Combined Proceedings IPPS 69:171-177. 2019.

A Genome Size Survey of the Blue and White Fruited Dogwoods (*Cornus* L.)

Joseph Pryzdia[1] and Kim Shearer[2]

[1]Plant Breeding Intern, The Morton Arboretum, 4100 Illinois Route 53, Lisle, Illinois 60532; [2]Tree and Shrub Breeder, The Morton Arboretum. USA

kshearer@mortonarb.org

Keywords: *Cornus*, genome size, biotechnology, research, flow cytometry

INTRODUCTION

Consisting of approximately 58 different species of shrubs, small trees, and to a lesser extent herbaceous perennial, dogwoods (*Cornus*) are considered to be a greatly valued landscape plant with a range covering much of the temperate and subtropical regions of the Northern Hemisphere. Species belonging to *Cornus* have been frequently cultivated, bred, and selected with respect to their pronounced four-season attributes including attractive flowers, fruit, bark, foliage, and form (Cappiello and Shadow, 2013) (Fig. 1). According to the Census of Horticultural Specialties (USDA, 2014) sales of dogwoods within the United States accounted for greater than $27.8 million.

Research resolving a species-level phylogeny in dogwoods has shown that four distinct clades exist within *Cornus*. These include the big-bracted dogwoods (BB), the dwarf dogwoods (DW), the cornelian cherries (CC), and the largest amongst the clades, the blue- or white-fruited dogwoods (BW) (Xiang et al., 2006). Within the BW group, there are further taxonomic divisions into subgenera including *Kraniopsis I, II* and *III*; *Mesomara*; and *Yinquania*. Reported ploidy for species of BW covered in this survey are diploid with a base chromosome number of x =11 in *Kraniopsis I* and *II* and x = 10 in *Mesomara* (Darlington and Wylie, 1956).

Genome size (nuclear DNA content) has been shown to support taxonomic relationships in *Cornus* (Shearer and Ranney, 2013). Shearer and Ranney found that relative genome sizes were taxonomically distinct in the BB, DW, and CC clades. It was also determined that all species belonging to these clades were diploid with the exception of a tetraploid *C. canadensis* and a triploid hybrid cultivar *C.* 'KN30-8' Venus® dogwood.

Figure 1. Inflorescence of *Cornus alba* (Frank Vincentz, Wikicommons) **(**top left)*;* blue fruit of *C. amomum* ssp. *obliqua*, ©The Morton Arboretum **(**top right)*;* red winter stems of *C. sanguinea*, (Jonathan Ballinger/*Glowing dogwoods*/CC BY-SA 2.0/cropped) **(**bottom left); white fruit of *C. racemosa* ©Deborah J.G. Brown **(**bottom right).

To complete the survey of *Cornus*, the objectives of this study were to complete a relative genome size survey for species of the BW clade, confirm ploidy is consistently diploid, and further explore the relationship between genome size and taxonomic classification.

Acknowledgements

Thanks to the Daniel P. Haerther Charitable Trust Foundation for their generous funding of the New Plant Development Program that supported the plant breeding internship and to Mike Yanny of JN Plant Select who provided plants to be used in this survey.

MATERIALS AND METHODS

Samples of expanding leaf tissue and vegetative buds were collected from 25 Morton Arboretum accessions and four selections provided by J.N. Plant Select. A total of 14 species are represented in addition to cultivars and hybrids of the BW clade of *Cornus*.

A 5 mm^2 sample from each of these individuals was co-chopped with 5 mm^2 of *Pisum sativum* 'Ctirad' (2C = 8.76 pg) in polystyrene petri dishes with a razor blade and 400 µl of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany). The nuclei suspension was filtered through 30-µm nylon filters and stained using 1.6 mL 4′, 6-diamidino-2-phenylindole (DAPI) staining buffer (Cystain ultraviolet Precise P Staining Buffer; Partec) (Fig. 2).

