Ploidy level, genome size, and the music of Magnolias

Kevin Parris, Arboretum Director, Spartanburg Community College, Spartanburg, SC

This article is adapted from Parris et al., (2010)

Dedication

This article is dedicated to the visionary breeders of *Magnolia* that have gone before us, and is presented to those that work today to expand the boundaries and fill in the gaps of a collective genome that surely inspires anyone with an appreciation of plants. The experiences of the recent 2011 MSI Annual Meeting in Providence, RI, were the inspiration I needed to turn the findings of this research into a format that I hope will be embraced by a wonderfully varied audience.

Introduction

Under the direction of Tom Ranney of North Carolina State University, I began a survey of genome sizes of a wide array of *Magnolia* taxa in 2008. The study was officially concluded in 2010 for statistical analyses, but will unofficially continue to be amended as additional species, hybrids, and cultivars of interest become available to study.

To understand the reproductive biology of *Magnolia* species, one must understand polyploidy. Polyploidy is the presence of multiple sets of chromosomes, above the diploid level (2n) within the somatic (vegetative or body) cells of an organism. Polyploidy is extremely rare in animals, but surprisingly common in plants. *Magnolias* naturally range in ploidy level, with species being either 2n, tetraploid (4n), or hexaploid (6n). Previous sources that include compilations of chromosome counts or ploidy levels used for this study include Callaway, 1994; Chen et al., 2000; Santamour, 1970; and Treseder, 1978. Since we know from chromosome counts that diploid magnolia species have 38 chromosomes, and the haploid gametes (n) have 19, we completely communicate the chromosomal content of a diploid with the expression $2n=2x=38$, tetraploid as $2n=4x=76$, and hexaploid as $2n=6x=114$, with the value before the x representing the complete sets of chromosomes present.

Polyploidy in Magnolias

Diploid species $2n=2x=38$	Base Number $1n=1x=19$
Triploid Hybrids $2n=3x=57$	Hexaploid Species $2n=6x=114$
Tetraploid Species $2n=4x=76$	Septaploid Hybrids $2n=7x=133$
Pentaploid Hybrids $2n=5x=95$	Induced Octaploids $2n=8x=152$

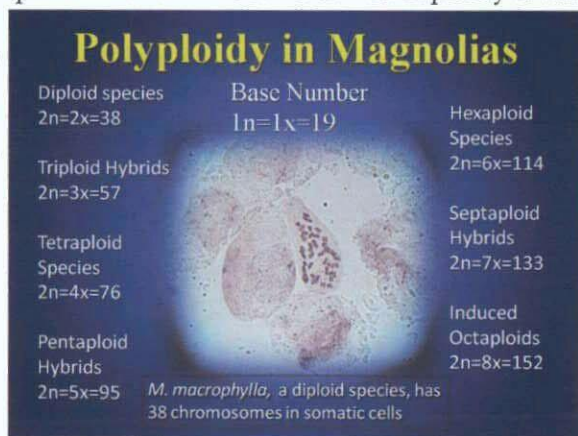*M. macrophylla*, a diploid species, has 38 chromosomes in somatic cells

Figure 1: Range of polyploidy in *Magnolia*.

This, of course, has significance to breeders because a cross between species of different ploidy levels will favor the higher ploidy parent. This is why the "Little Girl" Hybrids (*Magnolia liliiflora* ($4x$) × *M. stellata* ($2x$)) most closely resemble the *M. liliiflora* parent. The greater the difference in ploidy level, the greater the offspring will favor the higher ploidy parent, as evidenced in the Freeman Hybrids (*M. grandiflora* ($6x$) x *M. virginiana* ($2x$)), which pass as a typical *M. grandiflora* to all but the most trained eye. When hybrids such as these are developed, we can typically confirm hybridity by closely observing morphological characters. To be certain, chromosome counts can be performed. Since many *Magnolia* species are polyploids with high chromosome numbers, traditional cytology based upon 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). A flow cytometer is essentially a cell counter which can illuminate the stained genetic material within cells. The measured fluorescence generated is displayed in the form of a histogram. We sampled over 300 accessions 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 4', 6-diamidino-2-phenylindole (DAPI),

Figure 2: Author running samples through a flow cytometer at the NCSU Mountain Horticultural Crops Research and Extension Center.

and then analyzed (minimum of 2500 nuclei per sample) using a flow cytometer (PA-I; Partec, Münster, Germany) to determine relative holoploid 2C DNA (whole genome) 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 picograms (one picogram = one trillionth (10^{-12}) of a gram) (Greilhuber et al., 2007). Holoploid, 2C DNA contents were calculated as: 2C = DNA content of standard x (mean fluorescence value of sample ÷ mean fluorescence value of the standard). Because tetraploid *Magnolia* taxa have similar genome sizes to *P. sativum* 'Ctirad', *Magnolia virginiana* 'Jim Wilson' (3.92 pg) was used as a secondary standard. 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 and 3) confirm hybridity of interploid and interspecific (when parents vary substantially in genome size) crosses.

