Ploidy Levels and Genome Sizes of *Magnolia* L. Species, Hybrids, and Cultivars

J. Kevin Parris[1], Thomas G. Ranney[2], W. Vance Baird[1], and Halina T. Knap[3]

[1]Clemson University, Department of Horticulture, 164 Poole Agricultural Center Clemson, SC 29634
[2]N.C. State University, Dept. of Horticultural Science, 455 Research Dr. Fletcher NC, 28732
[3]Clemson University, Dept. of Entomology, Soils, and Plant Sciences and Dept. of Genetics and Biochemistry, 276 P&AS Bldg., Clemson, SC 29634

parrisk@sccsc.edu

Index Words: Cytology, Flow Cytometry, Genome Size, Magnolia, Polyploidy

Significance to Industry: The genus *Magnolia* L. includes a broad range of valuable nursery and landscape plants. In 1980, The Journal of the American Magnolia Society posthumously quoted E.H. Wilson, the great 19th century British plant explorer as saying: “No group of trees and shrubs is more favorably known or more highly appreciated in gardens than magnolias, and no group produces larger or more abundant blossoms.” (9). Considerable progress has been made breeding improved Magnolias; however, a greater understanding of polyploidy in this genus would greatly enhance future breeding efforts. Polyploidy is an important factor in plant breeding as it can influence reproductive compatibility, fertility, and gene expression (7). This research provides an extensive survey of polyploidy and determination of genome sizes in the genus *Magnolia* and will provide a valuable database for Magnolia breeders.

Nature of Work: The genus *Magnolia* contains more than 250 species belonging to various sections within three subgenera (3). Although basic information on chromosome counts and ploidy levels of different magnolia species have been compiled (1, 2), sampling has been limited and little is known about ploidy levels of specific hybrids and cultivars. The base chromosome number for Magnolia is 1*n*=1*x*=19. However, different subgenera contain species with a variety of ploidy levels from 2n=2x=38 to 2n=6x=114. Crosses of species with varying ploidy levels may yield hybrids with odd ploidy levels, which often result in reduced fertility or sterility (7). Because of these constraints, Magnolia breeders with a desire to incorporate the best features of these hybrids 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 of distant hybrids, based on chromosome number and genome size, when parents differ in ploidy levels.

Because many species with significant ornamental appeal are polyploids with high chromosome counts, traditional cytology with light microscopy is extremely difficult. Diploid counts are more feasible, but still require considerable skill and effort. Flow cytometry has proved to be an efficient means of estimating genome size and allows for

elucidation of ploidy level (5). 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* by using flow cytometry in order to: 1) increase sampling among and within species to develop an extensive database for use by magnolia breeders; 2) determine the ploidy level of suspected, but unconfirmed, polyploid taxa (both naturally occurring and chemically induced); and 3) confirm hybridity in interploid crosses and interspecific hybrids that vary in genome size.

Over 275 diverse species and cultivars were sampled from various sources that included taxa from each subgenera of *Magnolia* as well as both species of genus *Liriodendron*. Nuclei from newly expanded leaf or tepal tissue, were extracted, stained (with DAPI), and then analyzed (minimum 2500 events) using a flow cytometer (PA-I, Partec, Münster, Germany) to determine relative DNA content. Genome sizes were determined by comparing mean relative fluorescence of each sample with an internal standard, *Pisum sativum* L. 'Ctirad', with a known genome size of 8.76 ρg (4). To increase resolution of genome size, tetraploid Magnolias which have similar genome sizes to *Pisum sativum* 'Ctirad', *M. virginiana* 'Jim Wilson' (3.73 ρg) and *M. grandiflora* 'Little Gem' (10.92 ρg) were used as secondary standards. Genome sizes for the secondary standards were calculated as the mean of 10 separate subsamples determined with the *Pisum sativum* 'Ctirad' as an internal standard. Holoploid, 2C DNA contents were calculated as: 2C = DNA content of standard × (mean fluorescence value of sample/ mean fluorescence value of standard). The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers. 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 were tested to derive a mean relative genome size for each taxa. Data were subjected to analysis of variance and means separation using the Waller procedure.

