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Ploidy Levels, Relative Genome Sizes, and Base Pair Composition in Magnolia

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Abstract. The genus *Magnolia* includes over 250 species that range in ploidy level from diploid to hexaploid. Although there is basic information on ploidy levels of various species, sampling has been limited and little information on specific cultivars and hybrids is available. The objective of this research was to determine relative genome sizes and relationships to ploidy levels among a diverse collection of species, hybrids, and cultivars using flow cytometry. Nuclei were extracted, stained with 4′, 6-diamidino-2-phenylindole (DAPI), and analyzed using a flow cytometer. Relative genome sizes were determined using *Pisum sativum* as the reference genome. Genome size was calibrated with ploidy level for species with documented chromosome numbers. Relative genome size for a given ploidy level varied significantly among most taxonomic sections indicating these groups have undergone considerable genomic divergence. These data also indicate it is desirable to calibrate ploidy level with relative genome size for each section separately. Within a section, relative 2C genome sizes, for a given ploidy level, had narrow ranges and could be used to clearly distinguish between euploid levels. Genome size estimates, determined with DAPI or propidium iodide fluorochromes, varied (by 0% to 14%) as a function of species and base pair (bp) composition. Both methods were suitable for determining euploid level. Base pair composition of representative *Magnolia* species ranged from 61.6% to 63.91% AT. Genome sizes and ploidy levels are presented for a broad range of species and hybrids within genus *Magnolia*. This information also provides further insight into reproductive biology, substantiation of numerous hybrids and induced polyploids, and comparison of methods for determining genome size that will help facilitate the development of improved hybrids in the future.

Polyploidy has been an important process in the evolution of plants that can contribute to reproductive isolation, novel gene expression, and ultimately divergence and speciation (Adams and Wendel, 2005; Comai, 2005; Hegarty and Hiscock, 2008; Soltis and Burleigh, 2009; Soltis et al., 2003). Polyploidy is also an important factor in plant breeding because it can influence reproductive compatibility, fertility, and phenotypic traits (Chen and Ni, 2006; Jones and Ranney, 2009; Ranney, 2006; Soltis et al., 2004). In some cases, the artificial induction of polyploidy in *Magnolia* also can enhance ornamental characteristics, including thicker leaves and larger flowers with thicker petals that persist longer (Kehr, 1985). As such, accurate and specific knowledge of ploidy levels of species and cultivars is important information for magnolia breeders.

The genus *Magnolia* comprises more than 250 species belonging to various sections within three subgenera (Figlar and Nooteboom, 2004). Although basic information on chromosome counts and ploidy levels of different *Magnolia* species have been compiled (Callaway, 1994; Chen et al., 2000), sampling has been limited and little is known about ploidy levels of specific hybrids and cultivars. The base chromosome number for *Magnolia* is $1n = 1x = 19$. However, different subgenera contain species with a variety of ploidy levels ranging from $2n = 2x = 38$ to $2n = 6x = 114$. Crosses between species with varying ploidy levels may yield hybrids with nonstandard chromosome numbers that can result in reduced fertility or sterility. Because of these constraints, *Magnolia* breeders have attempted to induce new polyploids to overcome these limitations, yet most of these putative polyploids have never been confirmed. The range in ploidy levels within this genus also provides an opportunity to indirectly substantiate hybridity when parents differ in ploidy levels.

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Because many *Magnolia* species are polyploids with high chromosome numbers, traditional cytology based on light microscopic examination is a difficult and time-consuming process. Flow cytometry has proved to be an efficient means of estimating genome size and associated ploidy level (Doležel et al., 2007; Jones et al., 2007). Therefore, the objectives of this study were to determine the genome sizes and relationships to ploidy levels of a diverse collection of species, hybrids, and cultivars of *Magnolia* to 1) develop an extensive database of ploidy levels for use by magnolia breeders; 2) determine the ploidy levels of plants that were chemically treated to artificially induce polyploidy; 3) confirm hybridity of interploid and interspecific (when parents vary substantially in genome size) crosses; and 4) compare estimates of genome size using DAPI (AT preferential) or propidium iodide (PI) (intercalating) fluorochrome stains and estimate bp composition for representative taxa from 10 taxonomic sections.

Materials and Methods

Relative genome size and ploidy level determination. Over 300 accessions were sampled from various sources that included 62 species, 125 hybrids, and 16 induced polyploids representing taxa from each subgenus of *Magnolia* as well as both species of *Liriodendron*, the only other genus in family Magnoliaceae per Figlar and Nooteboom (2004). Nuclei from newly expanded leaf or tepal tissue were extracted, stained with DAPI, and then analyzed (minimum of 2500 nuclei per sample) using a flow cytometer (PA-I; Partec, Münster, Germany) to determine relative holoploid 2C DNA content following the methods of Jones et al. (2007). Genome sizes were determined by comparing mean relative fluorescence of each sample with an internal standard, *Pisum sativum* 'Ctirad', with a known genome size of 8.76 pg (Greilhuber et al., 2007). Because tetraploid *Magnolia* taxa have similar genome sizes to *P. sativum* 'Ctirad', *Magnolia virginiana* 'Jim Wilson' [NCSU 2004-24 (3.92 pg)] was used as a secondary standard. Absolute genome size for the secondary standard was calculated as the mean of 10 separate subsamples determined with *P. sativum* 'Ctirad' as an internal standard and PI as the fluorochrome stain (see procedure subsequently in "Comparison of fluorochromes and estimate of base pair composition"). Holoploid, 2C DNA contents were calculated as: 2C = DNA content of standard × (mean fluorescence value of sample ÷ mean fluorescence value of the standard).

The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers (Chen et al., 2000). Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 19) was calculated as 2C genome size ÷ ploidy level to assess variability in base genome size. A minimum of two subsamples was tested to derive a mean relative genome size for each accession. Data for species were subjected to analysis of variance and means separation using the Waller procedure (Proc GLM, SAS Version 9.1; SAS Institute, Cary, NC). Ploidy levels for hybrid taxa and suspected aneuploid hybrids were derived in the following manner: ploidy level = mean 2C genome size ÷ weighted average 1Cx genome size of the reported parental species.

Comparison of fluorochromes and estimate of base pair composition. Ten species were sampled that included taxa from each subgenus of *Magnolia*. Nuclei were extracted, stained, and analyzed as described previously using a minimum of 3000 nuclei per sample. Sample preparation was similar to methods described for DAPI with the exception that the staining solution consisted of 2 mL staining buffer, 6 µL RNase A, and 12 µL PI (CyStain PI absolute P; Partec) and the samples were maintained at 4 °C for 1 h before flow cytometry analysis using a 488-nm laser for excitation (PA-II; Partec). The experimental design was a split-plot design with fluorochrome (DAPI versus PI) as the whole plot and species as the subplot. Samples were collected and analyzed over time in complete blocks. Data were subjected to analysis of variance and mean separation using Fisher's least significant difference specifically calculated for comparing two whole plot (fluorochrome) factors for a given subplot (species). Base pair composition was calculated following the equation: AT% = AT% for internal standard × [(fluorescence internal standard, DAPI/fluorescence sample, DAPI) ÷ (fluorescence internal standard, PI/fluorescence sample, PI)]$^{(1/\text{binding length})}$ (Godelle et al., 1993), where AT% of the internal standard, *Pisum sativum* = 61.50% and binding length of DAPI ≈3.5 bp (Meister and Barrow, 2007).

Cytology. Actively growing root tips of container-grown seedlings of putative octaploid *M. cylindrica* were collected at midday and placed in the mitotic inhibitor 8-hydroxyquinoline for 2 h at 5 °C in dark conditions. They were then transferred to a fixative solution of three parts 95% ethanol:one part glacial acetic acid (v/v) for 24 h while remaining at 5 °C in dark conditions. Tissue was excised from just behind the root tip and placed in 12 N HCl for 10 s. Squashes were prepared with a small amount of this tissue and a drop of modified Fuelgen stain on a slide with a coverslip.

Results and Discussion

Relative genome size and ploidy level among species. Relative genome sizes and ploidy levels were determined for 175 accessions, representing 62 species of Magnoliaceae and arranged by taxonomic sections following Figlar and Nooteboom (2004) (Tables 1 and 2). Base, 1Cx genome size varied significantly among plants sampled from different taxonomic sections indicating these groups have undergone considerable genome size divergence (Table 1). This variation indicates it is necessary to calibrate ploidy level with genome size for each section to estimate ploidy level from genome size in *Magnolia*. However, within a section, genome sizes for a given ploidy level had sufficiently narrow ranges that they could be used to clearly determine ploidy levels. Diploidy was prevalent throughout taxonomic sections, but variation in ploidy level occurred among species within several sections. Section *Magnolia* in subgenus *Magnolia* had both diploid and hexaploid members, whereas section *Yulania* in subgenus *Yulania* was represented by diploid, tetraploid, and hexaploid species. The two species tested in section *Gynopodium*, subgenus *Gynopodium*, were both hexaploid.