Figure 3: Typical histogram with peaks generated by two samples of differing genome size.
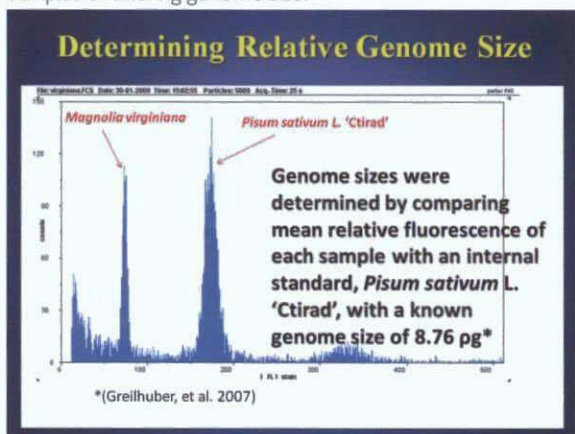
Resources

The collection of samples for this study was an extremely rewarding exercise. The NCSU Mountain Crop Improvement Lab was a primary source of accessions. Multiple trips were made to Magnolian Grove Arboretum, the garden of Dick and Anita Figlar. Dick eagerly advised me as I worked my way through samples obtained from the various taxonomic sections of Magnolia. Pat McCracken also generously hosted me on a collection trip and provided samples from numerous taxa. Another substantial contributor of samples was Greg Paige of the Bartlett Tree Research Laboratory, and with the help of David Kruse-Pickler of the San Francisco Botanic Garden I was able to turn a family vacation into a collection opportunity. On another family trip to Washington DC, I was privileged to spend time with Richard Olsen of the U.S. National Arboretum and take a glimpse at the hand-written notes of breeders such as Frank Santamour, William Kosar, and Francis de Vos. Richard also tracked down some significant accessions that led to key findings. Other significant contributions were made from Charles Tubesing of the Holden Arboretum and breeders Dennis Ledvina of Green Bay, Wisconsin, and Bill Smith of Richmond, Virginia. In the past year I have been able to meet both Dennis and Bill and I now know what has driven the likes of these gentlemen and so many others to keep pushing the boundaries of magnolia diversity.

The collection and testing of so many samples may have turned into a painfully repetitive exercise if it were not for the immense respect I have developed for the individuals that brought species into cultivation and

escorted scores of hybrids into existence. Without their work, this study would have never been possible. Each sample I held was a piece of living history. Nevertheless, I was using modern technology to measure the relative weight of the genetic material present in the average cell of each named plant, thus turning the product of a plant explorer or breeder's work

into a cold number derived from a mathematical equation. Having adventured with friends from the Magnolia Society International to some of the world's finest assemblies of *Magnolia*, I recognize these plants are not the outcome of equations. They are the results of nature, combined with the human qualities of hope and frustration, anticipation and tenacity, passion and heartache. But the numbers have consequence, and by better understanding them, greater hope with less heartache may be realized.

Bill Smith and Kevin Parris with some of Bill's new hybrid seedlings at Lewis Ginter Botanic Garden, May 2011.

Data analysis

The first step in analyzing data was to determine the mean relative genome size of each species and the section they represent (Tables 1 and 2). As expected, a statistical difference in genome size occurs between taxa of differing ploidy levels. This allows us to clearly illustrate the ploidy level of a sample <u>without</u> performing a chromosome count. Also important was the fact that genome size <u>within</u> each species and taxonomic section had insignificant variation. Another important detail to draw from Table 1 is that a statistic difference in genome size can occur <u>between</u> species of different taxonomic sections within the <u>same</u> ploidy level. For example, hybridity can be confirmed in a group of seedlings from a cross between *Magnolia virginiana* (Section *Magnolia*) and *Magnolia insignis* (Section *Manglietia*) when the plants are large enough to spare just a small portion of one leaf, before intermediate morphological characters become pronounced. This can save breeders the time and expense of cultivating errant progeny to maturity.

Table 2 is condensed in this article to display only the means for each species, rather than reporting all cultivars surveyed. In the case of *M. virginiana* and *M. grandiflora*, numerous cultivars were surveyed, with no significance in genome size found. In other species with obscure availability, such as *M. sinica*, the mean genome size reported is derived from only one accession. Under *M. grandiflora*, several cultivars previously

reported to be interploid hybrids are listed to clarify their confirmed ploidy level and species designation. The cultivar 'Charles Dickens' has been suggested to have been the result of a cross between *M. grandiflora* and *M. macrophylla* (2n=2x=38), but it is hexaploid, aligning it entirely with *M. grandiflora*. 'Griffin', 'Riegel', and 'Sweet Summer' are three cultivars which were also thought to have been of hybrid origin, but their genome size is consistent with other *M. grandiflora* cultivars. Had they been the product of a cross with *M. virginiana*, their genome size would have been that of a tetraploid.