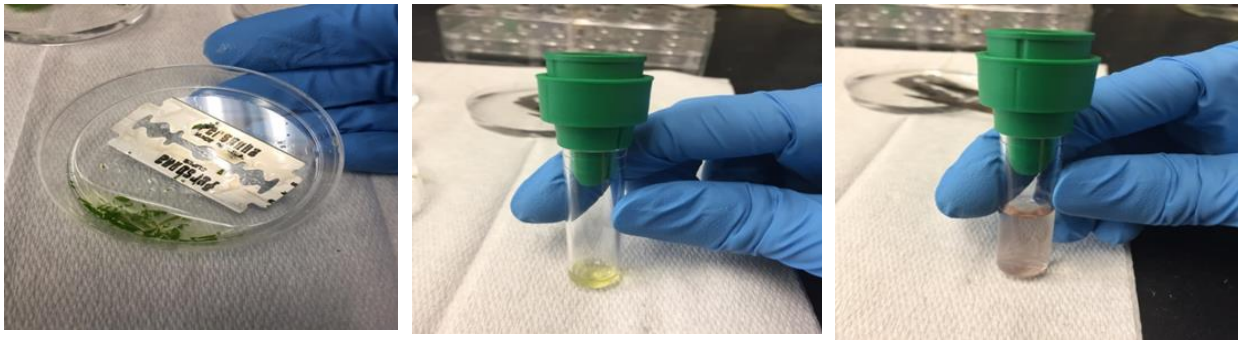

Figure 2. Sample preparation of *Cornus racemosa.* Chopped leaf tissue from both *C. racemosa* and the internal standard *Pisum sativum* 'Ctirad' immersed in 0.4 ml of nuclei extraction buffer (left). Contents of chopped leaf tissue from *C. racemosa* and the internal standard *Pisum sativum* 'Ctirad' filtered through a 50-µm nylon filter center). Addition of 1.6 ml of DAPI staining buffer to filtered chopped leaf tissue from *C. racemosa* and the internal standard *Pisum sativum* 'Ctirad' (right).

Relative 2C genome sizes were determined using flow cytometry equipped with a UV excitation lamp. A minimum of 3000 nuclei were analyzed for each sample and for each individual, three samples were prepared. Relative genome sizes were calculated using the equation

$$2C = DNA\ content\ of\ standard\ X\ \frac{Mean\ fluorescense\ value\ of\ sample}{Mean\ fluorescense\ value\ of\ the\ standard}$$

Statistical Analysis

One-Way ANOVA was used to determine statistical significance of monoploid genome size (1Cx) data at the species and subgenus levels with *Kraniopsis* separated into the two subgenera *Kraniopsis I* and *Kraniopsis II*. Tukey's honestly significant difference (HSD) was used for means separation. R package agricolae was used for statistical analysis (de Mendiburu, 2019).

RESULTS AND DISCUSSION

All individuals surveyed were diploid (*Kraniopsis*, 2*n* = 2*x* = 22; *Mesomara*, 2*n* = 2*x* = 20). Relative 2C genome sizes for the BW clade ranged from 1.70 pg (*C. controversa*) to 2.51 pg (*C. sanguinea*) (Figs. 3 and 4). Mean monoploid genome size of the three subgenera *Kraniopsis I* (1C*x* = 1.15 pg), *Kraniopsis II* (1C*x* = 1.07 pg) and *Mesomara* (1Cx = 0.92 pg) were found to be significantly different (Table 1). However, when considering mean 1Cx values of individuals in *Kraniopsis I* and *Kraniopsis II*, there is overlap between the two subgenera at the species level, suggesting that they are not necessarily significantly distinct in genome size as two separate subgenera.