Ploidy levels of species were generally consistent with past reports (Chen et al., 2000; Treseder, 1978; Xia et al., 2008) with some new additions and clarifications. Samples from wild-collected *M. cylindrica* (Bartlett 193, Holden 96-111A, Holden 96-115B, and MGA 216/Holden 87-86-93) were found to be tetraploid, having relative 2C genome sizes ranging from 8.82 to 9.11 (Table 2), in agreement with Xia et al. (2008) but not with prior reports (Treseder, 1978) that indicated *M. cylindrica* was diploid. Earlier reports may have varied as a result of lack of confirmed, wild-collected accessions in gardens of Europe and North America as stated by Callaway (1994). Chromosome counts have not been published for *M. zenii*, a species recently introduced into cultivation. The three accessions of *M. zenii*

Table 1. Summary of means and ranges for 2C, holoploid genome size (pg), and 1Cx monoploid genome size (pg) of *Magnolia* species grouped by section and ploidy level.

	Ploidy level[z]		
Classification	$2n = 2x = 38$	$2n = 4x = 76$	$2n = 6x = 114$
Subgenus *Magnolia*			
Section *Magnolia* (5/41[y])	2C = 3.80[x]E[w] (3.43–4.40)[u] $1C_X$ = 1.90[t] (1.72–2.20)[s]	N[v]	2C = 11.18 C (10.83–11.86) $1C_X$ = 1.86 (1.81–1.98)
Section *Gwillimia* (4/6)	2C = 5.32 A (5.10–5.63) $1C_X$ = 2.66 (2.41–2.82)	N	N
Section *Rhytidospermum* (5/18)	2C = 4.27 CD (3.66–4.69) $1C_X$ = 2.14 (1.83–2.35)	N	N
Section *Manglietia* (10/17)	2C = 4.87 B (4.65–5.25) $1C_X$ = 2.44 (2.33–2.63)	N	N
Section *Macrophylla* (1/5)	2C = 4.57 BC (4.41–4.87) $1C_X$ = 2.28 (2.21–2.44)	N	N
Section *Auriculata* (1/3)	2C = 3.83 E (3.74–3.96) $1C_X$ = 1.94 (1.87–1.98)	N	N
Section *Kmeria* (1/1)	2C = 5.51 A (5.51–5.51) $1C_X$ = 2.76 (2.76–2.76)	N	N
Subgenus *Yulania*			
Section *Yulania* (14/43)	2C = 4.05 DE (3.84–4.26) $1C_X$ = 2.02 (1.92–2.13)	2C = 8.56 A (8.08–9.34) $1C_X$ = 2.14 (2.02–2.34)	2C = 12.68 A (11.49–13.47) $1C_X$ = 2.11 (1.92–2.25)
Section *Michelia* (17/31)	2C = 4.56 BC (4.23–4.92) $1C_X$ = 2.28 (2.11–2.46)	N	N
Subgenus *Gynopodium*			
Section *Gynopodium* (2/3)	N	N	2C = 11.93 B (11.57–12.50) $1C_X$ = 1.99 (1.93–2.08)
Section *Manglietiastrum* (1/1)	2C = 4.21 D (4.21–4.21) 1CX = 2.11 (2.11–2.11)	N	N
Genus *Liriodendron* (2/2)	2C = 3.41 F (3.35–3.47) $1C_X$ = 1.71 (1.68–1.74)	N	N

[z]Taxa assigned to given ploidy level based on estimated genome sizes and in agreement with published chromosome counts, if available.
[y]Numbers in parentheses, after classifications, indicate the number of species sampled and the total number of taxa within those species sampled.
[x]Relative 2C genome sizes (pg) were determined using 4′,6-diamidino-2-phenylindole as the flourochrome stain.
[w]Means separation using the Waller Procedure (Proc GLM, SAS Version 9.1; SAS Institute, Cary, NC) at $P < 0.05$.
[v]N = no genome size reported; indicates given ploidy level was not reported or observed in this section.
[u]Values represent ranges of 2C genome size for all *Magnolia* species sampled in each section.
[t]Relative 1Cx mean genome sizes (pg) were calculated as: (2C mean/ploidy level).
[s]Values represent ranges of $1C_X$ genome size means for all *Magnolia* species sampled in each section.

(MGA 440/Arnold 1545-80-B, Chollipo Form, and 'Pink Parchment') tested here were diploid with a mean relative genome size of 4.16 pg. *Magnolia biondii* has been reported to be tetraploid (Xia et al., 2008), although we found two *M. biondii* accessions (MGA 027 and Bartlett 2002-056) to be diploid with a mean relative genome size of 4.11 pg. In our study, no natural variation in ploidy level was found among accessions within a given species.

Relative genome size and ploidy level among hybrids. Genome sizes and ploidy levels were determined for a broad range of reported interspecific, intra- and interploid hybrids (Table 3). In certain cases, analysis of genome size helped to substantiate or refute the authenticity of the hybrids. For example, the intersectional, intraploid hybrid *Magnolia* 'Katie-O' (NCSU 2004-012, MGA 307) had a mean 2C genome size of 4.30 pg, intermediate between the reported parents of *M. insignis* (2C = 4.94 pg) × *M. virginiana* (2C = 3.72 pg), supporting hybridity. Additional interspecific, intraploid hybrids strongly supported by genome size analysis include *M. yuyuanensis* × *M. virginiana*, (NCSU 2009-131), *M. virginiana* 'Havener' × *M. insignis* Red Form, 111/7, (McCracken), and [(*M. tripetala* × *M. obovata*) × *M. tripetala*] 'Silk Road' × *M. insignis* (MGA). Flow cytometry did not typically allow for distinguishing interspecific hybrids within a given section and ploidy level as a result of conserved genome sizes within sections. Taxa including *M.* ×*kewensis*, *M.* ×*loebneri*, *M.* ×*brooklynensis*, and *M.* ×*veitchii* fall into this category.

Evidence for successful hybridization between plants of different ploidy levels was apparent based on analysis of genome sizes. In many cases, interploid hybrids were substantiated. These include the following within subgenus *Magnolia*: [*M. grandiflora* (6*x*) × *M. virginiana* (2*x*)] 'Maryland' (MGA 077, McCracken) with an intermediate genome size of 7.49 pg, and also a seedling of 'Maryland' (MGA 325), which was likely open-pollinated by *M. grandiflora* that had a genome size of 9.00 pg, consistent with a pentaploid derived from a tetraploid by hexaploid cross. An unnamed plant at the U.S. National Arboretum (USNA 2) with morphological similarity to *Magnolia*

Table 2. Relative genome size and estimated ploidy level for a diverse collection of Magnoliaceae representing 62 species.