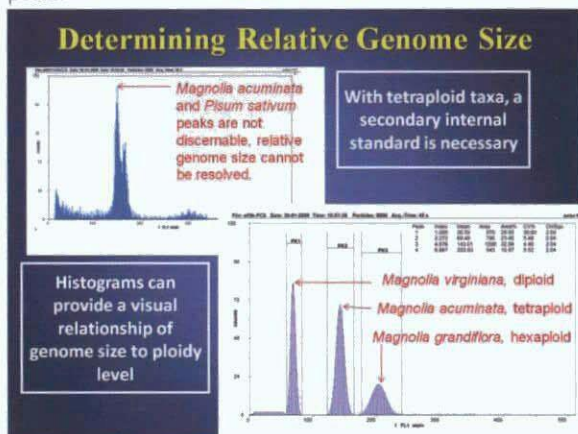Figure 4: Histograms illustrating discernable and indiscernible peaks.

Table 3 summarizes the results of many hybrids that were surveyed. The results were enlightening. 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* (6x) × *M. virginiana* (2x)) 'Maryland' with an intermediate genome size of 7.49 pg, and also a seedling of 'Maryland' from Magnolian Grove Arboretum which was likely open pollinated by *M. grandiflora*. This accession had a genome size of 9.00 pg, consistent with a pentaploid derived from a (4x × 6x) cross. An unnamed plant at the U.S. National Arboretum with similar appearance to *M.* 'Maryland' was found to have a genome size of 5.62 pg, consistent with a triploid, suggesting a hybrid of *M. grandiflora* (6x) x *M. virginiana* (2x) had been backcrossed to *M. virginiana*. An intermediate tetraploid condition was determined for *M. insignis* (2x) × *M. grandiflora* 'Kay Parris' (6x) which had an 8.50 pg relative genome size. In addition to the *M. virginiana* × *M. insignis* cross mentioned earlier, the following

interspecific, intraploid hybrids were confirmed by intermediate size; *M. yuyuanensis* × *M. virginiana*, *M.* 'Silk Road' × *insignis*, and very recently (not included in Table 3) *M. insignis* × *M. fraseri*.

Within subgenus *Yulania*, confirmed interploid hybrids were numerous. Verification of hybridity was readily confirmed for the U.S. National Arboretum'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

Flow cytometry was used to verify this seedling was tetraploid (8.50 pg), confirming the first successful cross between *M. insignis* and *M. grandiflora*.

also verified. These hybrids 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 septaploid (7*x*) or higher. Other taxa in Table 3 of approximately septaploid genome size include *M.* 'Andre Leroy', *M.* 'Manchu Fan', *M.* 'Sunsation', and *M.* 'Todd Gresham'. Each of these hybrids has a parental combination that theoretically could yield 7*x* offspring. 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* (4*x*) × *M. stellata* (2*x*) 'Gold Star' 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 realization that *M.* 'Gold Star' is tetraploid explains why it has been successfully used as a fertile parent in several crosses. No triploid hybrids were found to be parents of any hybrid surveyed in this study, indicating triploids may typically not be fertile.

Table 4 documents the confirmation of several induced polyploids. 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). 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 most notable clarification provided by this study was the ploidy level of *M. sieboldii* 'Colossus', long thought to be hexaploid, yet multiple accessions from multiple sources were found to be diploid. This revelation, coupled with the discovery that *M.* 'Sweet Summer' is not a tetraploid hybrid, sheds light on the reason a cross between *M. sieboldii* 'Colossus' and *M.* 'Sweet Summer' (genome size documented in Table 2) so closely resembles *M. grandiflora*. Instead of a $6x \times 4x$ cross that should have resulted in $5x$ hybrid with more intermediate characteristics, this was really a $2x \times 6x$ cross heavily favoring the higher ploidy level of *M. grandiflora*. Another most interesting discovery was a cytochimera (tissue composed of cells with differing ploidy levels) of *M. grandiflora* 'Little Gem', created by Bob Head of Seneca, SC, by treating young rooted cuttings with oryzalin. The specimen was induced 10 years prior, and 55% of the cells in the examined tissue had remained dodecaploid ($2n=12x=228$). Multiple accessions of *M. cylindrica* ($8x$) and *M. stellata* ($4x$) from the Holden Arboretum were also confirmed as induced polyploids.

In order to demonstrate the reliability of flow cytometry as a means to discern ploidy level, standard cytology was performed on a seedling of uncertain parentage. 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. The source of this seed was the Holden Arboretum via the Seed Counter of the Magnolia Society International. They were then transferred to a fixative solution of 3 parts 95% ethanol: 1 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 12N 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 cover slip. A chromosome count of one of these seedlings, SCC 2009-004, identified approximately 133 chromosomes ($2n=7x=133$) (Fig. 5), in close agreement with genome size data (14.92 pg) which was determined to be approximately $7x$ ($6.7x$). This supports the

assertion of Charles Tubesing (in MSI Seed Counter information) that the octoploids could have outcrossed with other magnolias with lower ploidy levels from their collections.