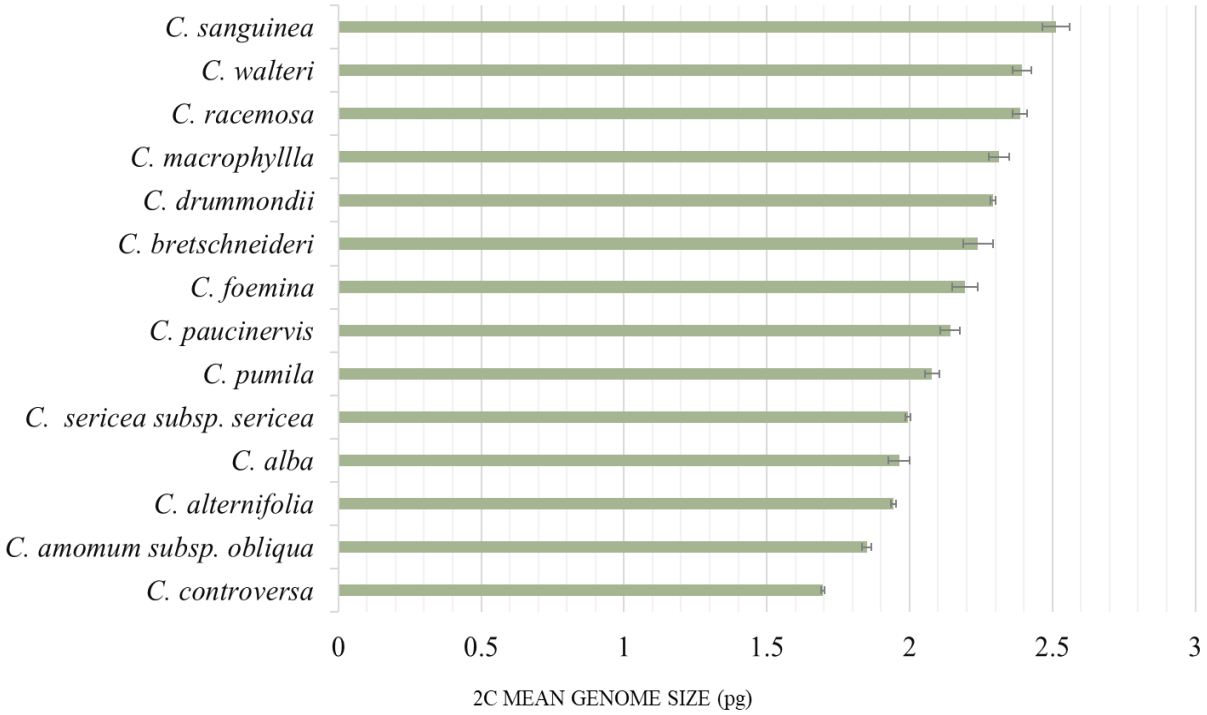

Figure 3. Relative 2C genome size of 14 *Cornus* species determined using flow cytometry analysis of nuclei stained 4′, 6-diamidino-2-phenylindole with *Pisum sativum* 'Ctirad' as an internal standard (2C = 8.76 pg).