Taxa	Cultivar/selection	Source/accession no.[z]	Relative 2C genome size [mean ± SE (pg)][y]	Mean relative 1Cx genome size by species (pg)[x]	Ploidy level (*x*)
Genus *Magnolia*					
Subgenus *Magnolia*					
Section *Magnolia*					
virginiana	NCSU Variegated	Bartlett in nursery	3.51 ± 0.06	1.86	2
	'Northern Belle'	Bartlett 2005-1177A	3.68 ± 0.02		2
	'Plena'	Bartlett 2007-0041	3.67 ± 0.03		2
	R14-397	McCracken	3.73 ± 0.01		2
	SCC Littleleaf	SCC	3.84 ± 0.07		2
virginiana var. *australis*	'Aiken County'	Bartlett 2004-644	3.69 ± 0.12		2
	'Coosa'	MGA 172	3.78 ± 0.06		2
	'Henry Hicks'	Bartlett 2003-603	3.68 ± 0.08		2
	'Jim Wilson'	NCSU 2004-24	3.75 ± 0.03		2
	'Santa Rosa'	Gilbert's Nursery	3.89 ± 0.07		2
	'Silver Savage'	MGA 255	3.71 ± 0.02		2
	'Tensaw'	McCracken	3.73 ± 0.01		2
	Texas/Lousiana Form	Bartlett 2002-269	3.43 ± 0.07		2
grandiflora	'24 Below'	NCSU	11.32 ± 0.03	1.87	6
	'Black Stem'	McCracken	11.18 ± 0.14		6
	'Bracken's Brown Beauty'	Milliken	11.07 ± 0.04		6
	'Carolina Compact'	McCracken	11.04 ± 0.02		6
	'Charles Dickens'	MGA 353	10.88 ± 0.01		6
	Charles Dickens Seedling	MGA	11.07 ± 0.00		6
	'Claudia Wannamaker'	Milliken	11.03 ± 0.02		6
	'Coco'	Forest St./Spartanburg	10.91 ± 0.06		6
	'D.D. Blanchard'	Gilbert's Nursery	11.13 ± 0.13		6
	'Edith Bogue'	Milliken	11.06 ± 0.06		6
	'Edith Bogue'	McCracken	11.16 ± 0.17		6
	'Gallisonier'	McCracken	11.47 ± 0.30		6
	'Harold Poole'	Head	11.64 ± 0.18		6
	'Kay Parris'	NCSU	11.10 ± 0.09		6
	'Little Gem'	NCSU 1998-406	11.16 ± 0.11		6
	'Main Street'	Bartlett 2006-0124A	10.83 ± 0.23		6
	'MGTIG' Greenback	Gilbert's Nursery	11.12 ± 0.17		6
	'Pat's Variegated'	Bartlett 2007-0566A	11.06 ± 0.02		6
	'Phyllis Barrow'	Milliken	11.14 ± 0.06		6
	'Reigel'	McCracken	11.49 ± 0.06		6
	'Samuel Sommer'	Strybing	11.86 ± 0.00		6
	'Scituate'	McCracken	10.98 ± 0.06		6
	'Smith Fogle'	McCracken	11.49 ± 0.13		6
	'Southern Charm'	SCC	10.84 ± 0.02		6
	USNA 1	USNA	11.09 ± 0.00		6
	USNA 3	USNA	11.32 ± 0.00		6
guatamalensis		Strybing 1992-0143	4.37 ± 0.02	2.19	2
sharpii		Strybing 1984-0182	4.40 ± 0.04	2.20	2
tamaulipana		MGA 191	11.01 ± 0.08	1.88	6
	'Bronze Sentinel'	Gilbert's Nursery	11.63 ± 0.15		6
Section *Gwillimia*					
Subsection *Gwillimia*					
coco		MGA in nursery	4.83 ± 0.04	2.42	2
delavayii		MGA 411	5.10 ± 0.05	2.64	2
		Strybing xy-0179	5.46 ± 0.02		2
Subsection *Blumiana*					
hodgsonii		Strybing	5.47 ± 0.14	2.73	2
		NCSU 2010-084	5.42 ± 0.01		2
liliifera		MGA in nursery	5.63 ± 0.01	2.82	2

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Table 2. Continued.

Taxa	Cultivar/selection	Source/accession no.[z]	Relative 2C genome size [mean ± SE (pg)][y]	Mean relative 1Cx genome size by species (pg)[x]	Ploidy level (*x*)
Section *Rhytidospermum*					
Subsection *Rhytidospermum*					
obovata (*hypoleuca*)		MGA 179	3.97 ± 0.01	1.99	2
officinalis var. *officinalis*		MGA 471	4.01 ± 0.01	1.89	2
officinalis var. *biloba*		MGA 111	3.78 ± 0.02		2
		Bartlett 2002-196	3.66 ± 0.03		2
		McCracken	3.68 ± 0.03		2
rostrata		NCSU	4.69 ± 0.07	2.35	2
tripetala		SCBG	4.05 ± 0.00	2.00	2
		MGA 135	3.94 ± 0.01		2
Subsection *Oyama*					
sieboldii	'Brusso' seedling	SCC 2008-101	4.41 ± 0.03	2.26	2
	'Colossus'	NCSU 2004-064	4.62 ± 0.01		2
	'Colossus'	Holden 98-173-99	4.43 ± 0.06		2
	'Colossus'	Holden 2005-337	4.59 ± 0.03		2
	'Colossus'	Holden 2005-336	4.58 ± 0.03		2
	'Colossus'	Holden 2001-223A	4.56 ± 0.06		2
	'Colossus'	Holden 89-518 A	4.56 ± 0.01		2
	'Colossus'	McCracken	4.35 ± 0.12		2
	'Halifax Hardy' Seedling	SCC 2008-100	4.56 ± 0.00		2
	ssp. sinensis	SCC 2008-102	4.47 ± 0.01		2
Section *Manglietia*					
aromatica		MGA in nursery	5.15 ± 0.05	2.58	2
changhungtana (*pachyphylla*)		MGA 300	4.69 ± 0.02	2.35	2
conifera var. *chingii*		MGA 378	4.67 ± 0.05	2.34	2
		Strybing	5.07 ± 0.10		2
fordiana		MGA 425	4.81 ± 0.01	2.41	2
garrettii		NCSU 2010-087	5.25 ± 0.01	2.63	2
hookeri		MGA 474	4.82 ± 0.01	2.41	2
insignis	Piroche Red Form	MGA 355	4.86 ± 0.04	2.47	2
		NCSU 2009-133	5.02 ± 0.05		2
		McCracken	4.80 ± 0.02		2
		Strybing Area 14	5.06 ± 0.01		2
kwangtungensis (*moto*)		MGA 435	4.65 ± 0.18	2.33	2
ovoidea		MGA in nursery	5.02 ± 0.06	2.51	2
yuyuanensis		McCracken	4.74 ± 0.01	2.37	2
		2002-041	4.73 ± 0.03		2
		MGA 160	4.73 ± 0.01		2
		Head	4.77 ± 0.02		2
Section *Macrophylla*					
macrophylla	White Form	Parris	4.52 ± 0.03	2.28	2
		MGA 110	4.51 ± 0.01		2
		Bartlett 2002-268	4.41 ± 0.14		2
macrophylla var. *ashei*		Parris	4.52 ± 0.03		2
macrophylla var. *dealbata*		Strybing 1986-1036	4.87 ± 0.00		2
Section *Auriculata*					
fraseri		SHR (wild in situ)	3.92 ± 0.04	1.94	2
		MGA (wild in situ)	3.96 ± 0.03		2
fraseri var. *pyramidata*		Bartlett 2007-0183B	3.74 ± 0.06		2
Section *Kmeria*					
thailandica		MGA in nursery	5.51 ± 0.02	2.76	2

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Table 2. Continued.

Taxa	Cultivar/selection	Source/accession no.[z]	Relative 2C genome size [mean ± SE (pg)][y]	Mean relative 1Cx genome size by species (pg)[x]	Ploidy level (*x*)
Subgenus *Yulania*					
Section *Yulania*					
Subsection *Yulania*					
amoena		MGA 304	4.26 ± 0.12	2.13	2
biondii		MGA 027	4.12 ± 0.02	2.06	2
		Bartlett 2002-056	4.10 ± 0.04		2
campbellii		MGA 032	12.46 ± 0.09	2.09	6
		Strybing 1981-0245	12.58 ± 0.09		6
		Strybing 1997-0354	12.67 ± 0.05		6
cylindrica		MGA 216/Holden 87-86-93	8.82 ± 0.06	2.23	4
		Holden 96-111A	9.11 ± 0.11		4
		Holden 96-115B	8.99 ± 0.06		4
		Bartlett 193	8.82 ± 0.15		4
dawsoniana		Strybing 1963-0386	13.12 ± 0.10	2.19	6
denudata		Riehle 010	13.01 ± 0.05	2.21	6
		Strybing xy-0919	13.47 ± 0.03		6
kobus		Bartlett 1994-2078	4.02 ± 0.04	2.02	2
	'Ballerina'	Strybing	4.14 ± 0.03		2
	'Esveld Select'	Bartlett 2004-271	3.84 ± 0.05		2
	'Spring Snow'	NCSU	4.16 ± 0.01		2
liliiflora		Strybing xy-0972	9.34 ± 0.14	2.28	4
	'Mini Mouse'	NCSU	9.24 ± 0.03		4
	'Nigra	Bartlett 1404	8.95 ± 0.07		4
	'O'Neill'	NCSU 2008-258	8.95 ± 0.12		4
sargentiana		Holden 96-114	11.49 ± 0.02	1.92	6
sprengeri	'Burncoose'	Bartlett 2003-251	12.57 ± 0.19	2.11	6
	'Diva'	MGA 024	12.52 ± 0.02		6
		Strybing 1963-0368	12.93 ± 0.11		6
salicifolia		MGA 470	3.91 ± 0.02	1.96	2
	'Miss Jack'	Bartlett 2003-281	3.91 ± 0.07		2
stellata		Bartlett 1392	3.91 ± 0.02	1.97	2
	'Chysanthemumiflora'	Riehle 002	4.05 ± 0.01		2
	'Kikuzaki'	USNA 57385-H	4.12 ± 0.00		2
	'Royal Star'	Bartlett 2003-270	3.88 ± 0.03		2
	'Two Stones'	Ledvina	4.04 ± 0.05		2
zenii		MGA 440/Arnold 1545-80-B	4.12 ± 0.03	2.08	2
	Chollipo Form	SCC in nursery	4.19 ± 0.03		2
	'Pink Parchment'	Johnston	4.13 ± 0.14		2
Subsection *Tulipastrum*					
acuminata	'Patriot'	Ledvina	8.21 ± 0.01	2.06	4
		SCC 2010-001	8.15 ± 0.19		4
		SCC 2010-002	8.24 ± 0.01		4
		SCC 2010-003	8.14 ± 0.03		4
		SCC 2010-004	8.08 ± 0.16		4
acuminata var. *subcordata*	'Brenda'	NCSU 2004-061	8.14 ± 0.03		4
	'Skylands Best'	MGA 231	8.32 ± 0.05		4
	'Steven's Creek'	MGA 152	8.26 ± 0.05		4
Section *Michelia*					
cavaleriei var. *platypetala*		Strybing area 14	4.40 ± 0.08	2.19	2
		Bartlett 2007-0372A	4.36 ± 0.01		2
champaca		Strybing area 14	4.76 ± 0.01	2.37	2