Results and Discussion: Flow cytometry was an efficient and effective method of estimating genome size. Genome size varied significantly among taxonomic sections (Table 1), indicating that these groups have undergone considerable evolutionary divergence (8). Furthermore, this indicates that it is necessary to calibrate ploidy level with genome size for each section, in order to estimate ploidy level from genome size in Magnolias. However, within a section, genome sizes for a given ploidy level had narrow ranges and could clearly be used to determine ploidy levels (Table 1). In general, the ploidy levels determined for different species was consistent with past reports, with a few exceptions. *Magnolia cylindrica* was found to be a tetraploid here, while past reports have indicated it is a diploid (1, 2).

The results of this study also verified that *M. stellata* and *M. cylindrica* accessions from the Holden Arboretum were induced polyploids. Phenotypic characteristics such as thickened foliage and increased width to length ratio in foliage (6,7) were suggestive of polyploidy in *M. seiboldii* 'Colossus', a reported hexaploid. In this study, samples of *M. seiboldii* 'Colossus' from multiple sources had genome sizes (2C =4.35 ρg -4.62 ρg)

consistent with a diploid. This unexpected lack of congruency between phenotypic characteristics and ploidy level will require further investigation.

Analysis of various putative interploid hybrids also had mixed findings. *Magnolia* (*virginiana* var. *australis* (2x) x *M. grandiflora* 'Samuel Sommer' (6x))'Sweet Summer' has been described as an allotetraploid cultivar since its introduction in 1990. Samples collected from two different sources were tested and had estimated genome sizes (2C =11.11 ρg and 11.54 ρg) consistent with a hexaploid, bringing the hybridity of *M.* 'Sweet Summer' into question. This result remains enigmatic given the reported direction of the cross indicatingthat *M. virginiana* var. *australis* was the maternal parent (1). Flow cytometry did provide confirmation of hybridity between taxa of varying genomic size in the case of *Magnolia* (*figo*(2x) *x acuminata* (4x)). This is an important cross that was once considered to be intergeneric before *Michelia* was given sectional status within subgenus *Yulania*. Relative genome size (2C =6.16 ρg) of this hybrid is consistent with a triploid condition, confirming it to be intermediate between the diploid and tetraploid parents. *M.* (*insignis x virginiana*) 'Katie-0' is another important intersectional cross that demonstrates pigmentation of tepals can be introgressed into white flowered species of subgenus *Magnolia*. Hybridity of *M.*'Katie-O' is suggested based on morphological appearance, but intermediate genome size (2C= 4.33 ρg) further substantiated its hybrid origin. The difference in mean relative genome size for diploids in section *Manglietia* (2C=4.78 ρg) and section *Magnolia* (2C=3.79 ρg) allow for this distinction to be made. Flow cytometry did not allow for distinction of cultivars or interspecific hybrids within a given section due to highly conserved genome sizes within sections.

Overall, flow cytometry provides an extremely useful tool to study polyploidy and provides an entry to the investigation of reproductive biology in the genus *Magnolia*. This research provides a foundation and database for breeders that will facilitate the development of hybrids in the future.

Literature Cited:

1. Callaway, D.J. 1994. The World of Magnolias. Timber Press, Portland.
2. Chen Zhong-yi, X. Huang, R. Wang, S. Chen. 2000. Chromosome Data of Magnoliaceae. Proceedings of The International Symposium on the Family Magnoliaceae. Beijing: Science Press. 192-201.
3. Figlar, R.B. 2004 Classification of Magnoliaceae. Magnolia Society International. Nov. 2008. <http://www.magnoliasociety.org/classifications_ndx.html.>
4. 4. Greilhuber, J., E.M. Temsch, and J.C.M. Loureiro. 2007. Nuclear DNA content measurement, 67-101. In: J. Doležel, J. Greilhuber, and J. Suda (eds.). *Flow cytometry with plant cells: Analysis of genes, chromosomes and genomes.* Wiley-VCH, Weinheim.
5. Jones, J.R., T.G. Ranney, N.P. Lynch, S.L. Krebs. 2007. Ploidy Levels and Genome Sizes of Diverse Species, Hybrids, and Cultivars of Rhododendron L. Journal of the American Rhododendron Society. 61(4):220-227.
6. Kehr, A.E. 1985. Inducing Polyploidy in Magnolias. The Journal of the American Magnolia Society. Vol. XX, No. 2 p.6-9.