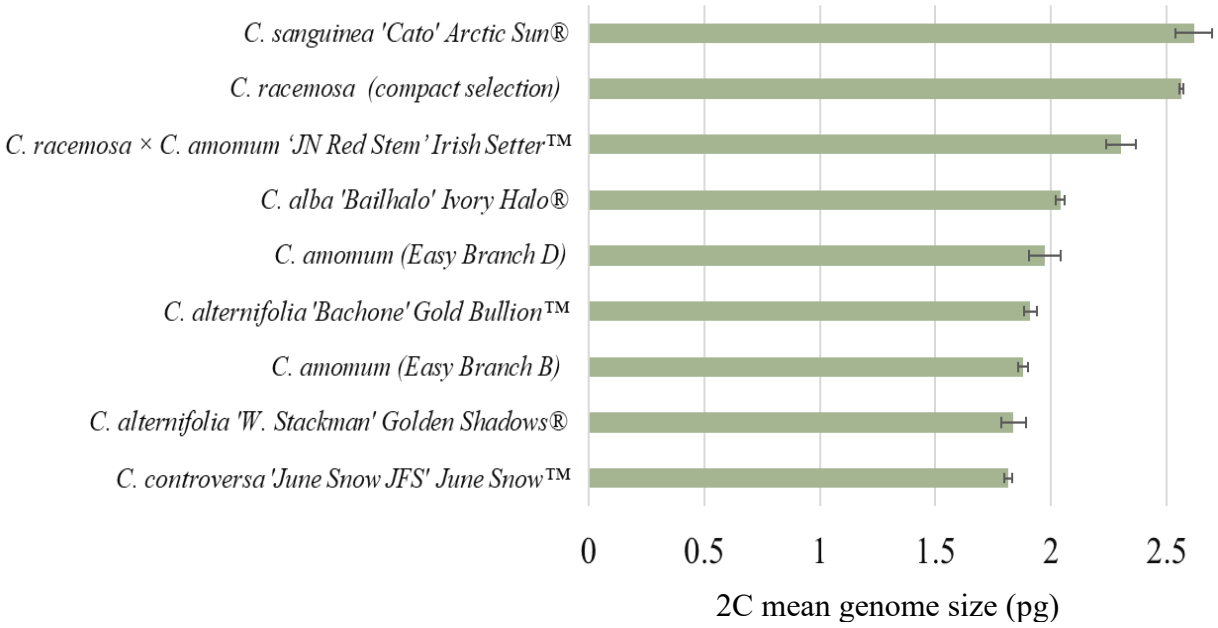

Figure 4. Relative 2C genome size of 9 *Cornus* cultivars determined using flow cytometry analysis of nuclei stained with 4′, 6-diamidino-2-phenylindole with *Pisum sativum* 'Ctirad' as an internal standard (2C = 8.76 pg).

Table 1. Relative genome sizes and ploidy levels for *Cornus* species, hybrids, and cultivars of the BW group determined using flow cytometry.

Subgenus	1C× (pg) / Taxon	Source/Accession[z]	1C×(pg)	Origin/Provenance[y]
Kraniopsis I x = 11	1.15±0.03 A[x]			
	Cornus drummondii	Mor117-24*1	1.15±0.00	Great Plains and Midwest US; W, Jasper, Missouri
	C. foemina	Mor279-84*3	1.07±0.04	Southeast and Eastern US; G
	C. foemina	Mor279-84*6	1.14±0.05	G
	C. foemina	Mor279-84*7	1.09±0.01	G
	C. racemosa	Mor181-92*2	1.19±0.01	Midwest and Eastern US; W, Will County, Illinois
	C. racemosa (compact)	JNPS01	1.28±0.00	G
Kraniopsis II	1.07± 0.01 B			
	Cornus alba	Mor105-79*9	0.98±0.02	Siberia/East Asia; W, Erfurt, German Democratic Republic
	C. alba 'Bailhalo', Ivory Halo® dogwood	Mor1319-2004*1	1.02±0.01	G
	C. amomum ssp. obliqua	Mor724-79*13	0.93±0.01	Midwest and Eastern US; W, Will County, Illinois
	C. amomum (Easy Branch B)	JNPS02	0.94±0.01	G
	C. amomum (Easy Branch D)	JNPS03	0.99±0.03	G
	C. bretschneideri	Mor303-90*5	1.12±0.03	Northeast China; W, Shanxi, China
	C. macrophylla	Mor503-2001*2	1.16±0.02	Eastern Asia; W, Mt. Maiji, Gansu, China
	C. paucinervis (*C. quinquenervis*)	Mor569-63*4	1.11±0.03	Central China; G
	C. paucinervis (*C. quinquenervis*)	Mor569-63*9	1.08±0.02	G
	C. paucinervis (*C. quinquenervis*)	Mor569-63*10	1.02±0.01	G
	C. pumila	Mor642-54*2	1.01±0.02	Unknown; G
	C. pumila	Mor642-54*3	1.05±0.03	G
	C. pumila	Mor719-79*4	1.06±0.01	G
	C. sanguinea	Mor196-81*3	1.26±0.02	Eurasia; W, Loire, France
	C. sanguinea 'Cato', Arctic Sun® dogwood	Mor270-2006*2	1.31±0.04	G
	C. sericea ssp. sericea	Mor464-85*6	1.00±0.00	North America; W, Lake County, Illinois
	C. walteri	Mor342-2002*1	1.20±0.02	East Asia; W, China
Putative hybrid	1.15±0.03 A			
	Cornus 'JN Red Stem' Irish Setter™ dogwood	JNPS04	1.15±0.03	G

