	ma (2)	py (2)	sh (1)	ta (2)	tr (2)
MA4-8	CGC TAA CGT ATC AAA TTC TTC AAA	ACGG	56					—	2	2	—	1	2	—	1	1	—
	ATG GAC GGC GCT GAT ATA AA																
	AAC GGA TTG GCT GAT GTA CC																

^aPrimers marked with an asterisk are those reported previously for the *M. ashei* population study (von Kohn et al., 2018).

^bAllelic statistics for loci were calculated using diploid samples only (i.e., excluding *M. acuminata* and *M. tamulipana*), and only for those loci that were present in all samples tested.

^cSpecies are abbreviated with the first two letters of the species name. A dash in the matrix indicates no amplification for any samples in that species.

Ho = observed heterozygosity; He = expected heterozygosity; PIC = polymorphic information content; K = number of alleles per locus.

Fig. 1. Unweighted pair group method with arithmetic mean dendrogram of 22 *Magnolia* samples based on Jaccard similarity data from 129 alleles from 21 simple sequence repeats loci. Numbers in boxes indicate the percentage of *M. ashei* primers that amplified a product in each species (of 50 primers listed in Table 2). Names following braces indicate the taxonomic section to which the species belongs. Cophenetic correlation coefficient (r) = 0.982.

in von Kohn et al. (2018). Briefly, SSRs were identified from Illumina sequence data (deposited in GenBank, accession no. PCNC00000000) using the MicroSatellite identification tool (Beier et al., 2017). Primers for 64 SSR loci with differing repeat units (Table 2) were identified using Primer 3 Plus (Untergasser et al., 2012). PCR primers were manufactured by Integrated DNA Technologies (Coralville, IA). The forward primers had an additional M13 (–21) universal sequence (TGTAACGACGCGCCAGT) attached to the 5' end to allow indirect fluorescent labeling of PCR products using a universal 6-carboxy-fluorescein (FAM)-labeled M13 primer (Schuelke, 2000). PCR was carried out in a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA). The 15-μL PCR mixture contained 30 ng template genomic DNA, 0.25 μM of each reverse and universal FAM-labeled M13 (–21) primer, and 0.0625 μM of the forward primer with 1× Bioline MangoMix and 3.77 mM Bioline MgCl₂ (Bioline Inc., Taunton, MA). PCR profiles consisted of initial denaturation at 94 °C for 5 min; followed by 30 cycles of 94 °C for 30 s, optimized annealing temperature of each primer pair (Table 2) for 30 s, and 72 °C for 60 s; followed by eight cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. Products were analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) using 2 μL PCR product, 10 μL formamide (Applied Biosystems), and 0.2 μL GeneScan 500 LIZ Size Standard (Applied Biosystems). Allele sizes and

number of alleles per locus were determined with GeneMarker version 2.6.3 (SoftGenetics, State College, PA). Reactions that resulted in unexpected, ambiguous, low, or no amplification were repeated.

Data analysis. Allele frequency analysis for 21 loci that showed consistent and predicted amplification products in all samples tested was performed using Cervus 3.0.7 (Kalinowski et al., 2007), based on allelic data from the 17 diploid samples (Table 1). Data from these 21 loci for all species were converted to a binary matrix (presence/absence of allele) and used to generate a similarity matrix based on the Jaccard coefficient using NYSYSpc version 2.02 (Rohlf, 1998). Accessions were then clustered using the unweighted pair group method with arithmetic mean (UPGMA) algorithm in NTSYSpc. Cophenetic matrices were constructed and compared with the similarity matrices using the MXCOMP program to test the goodness of fit of a cluster (Rohlf, 1998).

Results

Of the 64 primer pairs tested, 21 amplified alleles in the expected size range in all samples of all 11 species tested (Table 2). Eleven primer pairs amplified clean products in most, but not all, species. An additional 18 primer pairs (below the dashed horizontal line in Table 2) consistently amplified a PCR product in the indicated species, but had either low peak height, ambiguous alleles, unexpected size of the repeated unit, or did not amplify a product in at least one sample in

a species that otherwise had that locus. Finally, the remaining 14 primers (not listed) showed excessive stutter, nonspecific amplification, or no amplification.