Conclusion

For me, this study has painted an abstract picture of the dance that takes place when gametes from *Magnolias* meet. Within species the match is so perfect that little or no genetic information is lost or gained that would lead to significant variation in genome size. The partners are well acquainted, the dance is well rehearsed, no toes are stepped on, and the performance is flawless. Yet, we have learned that *Magnolia* species may dance with different partners. Though they may have been separated by mountains, plains, or oceans, and eons of time, there is an affinity that still exists. The harmony of the genetic sequences rings like a musical composition. Therefore, the tune is familiar, and though the partners may stumble, the jubilation of the reunion often shines through in the dance.
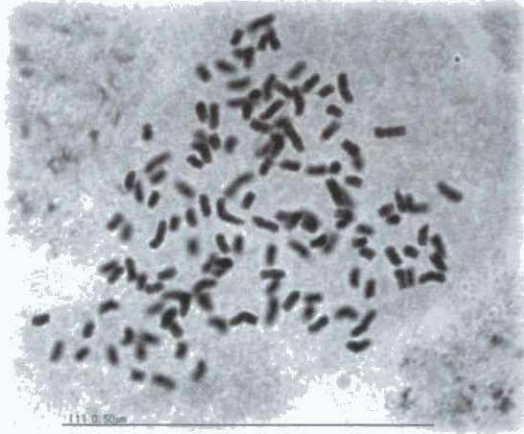

Figure 5: Photomicrograph of a root tip cell of *Magnolia* SCC 2009-004 in early metaphase, with approximately 133 chromosomes. Maternal parent *Magnolia cylindrica* (2n=8x=152), paternal parent unknown, but likely (2n=6x=114), resulting in a plant that is 7x

For breeders, the revised taxonomy by Figlar and Nooteboom (2004), along with molecular data presented by Azuma et al. (1999, 2000, 2001) and Kim et al. (2001), provides a greater understanding of the relatedness and potential for interspecific hybridizations among closely allied species that is often supported empirically in Table 3 of this paper. 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. The results of this study have provided data on genome sizes and ploidy levels of *Magnolia*, confirmation of hybrids and induced polyploids, comparison of methods for determining genome size, and insights into reproductive biology that will help facilitate the development of improved hybrids in the future. If plants are developed with the inspiration of sound science

and a creative eye, they will grace gardens beyond the life of the breeder, just like the melody of a classic song can transcend generations.

Below is some of the sheet music, let us continue the song and dance.

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

Classification	Ploidy level [z]		
	$2n = 2x = 38$	$2n = 4x = 76$	$2n = 6x = 114$
Subgenus *Magnolia*			
Section *Magnolia* (5/41[y])	2C = 3.80×E[w]	N[v]	2C = 11.18 C
	(3.43 - 4.40)[u]		(10.83 - 11.86)
	1Cx = 1.90[t]		1Cx = 1.86
	(1.72 - 2.20)[s]		(1.81 - 1.98)
Section *Gwillimia* (4/6)	2C = 5.32 A	N	N
	(5.10 - 5.63)		
	1Cx = 2.66		
	(2.41 - 2.82)		
Section *Rhytidospermum* (5/18)	2C = 4.27 CD	N	N
	(3.66 - 4.69)		
	1Cx = 2.14		
	(1.83 - 2.35)		
Section *Manglietia* (10/17)	2C = 4.87 B	N	N
	(4.65 - 5.25)		
	1Cx = 2.44		
	(2.33 - 2.63)		
Section *Macrophylla* (1/5)	2C = 4.57 BC	N	N
	(4.41 - 4.87)		
	1Cx = 2.28		
	(2.21 - 2.44)		

Section *Auriculata* (1/3)	2C = 3.83 **E**	N	N
	(3.74 - 3.96)		
	1Cx = 1.94		
	(1.87 - 1.98)		
Section *Kmeria* (1/1)	2C = 5.51 **A**	N	N
	(5.51 - 5.51)		
	1Cx = 2.76		
	(2.76 - 2.76)		
Subgenus *Yulania*			
Section *Yulania* (14/43)	2C = 4.05 **DE**	2C = 8.56 **A**	2C = 12.68 **A**
	(3.84 - 4.26)	(8.08 - 9.34)	(11.49 - 13.47)
	1Cx = 2.02	1Cx = 2.14	1Cx = 2.11
	(1.92 - 2.13)	(2.02 - 2.34)	(1.92 - 2.25)
Section *Michelia* (17/31)	2C = 4.56 **BC**	N	N
	(4.23 - 4.92)		
	1Cx = 2.28		
	(2.11 - 2.46)		
Subgenus *Gynopodium*			
Section *Gynopodium* (2/3)	N	N	2C = 11.93 **B**
			(11.57 - 12.50)
			1Cx = 1.99
			(1.93 - 2.08)
Section *Manglietiastrum* (1/1)	2C = 4.21 **D**	N	N
	(4.21 - 4.21)		
	1Cx = 2.11		
	(2.11 - 2.11)		

Magnolia

Genus *Liriodendron* (2/2)	2C = 3.41 F	N	N
	(3.35 - 3.47)		
	$1C_x = 1.71$		
	(1.68 - 1.74)		

[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, following 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] Letters following Relative 2C genome sizes, within a column, are significantly different, using the Waller Procedure(Proc GLM, SAS version 9.1; SAS Institute, Cary, NC) for means separation, 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* spp. sampled in each section.