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Table 2. Continued.

Taxa	Cultivar/selection	Source/accession no.[z]	Relative 2C genome size [mean ± SE (pg)][y]	Mean relative 1Cx genome size by species (pg)[x]	Ploidy level (*x*)
	Orange Form	Stowe Conservatory	4.72 ± 0.06		2
chapensis		Strybing 99-0128	4.92 ± 0.02	2.46	2
doltsopa		MGA 406	4.44 ± 0.10	2.26	2
		Strybing	4.61 ± 0.01		2
ernestii		MGA 211	4.50 ± 0.03	2.25	2
figo		SCBG	4.82 ± 0.01	2.29	2
		MGA 397	4.52 ± 0.02		2
	'Port Wine'	NCSU 2009-045	4.66 ± 0.01		2
	'Port Wine'	Bartlett 2006-0124	4.30 ± 0.03		2
	var. skinneriana	Parris	4.48 ± 0.08		2
	var. crassipes	SCC in nursery	4.71 ± 0.06		2
floribunda		MGA in nursery	4.51 ± 0.02	2.26	2
foveolata	Shibamichi Form	MGA 356	4.23 ± 0.07	2.16	2
	var. cinerascens	MGA 426 TH2285	4.42 ± 0.06		2
fulva var. *calcicola*		MGA in nursery	4.61 ± 0.13	2.31	2
laevifolia		MGA 424	4.63 ± 0.02	2.28	2
	'Bubbles'	McCracken	4.52 ± 0.01		2
	'Copperstop'	NCSU 2008-296	4.58 ± 0.03		2
	'Gail's Favorite'	NCSU 2008-268	4.45 ± 0.07		2
	Heronswood Selection	MGA 432	4.64 ± 0.02		2
	'Willlowleaf'	McCracken	4.42 ± 0.07		2
lanuginosa		MGA 454	4.80 ± 0.07	2.40	2
maudiae		Head	4.41 ± 0.03	2.28	2
		NCSU 2009-092	4.45 ± 0.03		2
	Yuyuan Form	MGA 188	4.87 ± 0.05		2
martinii		MGA in nursery	4.75 ± 0.04	2.38	2
odora		MGA 472	4.54 ± 0.01	2.27	2
shiluensis		MGA 385	4.49 ± 0.02	2.25	2
sirindhorniae		MGA in nursery	4.53 ± 0.16	2.27	2
Subgenus *Gynopodium*					
Section *Gynopodium*					
lotungensis	Small Leaf Form	MGA 380	11.44 ± 0.06	1.93	6
	Small Leaf Form	MGA 260	11.72 ± 0.17		6
	Large Leaf Form	MGA 367	11.57 ± 0.09		6
yunnanensis	Vietnam origin	MGA (07-SM-051)	12.50 ± 0.00	2.08	6
Section *Manglietiastrum*					
sinica		MGA in nursery	4.21 ± 0.02	2.11	2
Genus *Liriodendron*					
chinensis		Strybing Area 4A	3.47 ± 0.09	1.74	2
tulipifera	'Arnold'	NCSU 1999-292	3.35 ± 0.02	1.68	2

[z]MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC; NCSU = North Carolina State University Mountain Horticultural Crops Research Station, Mills River, NC; McCracken = P. McCracken, Zebulon, NC; Strybing = Strybing Arboretum, San Francisco, CA; Bartlett = Bartlett Tree Research Facility, Charlotte, NC; SCC = Spartanburg Community College Arboretum, Spartanburg SC; USNA = U.S. National Arboretum, Washington, DC; Head = R. Head, Seneca, SC; Parris = J.K. Parris' residential garden, Spartanburg, SC; Ledvina = D. Ledvina, Green Bay, WI; Holden = Holden Arboretum, Kirtland, OH; Gilbert's = Gilbert's Nursery, Chesnee, SC; SHR = Southern Highlands Reserve, Lake Toxaway, NC; Milliken = Milliken Arboretum, Spartanburg, SC; Riehle = R. Riehle Garden, Spartanburg, SC; SCBG = South Carolina Botanical Garden, Clemson, SC; KP = J.K. Parris' plants in greenhouse, Spartanburg, SC; Johnston = J. Johnston, Clayton, GA.
[y]Genome sizes were determined using 4′,6-diamidino-2-phenylindole as the flourochrome stain.
[x]$1C_X$ values were calculated as 2C value/ploidy level.

'Maryland' was found to have a genome size of 5.62 pg, consistent with a triploid, suggesting a *M. grandiflora* (6*x*) × *M. virginiana* (2*x*) backcrossed to *M. virginiana*. An intermediate tetraploid condition was determined for *M. insignis* (2*x*) × *M. grandiflora* 'Kay Parris' (6*x*) (NCSU H2010-026-001), which had an 8.50 pg relative genome size.

Within subgenus *Yulania*, confirmed interploid hybrids were numerous. Verification of hybridity was readily confirmed for

Table 3. Relative genome size and estimated ploidy level for interspecific hybrids of *Magnolia* arranged by reported parentage ploidy levels.

Reported parentage	Cultivar/selection	Source/accession no.[z]	Relative 2C Genome size [mean ± SE (pg)][y]	Weighted $1C_X$ genome size (pg)[x]	Reported parental ploidy levels (x)[w]	Estimated ploidy level (x)[v]
Subgenus *Magnolia*						
Intraploid hybrids						
$2n = 2x = 38$						
insignis × *virginiana*	'Katie-O'	NCSU 2004-012	4.33 ± 0.04	2.17	2 × 2	2
insignis × *virginiana*	'Katie-O'	MGA 307	4.27 ± 0.04	2.17	2 × 2	2
macrophylla × *tripetala*		MGA in nursery	3.68 ± 0.01	2.12	2 × 2	2
obovata × *virginiana*	'Nimbus'	NCSU 2003-041	3.79 ± 0.04	1.93	2 × 2	2
officinalis × *tripetala*		MGA 457	3.96 ± 0.01	1.95	2 × 2	2
sieboldii 'Colossus' × *insignis*		MGA in nursery	4.60 ± 0.03	2.37	2 × 2	2
sieboldii 'Colossus' × *insignis*		McCracken	4.63 ± 0.06	2.37	2 × 2	2
sieboldii 'Genesis' × *virginiana*	R10-24	Riehle 009	4.06 ± 0.01	2.06	2 × 2	2
sieboldii 'Genesis' × *virginiana*	R10-24	Bartlett 2007-0045A	3.93 ± 0.13	2.06	2 × 2	2
sieboldii 'Genesis' × *virginiana*	R10-24	McCracken	4.10 ± 0.01	2.06	2 × 2	2
×*thompsoniana* (=*virginiana* × *tripetala*)		Strybing 1963-0522	3.95 ± 0.02	1.93	2 × 2	2
×*thompsoniana* (=*virginiana* × *tripetala*)	'Cairn Croft'	Bartlett 2007-0019	3.67 ± 0.02	1.93	2 × 2	2
[(*tripetala* × *obovata*) × *tripetala*)] 'Silk Road' × *insignis*		MGA in nursery	4.35 ± 0.04	2.23	2 × 2	2
yuyuanensis × *insignis*		McCracken	4.53 ± 0.07	2.43	2 × 2	2
yuyuanensis × *sieboldii*	104/1	McCracken	4.67 ± 0.11	2.32	2 × 2	2
yuyuanensis × *virginiana*		NCSU 2009-131	4.41 ± 0.04	2.12	2 × 2	2
virginiana 'Havener' × *insignis* (Red Form)	111/7	McCracken	4.23 ± 0.00	2.17	2 × 2	2
Interploid hybrids						
$2n = 3x = 57$						
(*grandiflora* × *virginiana*) × *virginiana*		USNA 2	5.62 ± 0.00	1.87	4 × 2	3
$2n = 4x = 76$						
grandiflora × *virginiana*	'Maryland'	MGA 077	7.52 ± 0.03	1.87	6 × 2	4
grandiflora × *virginiana*	'Maryland'	McCracken	7.45 ± 0.04	1.87	6 × 2	4
grandiflora × *virginiana*	'Monland'	SCBG	11.29 ± 0.07	1.87	6 × 2	6
insignis × *grandiflora* 'Kay Parris'		KP 2009-005	8.53 ± 0.11	2.02	2 × 6	4
insignis × *grandiflora* 'Kay Parris'		NCSU 2010-026-001	8.50 ± 0.09	2.02	2 × 6	4
sieboldii 'Colossus' × *grandiflora* 'Bracken's Brown Beauty'		McCracken	7.87 ± 0.01	1.97	2 × 6	4
sieboldii 'Colossus' × *grandiflora* 'Kay Parris'		KP 2008-001	8.23 ± 0.02	1.97	2 × 6	4
sieboldii 'Colossus' × 'Sweet Summer'		MGA 280	8.02 ± 0.10	1.97	2 × 6	4
sieboldii 'Pride of Norway' × 'Sweet Summer'		MGA 417	7.99 ± 0.04	1.97	2 × 6	4
virginiana var. *australis* × *grandiflora* 'Samuel Sommer'	'Sweet Summer'	MGA 327	11.51 ± 0.04	1.87	2 × 6	6