7. Kehr, A. E. 1996. Woody plant polyploidy. American Nurseryman 183, no. 3: 38. *Business Source Premier*, EBSCO*host* (accessed February 8, 2009).
8. Soltis, D. E., P. S. Soltis, M. D. Bennett, and I. J. Leitch. 2003. Evolution of Genome Size in the Angiosperms. American Journal of Botany 90(11): 1596–1603.
9. Wilson, E.H. 1980. Magnolias. The Journal of the American Magnolia Society. Vol. XVI No. 2 p. 5-8.

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

	Ploidy Level			
Classification	**2n=2x=38**	**2n=4x=76**	**2n=6x=114**	**2n=8x=152**
Subgenus *Magnolia*				
Section *Magnolia* (including *Theorhodon,5,40*[1])	2C=3.79[2] **D** (3.43-4.40) $1C_X$=1.90 (1.72-2.20)	NA	2C=11.16 **C** (10.83-11.63) $1C_X$=1.86 (1.81-1.94)	NA
Section *Gwillimia (2,3)*	2C=5.34 **A** (5.1-5.47) $1C_X$=2.67 (2.55-2.73)	NA	NA	NA
Section *Oyama (1,7)*	2C=4.52 **C** (4.35-4.62) $1C_X$=2.26 (2.17-2.31)	NA	NA	NA
Section *Manglietia (6,12)*	2C=4.78 **B** (4.65-5.07) $1C_X$=2.39 (2.33-2.53)	NA	NA	NA
Section *Rhytidospermum (4,8)*	2C=3.96 **D** (3.66-4.69) $1C_X$=1.98 (1.83-2.35)	NA	NA	NA
Section *Macrophylla (1,5)*	2C=4.56 **BC** (4.41-4.87) $1C_X$=2.28 (2.20-2.43)	NA	NA	NA
Section *Auriculata (1,2)*	2C=3.83 **D** (3.74-3.92) $1C_X$=1.92 (1.87-1.96)	NA	NA	NA
Section *Kmeria (1,1)*	2C=5.51 **A** (5.48-5.54) $1C_X$=2.76	NA	NA	NA
Subgenus *Yulania*				
Section *Yulania* (including *Beurgaria and M. liliiflora, 13,46)*	2C=4.07 **D** (3.84-4.16) $1C_X$=2.04 (1.92-2.08)	2C=8.43 **A** (7.71-8.88) $1C_X$=2.11 (1.93-2.22)	2C=12.74 **A** (11.49-13.22) $1C_X$=2.12 (1.92-2.20)	2C=17.34 (17.07-17.49 $1C_X$=2.17 (2.13-2.19)
Section *Tulipastrum (1,3)*	NA	2C=8.01 **A** (7.86-8.26) $1C_X$=2.00 (1.97-2.07)	NA	NA
Section *Michelia (14,22)*	2C=4.55 **BC** (4.27-4.87) $1C_X$=2.28 (2.14-2.44)	NA	NA	NA
Subgenus *Gynopodium*				
Section *Gynopodium (1,3)*	NA	NA	2C=11.57 **B** (11.44-11.72) $1C_X$=1.93 (1.91-1.95)	NA
Genus *Liriodendron (2,2)*	2C=3.39 **E** (3.35-3.43) $1C_X$=1.70 (1.68-1.72)	NA	NA	NA

[1]Numbers in parenthesis, following classifications, indicated the number of species sampled, and the total number of taxa within those species sampled.
[2]Values represent means followed by (ranges) for all magnolia species sampled. Means for 2C genome size followed by different letters, within a column, are significantly different, P<0.05.