[t] Relative 1Cx mean genome sizes (pg) were calculated as: (2C mean / ploidy level).

[s] Values represent ranges of 1Cx genome size means for all *Magnolia* spp. sampled in each section.

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

Taxa	Cultivar/selection	Mean Relative 2C genome size (pg) ^{z}	Mean relative 1Cx genome size by species (pg) ^{y}	Ploidy level (x)
Subgenus *Magnolia*				
Section *Magnolia*				
virginiana var. *virginiana*	including	3.72	1.86	2
	R14-397 (thought to be tetraploid)			
virginiana var. *australis*		3.73		
grandiflora	including	11.22	1.87	6
	'Charles Dickens' (suggested			
	hybrid w/ *macrophylla*)			
	'Griffin' (suggested hybrid w/			
	virginiana)			
	'Riegel' (suggested hybrid w/			
	virginiana)			
	'Sweet Summer' (suggested hybrid			
	w/ *virginiana*)			
guatamalensis		4.37	2.19	2
sharpii		4.40	2.20	2
tamaulipana		11.28	1.88	6
Section *Gwillimia*				
Subsection *Gwillimia*				
coco		4.83	2.42	2
delavayii		5.28	2.64	2
Subsection *Blumiana*				
hodgsonii		5.47	2.73	2
liliifera		5.63	2.82	2
Section *Rhytidospermum*				
Subsection *Rhytidospermum*				
obovata (hypoleuca)		3.97	1.99	2

Magnolia

officinalis var. *officinalis*	4.01	2.00	2
officinalis var. *biloba*	3.72	1.86	2
rostrata	4.69	2.35	2
tripetala	4.00	2.00	2
Subsection *Oyama*			
sieboldii including 'Colossus'	4.50	2.25	2
Section *Manglietia*			
aromatica	5.15	2.58	2
changhungtana (pachyphylla)	4.69	2.35	2
conifera var. *chingii*	4.87	2.34	2
fordiana	4.81	2.41	2
garrettii	5.25	2.63	2
hookeri	4.82	2.41	2
insignis	4.94	2.47	2
kwangtungensis (moto)	4.65	2.33	2
ovoidea	5.02	2.51	2
yuyuanensis	4.74	2.37	2
Section *Macrophylla*			
macrophylla var. *macrophylla*	4.56	2.28	2
macrophylla var. *ashei*	4.52	2.26	2
macrophylla var. *dealbata*	4.87	2.44	2
Section *Auriculata*			
fraseri var. *fraseri*	3.94	1.97	2
fraseri var. *pyramidata*	3.74	1.87	2
Section *Kmeria*			
thailandica	5.51	2.76	2
Subgenus *Yulania*			
Section *Yulania*			
Subsection *Yulania*			
amoena	4.26	2.13	2
biondii	4.12	2.06	2
campbellii	12.54	2.09	6

cylindrica	8.92	2.23	4
dawsoniana	13.12	2.19	6
denudata	13.26	2.21	6
kobus	4.04	2.02	2
liliiflora	9.34	2.28	4
sargentiana	11.49	1.92	6
sprengeri	12.66	2.11	6
salicifolia	3.91	1.96	2
stellata including 'Two Stones' (reported tetraploid)	3.94	1.97	2
zenii	4.16	2.08	2
Subsection *Tulipastrum*			
acuminata	8.24	2.06	4
acuminata var. *subcordata*	8.27	2.07	4
Section *Michelia*			
cavaleriei var. *platypetala*	4.40	2.19	2
champaca	4.74	2.37	2
chapensis	4.92	2.46	2
doltsopa	4.52	2.26	2
ernestii	4.50	2.25	2
figo var. *figo*	4.58	2.29	2
figo var. *skinneriana*	4.48	2.24	2
figo var. *crassipes*	4.71	2.36	2
floribunda	4.51	2.26	2
foveolata var. *foveolata* 'Shibamichi'	4.23	2.12	2
foveolata var. *cinerascens*	4.42	2.21	2
fulva var. *calcicola*	4.61	2.31	2
laevifolia	4.56	2.28	2
lanuginosa	4.80	2.40	2
maudiae	4.56	2.28	2
martini	4.75	2.38	2
odora	4.54	2.27	2

Magnolia

shiluensis		4.49	2.25	2
sirindhorniae		4.53	2.27	2
Subgenus *Gynopodium*				
Section *Gynopodium*				
lotungensis		11.54	1.93	6
yunnanensis	Vietnam origin	12.50	2.08	6
, Section *Manglietiastrum*				
sinica		4.21	2.11	2
Genus *Liriodendron*				
chinensis		3.47	1.74	2
tulipifera		3.35	1.68	2

ᶻ Genome sizes were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain. Values are means of

multiple samples, and often from multiple cultivars, accessions or sources.