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Table 3. Continued.

Reported parentage	Cultivar/selection	Source/accession no.[z]	Relative 2C Genome size [mean ± SE (pg)][y]	Weighted $1C_X$ genome size (pg)[x]	Reported parental ploidy levels (x)[w]	Estimated ploidy level (x)[v]
virginiana var. *australis* × *grandiflora* 'Samuel Sommer'	'Sweet Summer'	McCracken	11.54 ± 0.13	1.87	2 × 6	6
virginiana × *grandiflora*		NCSU 2001-233	11.58 ± 0.09	1.87	2 × 6	6
$2n = 5x = 95$						
(*grandiflora* × *virginiana*) 'Maryland' × *grandiflora*	(Maryland Seedling)	MGA 325	9.00 ± 0.01	1.87	4 × 6	5
Subgenus *Yulania*						
Intraploid hybrids						
$2n = 2x = 38$						
×*kewensis* (=*kobus* × *salicifolia*)	'Wada's Memory'	NCSU/MHCREC	4.05 ± 0.04	1.99	2 × 2	2
	'Wada's Memory'	Bartlett 2007-0131	3.83 ± 0.02	1.99	2 × 2	2
×*loebneri* (=*kobus* × *stellata*)	'Donna'	Bartlett 2007-0281B	5.86 ± 0.04	2.00	2 × 2	3
	'Pink Superstar'	MGA 076	4.02 ± 0.01	2.00	2 × 2	2
	'Leonard Messel'	NCSU 1998-348	4.40 ± 0.12	2.00	2 × 2	2
	'Leonard Messel'	Milliken 6-0043-01-89-003	4.00 ± 0.08	2.00	2 × 2	2
	'Mag's Pirouette'	Bartlett	3.97 ± 0.04	2.00	2 × 2	2
	'Merril'	MGA 085	3.86 ± 0.01	2.00	2 × 2	2
	'Spring Snow'	Bartlett 2004-0126A	3.86 ± 0.00	2.00	2 × 2	2
	'Wildcat'	MGA 248	3.71 ± 0.22	2.00	2 × 2	2
		Bartlett 1406	3.98 ± 0.03	2.00	2 × 2	2
×*alba* (=*champaca* × *montana*)		Stowe Conservatory	4.81 ± 0.02	2.28	2 × 2	2
laevifolia × *figo*	(Clifford Parks)	MGA 456	4.46 ± 0.04	2.28	2 × 2	2
×*foggii* (=*figo* × *doltsopa*)		MGA 144	4.53 ± 0.02	2.27	2 × 2	2
$2n = 4x = 76$						
acuminata 'Busey' × *acuminata sub.* 'Miss Honeybee'	'Miranja'	Bartlett 2004-313	18.25 ± 0.52	2.10	4 × 4	≈8.6
liliiflora 'O'Neill' × *kobus* 'Norman Gould'	'Roseanne'	Ledvina	8.53 ± 0.08	2.15	4 × 4	4
×*brooklynensis* 'Woodsman' × (*acuminata* 'Miss Honeybee' × *stellata*) 'Gold Star'	'Solar Flair'	NCSU 2001-239	8.19 ± 0.06	2.13	4 × 4	4
×*brooklynensis* 'Woodsman' × (*acuminata* 'Miss Honeybee' × *stellata*) 'Gold Star'	'Sunburst'	NCSU 2000-065	8.07 ± 0.02	2.13	4 × 4	4
×*brooklynensis* 'Woodsman' × (*acuminata* 'Miss Honeybee' × *stellata*) 'Gold Star'	'Tranquility'	Bartlett 2004-308-A	8.15 ± 0.01	2.13	4 × 4	4
×*brooklynensis* (=*acuminata* × *liliiflora*)	'Woodsman'	SCBG	8.21 ± 0.05	2.17	4 × 4	4
$2n = 6x = 114$						
denudata × *sprengeri* 'Diva'	'Legacy'	NCSU 1998-260	13.11 ± 0.16	2.16	6 × 6	6
sargentii var. *robusta* × *campbellii*	'Hawk'	Bartlett 2007-0288A	12.67 ± 0.25	2.01	6 × 6	6
×*veitchii* (=*campbellii* × *denudata*)		Strybing 1963-0387	12.96 ± 0.04	2.15	6 × 6	6
Interploid hybrids						
cylindrica × ×*veitchii* 'Peter Veitch'	'Albatross'	MGA 004	11.14 ± 0.05	2.18	4 × 6	5

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Table 3. Continued.