A total of 129 alleles were scored from the 21 primer pairs that resulted in “complete” data. Allelic data from the 17 diploid samples were used to calculate allelic frequencies and polymorphic information content (PIC) for these 21 loci. PIC values ranged from 0.16 to 0.83 (Table 2). The 129 alleles were also used to generate a UPGMA dendrogram based on the Jaccard similarity coefficient (Fig. 1). Although the purpose of this study was not to confirm interspecific diversity or taxonomic classification in *Magnolia*, the phenogram provides strong evidence that the primers developed in *M. ashei* are representative of the allelic diversity in the genus. The cophenetic correlation coefficient for the dendrogram was 0.982, indicating that the dendrogram is a very good fit to the data set (Rohlf, 1998). Equally significant, the major clusters in the dendrogram followed the taxonomic relationships reported previously (Figlar and Nooteboom, 2004; Kim et al., 2001; Kim and Suh, 2013). Specifically, all samples grouped together by species, and all species clustered together by section (Table 1).

Discussion

Many studies have reported the utility and limitations of cross-species amplification of SSR primers in plants [e.g., reviewed by Barbara et al. (2007), Varshney et al. (2005), Wang et al. (2009)]. As would be expected and has been confirmed in other studies, the success of SSR primer transferability across species is correlated with how closely related the species are (Brueggmann and Fladung, 2013; Buzatti et al., 2016). We observed similar results in our study (Fig. 1), in which only one primer did not amplify in *M. macrophylla* Michx. and two primers did not amplify in *M. dealbata* Zucc. (Table 2), both of which are in the same section (sec. *Macrophylla* Figlar & Noot.) as *M. ashei* (Table 1). Conversely, an average of 5.75 primers did not amplify in species outside the section.

The real test of how useful SSR markers will be in related species or genera is not whether they amplify, but how polymorphic the markers are (Barbara et al., 2007). Because our study used only one to three samples of each species, it is not possible to determine whether the SSR loci that showed only one allele in a species are truly monomorphic in that species. Some studies indicate that genomic SSRs may have more nonspecific amplification or stuttering than genic SSRs (Varshney et al., 2005), although they may be more polymorphic in closely related accessions [such as within a species (Song et al., 2012)]. The SSRs described here were developed to study population structure within one species (*M. ashei*) and were derived from genomic DNA, so they may prove to be less polymorphic, less transferable, or less clearly amplified in more distantly related taxa. Comparative studies indicate that successful cross-species amplification and polymorphism

are more likely within genera such as *Magnolia*, which are perennial (vs. annual) and are outcrossing (vs. selfing) (Barbara et al., 2007).

The SSR primers reported in this article and those from other *Magnolia* studies contribute to an increasingly valuable set of tools to gather genetic and population data on *Magnolia* species. Of the 304 Magnoliaceae species studied in the 2016 BGCI report, 20% could not be assessed as a result of a lack of data. As development of SSR markers becomes more economical (Zalapa et al., 2012), it may be feasible to develop markers specifically for data-deficient species with pressing conservation needs. However, in many cases, especially when quick action is needed and resources are limited, the use of SSR markers developed for other species will be critical in obtaining sufficient data to guide effective conservation of threatened species. The Global Strategy for Plant Conservation, adopted as part of the Convention on Biological Diversity in 2002, includes as one of its targets the conservation of at least 75% of the world’s threatened plants in ex situ collections by 2020 (Hird and Kramer, 2013). Currently, only 43% of threatened *Magnolia* species are preserved in ex situ collections (Rivers et al., 2016). Many *Magnolia* species have recalcitrant seeds, so must be conserved via resource- and space-intensive plantings. Data derived from SSR markers can be used to determine population structure or genetic diversity, which enables maximizing the amount of genetic diversity held in ex situ collections while minimizing the number of individuals required. This information will allow curation of ex situ collections to capture and reflect the genetic diversity of the wild populations (Cires et al., 2013; IUCN/SSC, 2014; Rivers et al., 2016). The SSR primers described here provide a valuable tool for developing conservation strategies for this important genus.

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