ᵞ1Cₓ values were calculated as: [(2C value / ploidy level)

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

Reported parentage	Cultivar/selection	Mean Relative 2C Genome size (pg)ᶻ	Weighted 1Cx Genome size (pg)ʸ	Reported parental Ploidy levels (x)ˣ	Estimated ploidy level (x)ʷ
Subgenus *Magnolia*					
Intraploid Hybrids					
2n=2x=38					
insignis × *virginiana*	'Katie-O'	4.30	2.15	2×2	2
macrophylla × *tripetala*		3.68	2.12	2×2	2
obovata × *virginiana*	'Nimbus'	3.79	1.93	2×2	2
officinalis × *tripetala*		3.96	1.95	2×2	2
sieboldii 'Colossus' × *insignis*		4.60	2.37	2×2	2
sieboldii 'Colossus' × *insignis*		4.63	2.37	2×2	2
sieboldii 'Genesis' × *virginiana*	R10-24	4.06	2.06	2×2	2
×*thompsoniana* (=*virginiana* × *tripetala*)		3.95	1.93	2×2	2
'Silk Road' ((*tripetala* × *obovata*) × *tripetala*))					
× *insignis*		4.35	2.23	2×2	2
yuyuanensis × *insignis*		4.53	2.43	2×2	2
yuyuanensis × *sieboldii*	104/1	4.67	2.32	2×2	2
yuyuanensis × *virginiana*		4.41	2.12	2×2	2
virginiana 'Havener' × *insignis* (Red Form)	111/7	4.23	2.17	2×2	2
Interploid Hybrids					
2n=3x=57					
(*grandiflora* × *virginiana*) × *virginiana*		5.62	1.87	4×2	3
2n=4x=76					
grandiflora × *virginiana*	'Maryland'	7.52	1.87	6×2	4
insignis × *grandiflora* 'Kay Parris'		8.53	2.02	2×6	4
sieboldii 'Colossus' × *grandiflora* 'Bracken's Brown Beauty'		7.87	1.97	2×6	4
sieboldii 'Colossus' × *grandiflora* 'Kay Parris'		8.23	1.97	2×6	4
sieboldii 'Colossus' × 'Sweet Summer'		8.02	1.97	2×6	4
sieboldii 'Pride of Norway' × 'Sweet Summer'		7.99	1.97	2×6	4
2n=5x=95					
'Maryland' (*grandiflora* × *virginiana*) × *grandiflora*	(Maryland Seedling)	9.00	1.87	4×6	5

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Intraploid Hybrids

2n=2x=38

×*kewensis* (=*kobus* × *salicifolia*)	'Wada's Memory'	3.83	1.99	2×2	2
×*loebneri* (=*kobus* × *stellata*)	'Donna'	5.86	2.00	2×2	3
	'Pink Superstar'	4.02	2.00	2×2	2
	'Leonard Messel'	4.08	2.00	2×2	2
	'Mag's Pirouette'	3.97	2.00	2×2	2
	'Merril'	3.86	2.00	2×2	2
	'Spring Snow'	3.86	2.00	2×2	2
	'Wildcat'	3.98	2.00	2×2	2
×*alba* (=*champaca* × *montana*)		4.81	2.28	2×2	2
laevifolia × *figo*	(Clifford Parks)	4.46	2.28	2×2	2
×*foggii* (=*figo* × *doltsopa*)		4.53	2.27	2×2	2

2n=4x=76

acuminata 'Busey' × *acuminata* sub. 'Miss Honeybee'	'Miranja'	18.25	2.10	4×4	~8.6
liliiflora 'O'Neill' × *kobus* 'Norman Gould'	'Roseanne'	8.53	2.15	4×4	4
×*brooklynensis* 'Woodsman' × 'Gold Star' (*acuminata* 'Miss Honeybee' × *stellata*)	'Solar Flair'	8.19	2.13	4×4	4
×*brooklynensis* 'Woodsman' ×'Gold Star' (*acuminata* 'Miss Honeybee' × *stellata*)	'Sunburst'	8.07	2.13	4×4	4
×*brooklynensis* 'Woodsman' ×'Gold Star' (*acuminata* 'Miss Honeybee' × *stellata*)	'Tranquility'	8.15	2.13	4×4	4
×*brooklynensis* (=*acuminata* × *liliiflora*)	'Woodsman'	8.21	2.17	4×4	4