Reported parentage	Cultivar/selection	Source/accession no.[z]	Relative 2C Genome size [mean ± SE (pg)][y]	Weighted $1C_X$ genome size (pg)[x]	Reported parental ploidy levels (x)[w]	Estimated ploidy level (x)[v]
×*soulangeana* (=*denudata* × *liliiflora*)	'Alexandrina'	Bartlett	10.70 ± 0.06	2.24	6 × 4	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Andre Leroy'	Milliken	14.60 ± 0.30	2.24	? × ?	≈6.5
cylindrica × *denudata* 'Sawada's Pink'	'Angelica'	Bartlett 2007-0287A	10.83 ± 0.21	2.22	4 × 6	5
stellata × *liliiflora* 'Nigra'	'Ann'	NCSU 2006-163	6.28 ± 0.01	2.18	2 × 4	3
liliiflora × *cambellii* 'Lanarth'	'Apollo'	Bartlett 2007-0287A	11.02 ± 0.14	2.17	4 × 6	5
(*campbellii* 'Lanarth' × *liliiflora*) 'Vulcan' × ×*soulangeana* 'Lennei'	ArborTree Select	NCSU 2000-119-001	16.97 ± 0.17	2.21	5 × ≈8	≈7.7
×*soulangeana* 'Lennei Alba' × (*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury'	'Athene'	Bartlett	14.96 ± 0.19	2.14	≈7.6 × 6	≈7
×*soulangeana* 'Lennei' × (*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury'	'Atlas'	MGA 156	12.82 ± 0.18	2.14	≈8 × 6	≈6
stellata 'Rosea' × *liliiflora* 'Nigra'	'Betty'	NCSU 2006-164	6.61 ± 0.04	2.18	2 × 4	3
(*acuminata* × ×*brooklynensis* 'Evamaria') 'Yellow Bird' × (*sargentiana* × *sprengeri* 'Diva') 'Caerhays Belle'	'Blushing Belle'	Bartlett 2007-0280B	10.32 ± 0.15	2.11	4 × 6	5
acuminata × *denudata* 'Sawada's Cream'	'Butterflies'	NCSU 1998-259	10.71 ± 0.01	2.15	4 × 6	5
'Legend' × 'Butterflies'	'Coral Lake'	Riehle 008	12.09 ± 0.02	2.15	5 × 5	≈5.6
×*veitchii* × ×*soulangeana*	'David Clulow'	Bartlett 2004-267	16.75 ± 0.31	2.19	6 × ?	≈7.6
×*brooklynensis* 'Woodsman' × (×*soulangeana* 'Lennei Alba' × ×*veitchii*) 'Tina Durio'	'Daybreak'	MGA 157	10.71 ± 0.01	2.20	4 × ≈6.9	≈4.9
acuminata × *denudata*	'Elizabeth'	NCSU 1998-272	10.59 ± 0.03	2.15	4 × 6	5
denudata × *stellata* 'Waterlily'	'Emma Cook'	MGA 197	10.26 ± 0.04	2.15	6 × 2	≈4.8
kobus 'Norman Gould' × ×*soulangeana* 'Lennei'	'Eskimo'	NCSU 2000-071	9.99 ± 0.04	2.14	4 × ≈8	≈4.6
sprengeri 'Diva' × 'Wada's Picture'	'Felicity'	Bartlett	10.75 ± 0.10	2.18	6 × 5	≈4.9
×*soulangeana* 'Deep Purple Dream' × ×*veitchii* 'Paul Cook'	'Frank's Masterpiece'	NCSU 2001-237	14.66 ± 0.14	2.19	? × 6	≈6.7
×*soulangeana* (=*denudata* × *liliiflora*)	'Fukuju'	Bartlett	19.02 ± 0.06	2.24	? × ?	≈8.5
liliiflora 'Nigra' × *sprengeri* 'Diva'	'Galaxy'	Bartlett 2002-724	10.45 ± 0.11	2.18	4 × 6	5
acuminata var. *sub.* 'Miss Honeybee' × *denudata* 'Sawada's Cream'	'Gold Finch'	NCSU 2000-261	10.81 ± 0.13	2.15	4 × 6	5.0
acuminata × *denudata*	'Golden Sun'	Bartlett 2007-0365A	13.59 ± 0.12	2.15	4 × 6	≈6.3
acuminata × *stellata*	'Gold Star'	NCSU 2004-063	8.22 ± 0.06	2.06	4 × 2	4
×*soulangeana* (=*denudata* × *liliiflora*)	'Grace McDade'	Bartlett 2004-238	17.35 ± 0.14	2.24	? × ?	≈7.8
(×*brooklynensis* 'Woodsman' × ×*soulangeana* 'Lennei') × (*acuminata* × *denudata*) 'Elizabeth'	'Green Snow'	Bartlett 2004-236	11.47 ± 0.15	2.20	(4 × ≈8) × 5	≈5.2
×*brooklynensis* 'Woodsman' × (*acuminata* × *denudata*) 'Elizabeth'	'Hot Flash'	Bartlett 2007-0367A	8.43 ± 0.07	2.15	4 × 5	≈3.9
(*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury' × ×*soulangeana* 'Lennei'	'Iolanthe'	MGA 407	13.62 ± 0.05	2.14	6 × ≈8	≈6.4

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Table 3. Continued.

Reported parentage	Cultivar/selection	Source/accession no.[z]	Relative 2C Genome size [mean ± SE (pg)][y]	Weighted $1C_X$ genome size (pg)[x]	Reported parental ploidy levels (x)[w]	Estimated ploidy level (x)[v]
acuminata × *denudata*	'Ivory Chalice'	NCSU 1998-262	10.76 ± 0.07	2.17	4 × 6	5
×*soulangeana* × ×*veitchii*	'Jon Jon'	NCSU 2000-258	15.16 ± 0.08	2.19	? × 6	≈6.9
×*loebneri* 'Encore' × ×*soulangeana* 'Alexandrina'	Kehr Seedling	NCSU 2001-143-001	10.92 ± 0.09	2.17	2 × ≈5	≈5
acuminata × *denudata*	'Legend'	NCSU 1998-261	10.77 ± 0.02	2.15	4 × 6	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Lennei'	Bartlett 1075	17.89 ± 0.16	2.24	? × ?	≈8
×*soulangeana* (=*denudata* × *liliiflora*)	'Lennei Alba'	Bartlett 1995-2153	16.91 ± 0.12	2.24	? × ?	≈7.6
acuminata var. *subcordata* × ×*soulangeana* 'Big Pink'	'Limelight'	Bartlett 2007-0495C	14.23 ± 0.13	2.18	4 × ?	≈6.5
acuminata × (*acuminata* × *denudata*)	'Lois'	Riehle 001	14.61 ± 0.28	2.10	4 × 5	≈7.0
×*soulangeana* × ×*veitchii*	'Manchu Fan'	Bartlett 2003-593	14.86 ± 0.04	2.19	? × 6	≈6.8
(*liliiflora* × *cylindrica*) × ×*soulangeana* 'Ruby'	'March till Frost'	NCSU 2001-257	12.89 ± 0.14	2.25	4 × ?	≈5.7
×*soulangeana* 'Lennei' × (×*soulangeana* 'Lennei' seedling × *sprengeri* 'Diva') 'Paul Cook'	'Millie Gaylon'	Bartlett 2007-0496A	14.20 ± 0.16	2.21	≈8 × (? × 6)	≈6.4
stellata × *liliiflora*	'Orchid'	Bartlett 2002-430	6.44 ± 0.24	2.18	2 × 4	3
acuminata × ×*veitchii* 'Peter Veitch'	'Pastel Beauty'	NCSU 2000-240	10.12 ± 0.06	2.13	4 × 6	5
acuminata × *sprengeri* 'Diva'	'Peachy'	Bartlett 2003-286	10.11 ± 0.11	2.11	4 × 6	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Pickard's Firefly'	Bartlett 2004-250	17.06 ± 0.39	2.24	? × ?	≈7.6
liliiflora 'Reflorescens' × *stellata* 'Rubra'	'Pinkie'	Bartlett 2003-714	6.47 ± 0.02	2.18	4 × 2	3
liliiflora × ×*soulangeana*	'Purple Prince	Bartlett 2003-285	10.91 ± 0.15	2.26	4 × ?	≈4.8
liliiflora 'Nigra' × *stellata*	'Randy'	Bartlett 2004-643	6.44 ± 0.04	2.18	4 × 2	3
acuminata × (×*soulangeana* 'Wada's Picture' × *sprengeri* 'Diva') 'Big Dude'	'Red Baron'	Bartlett 2004-311	13.19 ± 0.23	2.14	4 × (? × 6)	≈6.2
liliiflora × ×*veitchii*	'Royal Crown'	Bartlett 2002-157	10.58 ± 0.01	2.20	4 × 6	5
liliiflora × ×*veitchii*	'Sayonara'	NCSU 2008-266	14.82 ± 0.10	2.20	4 × 6	≈6.7
liliiflora × (*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury'	'Serene'	Bartlett 2003-263	10.59 ± 0.06	2.12	4 × 6	5
denudata × (*campbellii* 'Lanarth' × *liliiflora*) 'Vulcan'	'Shiraz'	Bartlett 2003-277	12.76 ± 0.16	2.19	6 × 5	≈5.8
liliiflora 'Nigra' × *sprengeri* 'Diva'	'Spectrum'	MGA	11.58 ± 0.02	2.18	4 × 6	5
campbellii × *liliiflora*	'Star Wars'	MGA 330	10.53 ± 0.08	2.17	6 × 4	5
acuminata × *denudata*	'Sunray'	Bartlett 2007-0358A	10.22 ± 0.17	2.17	4 × 6	5
×*brooklynensis* 'Woodsman' × (*acuminata* × *denudata*) 'Elizabeth'	'Sunsation'	SCC nursery	14.73 ± 0.33	2.18	4 × 5	≈6.8
liliiflora × *stellata* 'Rosea'	'Susan'	Bartlett 2002-433	6.58 ± 0.01	2.18	4 × 2	3
×*soulangeana* 'Lennei Alba' × ×*veitchii*	'Tina Durio'	MGA 087	15.23 ± 0.02	2.20	≈7.6 × 6	≈6.9
×*veitchii* × ×*soulangeana* 'Rustica Rubra'	'Todd Gresham'	Bartlett 2002-641	14.75 ± 0.33	2.19	6 × ?	≈6.7
campbellii 'Lanarth' × *liliiflora* hybrid	'Vulcan'	Riehle 004	10.54 ± 0.11	2.17	6 × 4	5

continued next page

Table 3. Continued.