2n=6x=114

denudata × *sprengeri* 'Diva'	'Legacy'	13.11	2.16	6×6	6
sargentii var. *robusta* × *campbellii*	'Hawk'	12.67	2.01	6×6	6
×*veitchii* (=*campbellii* × *denudata*)		12.96	2.15	6×6	6

Interploid Hybrids

cylindrica × ×*veitchii* 'Peter Veitch'	'Albatross'	11.14	2.18	4×6	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Alexandrina'	10.70	2.24	6×4	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Andre Leroy'	14.60	2.24	? ×?	~6.5
cylindrica × *denudata* 'Sawada's Pink'	'Angelica'	10.83	2.22	4×6	5

Parentage	Name				
stellata × *liliiflora* 'Nigra'	'Ann'	6.28	2.18	2×4	3
liliiflora × *cambellii* 'Lanarth'	'Apollo'	11.02	2.17	4×6	5
'Vulcan' (*campbellii* 'Lanarth' × *liliiflora*) × ×*soulangeana* 'Lennei'	ArborTree Select	16.97	2.21	5×~8	~7.7
×*soulangeana* 'Lennei Alba' × 'Mark Jury' (*campbellii* 'Lanarth' × *sargentiana*)	'Athene'	14.96	2.14	~7.6×6	~7
×*soulangeana* 'Lennei' × 'Mark Jury' (*campbellii* 'Lanarth' × *sargentiana*)	'Atlas'	12.82	2.14	~8×6	~6
stellata 'Rosea' × *liliiflora* 'Nigra'	'Betty'	6.61	2.18	2×4	3
'Yellow Bird' (*acuminata* × ×*brooklynensis* 'Evamaria') × 'Caerhays Belle' (*sargentiana* × *sprengeri* 'Diva')	'Blushing Belle'	10.32	2.11	4×6	5
acuminata × *denudata* 'Sawada's Cream'	'Butterflies'	10.71	2.15	4×6	5
'Legend' × 'Butterflies'	'Coral Lake'	12.09	2.15	5×5	~5.6
×*veitchii* × ×*soulangeana*	'David Clulow'	16.75	2.19	6×?	~7.6
×*brooklynensis* 'Woodsman' × 'Tina Durio' (×*soulangeana* 'Lennei Alba' × ×*veitchii*)	'Daybreak'	10.71	2.20	4×~6.9	~4.9
acuminata × *denudata*	'Elizabeth'	10.59	2.15	4×6	5
denudata × *stellata* 'Waterlily'	'Emma Cook'	10.26	2.15	6×2	~4.8
kobus 'Norman Gould' × ×*soulangeana* 'Lennei'	'Eskimo'	9.99	2.14	4×~8	~4.6
sprengeri 'Diva' × ×*soulangeana* 'Wada's Picture'	'Felicity'	10.75	2.18	6×5	~4.9
×*soulangeana* 'Deep Purple Dream' × 'Paul Cook'	'Frank's Masterpiece'	14.66	2.19	?×6	~6.7
×*soulangeana* (=*denudata* × *liliiflora*)	'Fukuju'	19.02	2.24	?×?	~8.5
liliiflora 'Nigra' × *sprengeri* 'Diva'	'Galaxy'	10.45	2.18	4×6	5
acuminata var. *sub.* 'Miss Honeybee' × *denudata* 'Sawada's Cream'	'Gold Finch'	10.81	2.15	4×6	5.0
acuminata × *denudata*	'Golden Sun'	13.59	2.15	4×6	~6.3
acuminata × *stellata*	'Gold Star'	8.22	2.06	4×2	4
×*soulangeana* (=*denudata* × *liliiflora*)	'Grace McDade'	17.35	2.24	?×?	~7.8
(×*brooklynensis* 'Woodsman' × ×*soulangeana* 'Lennei') × 'Elizabeth' (*acuminata* × *denudata*)	'Green Snow'	11.47	2.20	(4×~8)×5	~5.2
×*brooklynensis* 'Woodsman' × 'Elizabeth' (*acuminata* × *denudata*)	'Hot Flash'	8.43	2.15	4×5	~3.9
'Mark Jury' (*campbellii* 'Lanarth' × *sargentiana*) × ×*soulangeana* 'Lennei'	'Iolanthe'	13.62	2.14	6×~8	~6.4

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acuminata × denudata	'Ivory Chalice'	10.76	2.17	4×6	5
×soulangeana × veitchii	'Jon Jon'	15.16	2.19	?×6	-6.9
×loebneri 'Encore' × *×soulangeana* 'Alexandrina'	Kehr Seedling	10.92	2.17	2×-5	-5
acuminata × denudata	'Legend'	10.77	2.15	4×6	5
×soulangeana (=*denudata × liliiflora*)	'Lennei'	17.89	2.24	?×?	-8
×soulangeana (=*denudata × liliiflora*)	'Lennei Alba'	16.91	2.24	?×?	-7.6
acuminata var. *subcordata* × *×soulangeana* 'Big Pink'	'Limelight'	14.23	2.18	4×?	-6.5
acuminata × (acuminata × denudata)	'Lois'	14.61	2.10	4×5	-7.0
×soulangeana × ×veitchii	'Manchu Fan'	14.86	2.19	?×6	-6.8
(*liliiflora × cylindrica*) × *×soulangeana* 'Ruby'	'March till Frost'	12.89	2.25	4×?	-5.7
×soulangeana 'Lennei' × 'Paul Cook' (*×soulangeana* 'Lennei' seedling × *sprengeri* 'Diva')	'Millie Gaylon'	14.20	2.21	-8×(?×6)	-6.4
stellata × liliiflora	'Orchid'		2.18	2×4	3
		6.44			
acuminata × ×veitchii 'Peter Veitch'	'Pastel Beauty'	10.12	2.13	4×6	5
acuminata × sprengeri 'Diva'	'Peachy'	10.11	2.11	4×6	5
×soulangeana (=*denudata × liliiflora*)	'Pickard's Firefly'	17.06	2.24	?×?	-7.6
liliiflora 'Reflorescens' × *stellata* 'Rubra'	'Pinkie'	6.47	2.18	4×2	3
liliiflora × ×soulangeana	'Purple Prince'	10.91	2.26	4×?	-4.8
liliiflora 'Nigra' × *stellata*	'Randy'	6.44	2.18	4×2	3
acuminata × 'Big Dude' (*×soulangeana* 'Wada's Picture' × *sprengeri* 'Diva')	'Red Baron'	13.19	2.14	4×(?×6)	-6.2
liliiflora × ×veitchii	'Royal Crown'	10.58	2.20	4×6	5
liliiflora × ×veitchii	'Sayonara'	14.82	2.20	4×6	-6.7
liliiflora × 'Mark Jury' (*campbellii* 'Lanarth' × *sargentiana*)	'Serene'	10.59	2.12	4×6	5
denudata × 'Vulcan' (*campbellii* 'Lanarth' × *liliiflora*)	'Shiraz'	12.76	2.19	6×5	-5.8
liliiflora 'Nigra' × *sprengeri* 'Diva'	'Spectrum'	11.58	2.18	4×6	5
campbellii × liliiflora	'Star Wars'	10.53	2.17	6×4	5
acuminata × denudata	'Sunray'	10.22	2.17	4×6	5
×brooklynensis 'Woodsman' × 'Elizabeth' (*acuminata × denudata*)	'Sunsation'	14.73	2.18	4×5	-6.8
liliiflora × stellata 'Rosea'	'Susan'	6.58	2.18	4×2	3
×soulangeana 'Lennei Alba' × *×veitchii*	'Tina Durio'	15.23	2.20	-7.6×6	-6.9
×veitchii × ×soulangeana 'Rustica Rubra'	'Todd Gresham'	14.75	2.19	6×?	-6.7

campbellii 'Lanarth' × *liliiflora* hybrid	'Vulcan'	10.54	2.17	6×4	5
acuminata var. subcordata × ×*soulangeana*					
'Alexandrina'	'Yellow Lantern'	14.43	2.18	4×5	~6.6
acuminata × *denudata*	'Yellow Sea'	8.68	2.17	4×6	4
'Pegasus' (*cylindrica* × *denudata*) × *campbellii*					
'Darjeeling'	'Zeal'	10.15	2.17	5×6	~4.6
acuminata × *figo*		6.16	2.16	4×2	3
'Galaxy' (*liliiflora* 'Nigra' × *sprengeri* 'Diva') ×					
campbellii var. *Mollicomata*		12.34	2.13	5×6	~5.8
×*veitchii* 'Isca' × *liliiflora*		10.84	2.13	6×4	5
cylindrica hybrid (Polly Hill)		13.35	2.23	4×?	6.0
cylindrica hybrid (Holden via MSI seed counter)		14.92	2.23	8×?	~6.7
cylindrica hybrid (Holden via MSI seed counter)		15.21	2.23	8×?	~6.8

[z] Genome sizes were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain. Values are means of multiple samples.

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

[x] Reported parental ploidy levels.

[w] Estimated ploidy levels were calculated as: 2C genome size / weighted 1Cx 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.

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Table 4. Relative genome sizes (pg) and estimated ploidy levels of artificially induced polyploid *Magnolia* spp.

Taxa	Relative 2C Mean genome size (pg) [z]	Estimated Ploidy level (x) [y]
cylindrica	17.3	8
grandiflora 'Little Gem' (cytochimera)	11.11	6
	21.80	12
kobus 'Norman Gould'	7.79	4
stellata	8.2	4

[z] Genome sizes were determined using 4',6-diamidino-2-phenylindole as

the flourochrome stain. Values are means of multiple samples.

[y] Estimated ploidy levels were calculated as: 2C genome size / 1Cx 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.

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