Reported parentage	Cultivar/selection	Source/accession no.[z]	Relative 2C Genome size [mean ± SE (pg)][y]	Weighted $1C_X$ genome size (pg)[x]	Reported parental ploidy levels (x)[w]	Estimated ploidy level (x)[v]
acuminata var. subcordata ×*soulangeana* 'Alexandrina'	'Yellow Lantern'	Bartlett 2003-266	14.43 ± 0.26	2.18	4 × 5	≈6.6
acuminata × *denudata*	'Yellow Sea'	Bartlett 2004-0495C	8.68 ± 0.01	2.17	4 × 6	4
(*cylindrica* × *denudata*) 'Pegasus' × *campbellii* 'Darjeeling'	'Zeal'	Bartlett 2005-0025	10.15 ± 0.26	2.17	5 × 6	≈4.6
acuminata × *figo*		MGA 120	6.16 ± 0.06	2.16	4 × 2	3
(*liliiflora* 'Nigra' × *sprengeri* 'Diva') 'Galaxy' × *campbellii* var. *mollicomata*		MGA 153	12.34 ± 0.01	2.13	5 × 6	≈5.8
×*veitchii* 'Isca' × *liliiflora*		MGA 109	10.84 ± 0.01	2.13	6 × 4	5
cylindrica hybrid (Polly Hill)		MGA 215	13.35 ± 0.04	2.23	4 × ?	6.0
cylindrica hybrid		SCC 2009-004	14.92 ± 0.27	2.23	8 × ?	≈6.7
cylindrica hybrid		SCC 2009-005	15.21 ± 0.11	2.23	8 × ?	≈6.8

[z]MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC; NCSU = North Carolina State University Mountain Horticultural Crops Research Station, Mills River, NC ; McCracken = P. McCracken, Zebulon, NC; Strybing = Strybing Arboretum, San Francisco, CA; Bartlett = Bartlett Tree Research Facility, Charlotte, NC; SCC = Spartanburg Community College Arboretum, Spartanburg, SC; USNA = U.S. National Arboretum, Washington, DC; Head = R. Head, Seneca, SC; Parris = J.K. Parris' residential garden, Spartanburg SC; Ledvina = D. Ledvina, Green Bay, WI; Holden = Holden Arboretum, Kirtland, OH; Gilbert's = Gilbert's Nursery, Chesnee, SC; SHR = Southern Highlands Reserve, Lake Toxaway, NC; Milliken = Milliken Arboretum, Spartanburg, SC; Riehle = R. Riehle Garden, Spartanburg, SC; SCBG = South Carolina Botanical Garden, Clemson, SC; KP = J.K. Parris' plants in greenhouse, Spartanburg, SC.

[y]Genome sizes were determined using 4′,6-diamidino-2-phenylindole as the flourochrome stain.

[x]Weighted $1C_X$ values were calculated as [($1C_X$ value of the female parent × ploidy level of the female parent/2) + ($1C_X$ value of the male parent × ploidy level of the male parent/2)]/[(ploidy level of the female parent + ploidy level of the male parent)/2] . When the $1C_X$ was not known for the exact parent, then an average for the parental species or section was used.

[w]Parental ploidy and genome sizes for *M.* ×*soulangeana* hybrids are unknown and marked as "?."

[v]Estimated ploidy levels were calculated as 2C genome size/weighted $1C_X$ value. If both parent species had even ploidy levels, then ploidy levels of the progeny were rounded to the nearest whole numbers if supported by an appropriate relative genome size. If either parent had an odd ploidy level, then ploidy levels of the progeny were rounded to the nearest 0.01 to reflect apparent aneuploidy.

the USNA's Kosar/de Vos hybrids. *M. liliiflora* (4*x*) × *M. stellata* (2*x*) had genome sizes ranging from 6.28 to 6.69 pg, consistent with triploids. Numerous putative pentaploid hybrid cultivars, derived from crosses of (6*x* × 4*x*) species or hybrids, were also verified. These hybrid cultivars include: Alexandrina, Angelica, Apollo, Blushing Belle, Butterflies, Elizabeth, Galaxy, Gold Finch, and Spectrum with 2C genome sizes ranging from 10.11 to 11.02 pg.

Hybrids arising from parents with odd ploidy levels (5*x* or aneuploids) were prevalent and had highly variable genome sizes. *Magnolia* ×*soulangeana*, a pentaploid hybrid between *M. denudata* (6*x*) and *M. liliiflora* (4*x*), exhibits fertility in initial F_1 hybrids and subsequent generations (McDaniel, 1968) and when used as parents gave rise to apparent aneuploid progeny ranging from ≈4.6 to ≈8.5*x* based on genome size. Fertility among *M.* ×*soulangeana* cultivars has been examined previously and it was found that pollen viability generally increased with increasing ploidy level above 5*x* (Santamour, 1970). Relative 2C genome sizes determined here support cytological findings by Santamour (1970) that the cultivars Lennei and Grace McDade are septaploids or higher. Other taxa in Table 3 of approximate septaploid genome size include *Magnolia* 'Andre Leroy' (Milliken), *Magnolia* 'Manchu Fan' (Bartlett 2003-593), *Magnolia* 'Sunsation' (SCC), and *Magnolia* 'Todd Gresham' (Bartlett 2002-641). Each of these hybrids has a parental combination that theoretically could yield 7*x* offspring. No triploid hybrids were found to be parents of any hybrid surveyed in this study indicating triploids may typically not be fertile.

In a number of cases, interploid hybridization was not validated. Two accessions of *Magnolia* 'Sweet Summer' [11.53 pg (McCracken, MGA 327)], a reported *M. virginiana* (2*x*) × *M. grandiflora* (6*x*) hybrid, and *Magnolia* 'Monland' [11.29 pg (SCBG)], a reported *M. grandiflora* (6*x*) ×*virginiana* (2*x*) hybrid (Langford, 1994), both had genome sizes consistent with a subgenus *Magnolia* hexaploid.

Unreduced gametes can lead to higher than expected genome sizes or ploidy levels in Magnolia hybrids (McDaniel, 1968; Santamour, 1970). In subgenus *Yulania*, the relative genome size of *M. acuminata* (*4x*) × *M. stellata* (*2x*) 'Gold Star' (NCSU 2004-063) was determined to be 8.22 pg, consistent with the genome size of a tetraploid. This suggests this cultivar is the result of pollination from an unintended source or the product of an unreduced gamete from *M. stellata*. The hybrids 'Miranja' and 'Sunsation' may also have resulted from stray pollination or unreduced gametes from at least one parent.

Determination of relative genome size and ploidy level among artificially induced polyploids. Attempts to develop artificially induced polyploids of *Magnolia* have met with varying degrees of success. *M. stellata* and *M. cylindrica* seedlings treated with colchicine at the Holden Arboretum (C. Tubesing, personal communication) were determined to be tetraploid and octoploid, respectively (Table 4). *Magnolia kobus* 'Norman Gould' [7.79 pg (USNA 59598-H)] was also confirmed to be tetraploid. Additonally, a *M. grandiflora* 'Little Gem' treated with colchicine at Head-Lee Nursery (R. Head, personal communication) was determined to be a 6*x* - 12*x* cytochimera. The plant was reported to be treated over 10 years ago and has stabilized as a cytochimera with ≈55% of the leaf tissue comprised of 12*x* cells. Phenotypic characteristics such as thickened foliage and increased width to length ratio of foliage (Kehr, 1985) were suggestive of polyploidy in *M. sieboldii* 'Colossus', a reported hexaploid. However, samples of *M. sieboldii* 'Colossus' from multiple sources had genome sizes (2C = 4.35 pg to 4.62 pg) consistent with a diploid. Hybrids with *Magnolia* 'Colossus', including *M. sieboldii* 'Colossus' × *M. grandiflora* 'Bracken's Brown Beauty' (McCracken), *M. sieboldii* 'Colossus' × *M. grandiflora* 'Kay Parris' (KP 2008-001), and *M. sieboldii* 'Colossus'× *Magnolia* 'Sweet Summer' (MGA 280) (Table 3), all had relative genome sizes consistent with a tetraploid, further confirming the ploidy level of the diploid and hexaploid parents. Other reported induced polyploids that were not confirmed include *M. stellata* 'Two Stones' and *M. acuminata* 'Patriot'. Seedlings SCC-2009-004 and SCC-2009-005, derived from open-pollinated octoploid *M. cylindrica* at the Holden Arboretum, were determined to be ≈7*x* based on genome sizes of 14.92 to 15.21 pg. This supports the assertion of Charles Tubesing (personal communication) that the octoploids probably outcrossed with other magnolias with lower ploidy levels from their collections. A chromosome count of one of these seedlings, SCC 2009-004, identified ≈133 chromosomes (Fig. 1), in close agreement with genome size data.

Table 4. Relative genome sizes and estimated ploidy levels of artificially induced polyploid *Magnolia* species.

Taxa	Source/accession no.[z]	Relative 2C genome size [mean ± SE (pg)][y]	Estimated ploidy level (*x*)[x]
cylindrica	Holden 92-443A	17.49 ± 0.01	8
	Holden 92-443F	17.42 ± 0.30	8
	Holden 92-443Q	17.40 ± 0.13	8
	Holden 92-443E	17.45 ± 0.58	8
	Holden 92-443P	17.36 ± 0.11	8
	Holden 92-443L	17.27 ± 0.04	8
	Holden 92-443J	17.28 ± 0.05	8
	Holden 92-443I	17.07 ± 0.11	8
	Holden 92-443G	17.31 ± 0.09	8
grandiflora 'Little Gem' (cytochimera)	Head	11.11 ± 0.09	6
		21.80 ± 0.32	12
kobus 'Norman Gould'	USNA 59598-H	7.79 ± 0.00	4
stellata	Holden 97-103F	8.31 ± 0.17	4
	Holden 97-103M	8.10 ± 0.12	4
	Holden 97-103C	8.17 ± 0.04	4
	Holden 97-103Q	8.23 ± 0.07	4
	Holden 97-103U	8.20 ± 0.00	4

[z]Holden = Holden Arboretum, Kirtland, OH; Head = R. Head, Seneca, SC; USNA = U.S. National Arboretum, Washington, DC.
[y]Genome sizes were determined using 4′,6-diamidino-2-phenylindole as the flourochrome stain.
[x]Estimated ploidy levels were calculated as 2C genome size/1C_X value (2.23 for *M. cylindrica*, 1.87 for *M. grandiflora*, 2.02 for *M. kobus*, and 1.97 pg for *M. stellata*) and rounded to the closest whole number.

Comparison of fluorochromes and estimate of base pair composition. Comparison of DAPI and PI stains showed there was a significant interaction between fluorochrome stain and species on the estimation of genome size ($P \leq 0.05$) (Table 5). For some species (e.g., *M. sinica*, *M. stellata* 'Royal Star', and *M. yuyuanensis*), there was no significant difference in genome size estimates between fluorochromes. In other cases, the

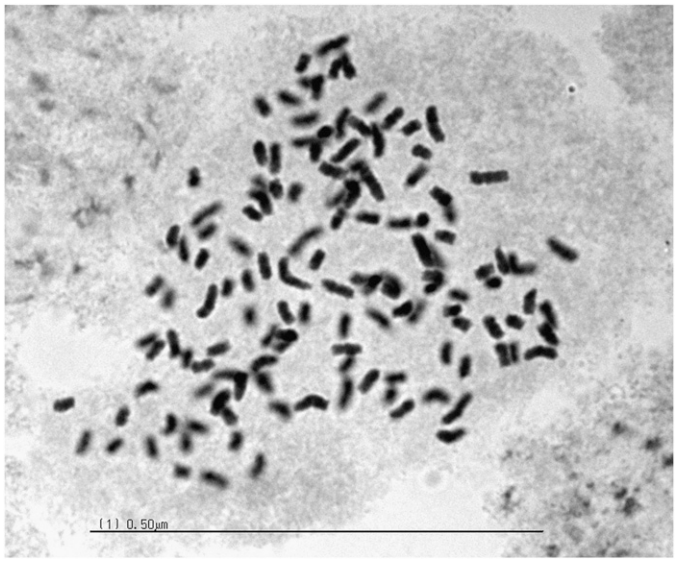

Fig. 1. Photomicrograph of a root tip cell of *Magnolia* SCC 2009-004 in early metaphase with ≈133 chromosomes. Maternal parent *Magnolia cylindrica* ($2n = 8x = 152$), paternal parent unknown, but likely ($2n = 6x = 114$), resulting in a plant that is $7x$.

difference in genome size estimates varied by as much as 0.73 pg or 14% for *M. delavayi*. This suggests that as bp composition of the sample deviates from the bp composition of the internal standard (in this case *P. sativum* = 61.50% AT), the estimate of genome sizes between methods diverges. However, for the purpose of determining euploid levels, either method was sufficiently accurate to provide proper classification and the DAPI procedure is faster, less expensive, uses less toxic compounds, and can have lower CV for mean nuclei fluorescence than the PI procedure. Base pair composition of representative *Magnolia* species ranged from 61.6% to 63.9% AT. Sequences of 8500 bases of cpDNA from seven different regions of 43 different species of *Magnolia* showed the relative frequency of AT ranging from 62.9% to 63.1% (H. Azuma, personal communication), similar to the range that we determined for the entire nuclear genome based on differential fluorochrome staining.

Implications of relative genome size for systematics and breeding. The most recent taxonomic revision of *Magnolia* (Figlar and Nooteboom, 2004) incorporates both morphological and molecular data (Azuma et al., 1999, 2000, 2001; Kim et al., 2001). In some cases, data on relative genome size support these revised taxonomic groupings. For example, establishment of section *Macrophylla* to include only *M. macrophylla* and botanical varieties *ashei* and *dealbata* is supported by the difference in 1Cx value (Table 2) of this group compared with other North American species (*M. fraseri* and *M. tripetala*) with which it was traditionally grouped (Treseder, 1978). However, in other cases, there is inconsistent variation in genome size within some sections (e.g., *M. rostrata* in section *Rhytidospermum*) and similarities in genome size among distantly related taxa (Table 2).

For breeders, the revised taxonomy by Figlar and Nooteboom (2004) provides a greater understanding of the relatedness and potential for interspecific hybridizations among closely allied species that is often supported empirically (Table 3). Yet, development of progeny from hybrids, beyond an F_1 generation, requires genome/chromosomal compatibility for meiosis to function properly. Thus, it is reasonable to expect that the greater the difference in genome size among parental species, the less likely hybrid progeny will be fertile.

Results from this study provide data on genome sizes and ploidy levels of a broad range of species and hybrids of *Magnolia*. This information also gives insight into reproductive biology, confirmation of hybrids and induced polyploids, and comparison of methods for determining genome size that will help facilitate the development of improved hybrids in the future.

Table 5. Comparison of differential staining of fluorochromes and DNA base pair content for selected species from 10 sections of *Magnolia*.

Taxa	Source/accession no.[z]	Genome size (pg)[y]			
		DAPI	PI	Difference[x]	AT%[w]
Subgenus *Magnolia*					
Section *Gwillimia*, *M. delavayii*	MGA 411	5.13	5.86	0.73*	63.91 A
Section *Auriculata*, *M. fraseri*	MGA wild in situ	3.85	4.01	0.16*	63.23 B
Section *Macrophylla*, *M. macrophylla*	Parris 1996-001	4.54	4.79	0.25*	62.46 B
Section *Magnolia*, *M. virginiana* 'Jim Wilson'	NCSU 2004-204	3.73	4.00	0.27*	62.68 B
Section *Rhytidospermum*, *M. rostrata*	NCSU 2008-028	4.51	4.67	0.16*	62.09 CD
Section *Manglietia*, *M. yuyuanensis*	NCSU 2002-041	4.77	4.90	0.13 NS	61.97 CD
Subgenus *Yulania*					
Section *Yulania*, *M. stellata* 'Royal Star'	NCSU 2008-157	3.93	4.04	0.11 NS	61.97 CD
Section *Michelia*, *M. laevifolia* 'Michelle'	NCSU 2008-244	4.35	4.55	0.20*	62.29 BC
Subgenus *Gynopodium*					
Section *Gynopodium*, *M. lotungensis*	Parris, 1997-001	12.27	12.94	0.67*	62.44 BC
Section *Manglietiastrum*, *M. sinica*	MGA, 2007 a	4.21	4.24	0.03 NS	61.60 D

[z]MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC; NCSU = North Carolina State University Mountain Horticultural Crops Research Station, Mills River, NC; Parris = J.K. Parris' residential garden, Spartanburg, SC.
[y]Genome size, n = 5, determined using either 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI).
[x]Difference between PI and DAPI methods. Fisher's least significant difference ($LSD_{0.05}$) (comparing DAPI with PI for a given taxa within a row) = 0.13; *, significant; NS, non-significant.
[w]% AT composition. Mean separation within column (among taxa) by $LSD_{0.05} = 0.56$.

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