Mesomara x = 10	0.92±0.02 C *Cornus alternifolia*	Mor295-2002*1	0.97±0.00	Eastern North America; W, Marinette, Wisconsin
	C. alternifolia 'Bachone' Gold Bullion™ dogwood	Mor695-2006*4	0.96±0.01	G
	C. alternifolia 'W. Stackman', Golden Shadows® dogwood	Mor595-2001*3	0.92±0.03	G
	C. controversa	Mor388-2007*2	0.85±0.00	East Asia; W, Liaoning, China
	C. controversa 'June Snow JFS', June Snow™ dogwood	Mor471-2009*1	0.91±0.01	G

There was one putative hybrid (*C.* 'JN Red Stem' Irish Setter™ dogwood) measured in this study. While the mean monoploid genome sizes of the putative parents (*C. racemosa* and *C. amomum*) are considered significantly different based on Tukey's HSD means separation (Table 2), the mean monoploid genome size of the putative hybrid was not found to be intermediate between the two by means separation. However, the putative hybrid was found to be intermediate with other taxa that fell within the Tukey's HSD Group E (Table 2). After personal correspondence with Mike Yanny, it has been determined that C. 'JN Red Stem' Irish Setter™ is most likely a hybrid between *C. racemosa* and *C. drummondii*.

The monoploid genome size of *Mesomara* was significantly smaller than that of *Kraniopsis I* and *II*. This reflects the smaller base chromosome number for this group.

CONCLUSIONS

Flow Cytometry is an efficient method for determining genome size in the BW group of *Cornus*. While cytological analysis of chromosome number was not completed, measured genome size seems to align with previous reports of diploid for taxa represented in this study. Monoploid genome size measured may support taxonomic relationships presented by Xiang et al. (2006) with further investigation and greater sampling.

The single putative hybrid measured in this study was found to be intermediate with two species with distinct relative genome sizes. As with the BB dogwoods, the potential for flow cytometry as a screening method for hybrids amongst the species of the BW could be of value for breeders.

Additional surveying of cultivated material may reveal unknown or unreported hybrids in the commercial industry. The smaller base chromosome number of *Mesomara* is evident in the relatively smaller monoploid genome size of 1Cx = 0.92 pg. Further investigation of cultivated material and species representative of taxonomic gaps including *Kraniopsis III* and *Yinquania* is needed to complete a genome size survey of *Cornus* L.

Table 2. Groupings of mean monoplid genome size values based on mean separating using Tukey's HSD at $\alpha = 0.05$.

Taxon	Mean monoploid genome size 1C× (pg)	Groups
Cornus sanguinea 'Cato' Arctic Sun®	1.31	a
C. racemosa (compact selection)	1.28	ab
C. sanguinea	1.26	abc
C.walteri	1.20	abcd
C. racemosa	1.19	abcd
C. macrophyllla	1.16	bcde
C. racemosa (putative hybrid)	1.15	bcde
C. drummondii	1.15	cde
C. bretschneideri	1.12	def
C. foemina	1.10	def
C. paucinervis	1.07	efg
C. pumila	1.04	fgh
C. alba 'Bailhalo' Ivory Halo® dogwood	1.02	fghi
C. sericea subsp. *sericea*	1.00	fghi
C. amomum (Easy Branch D)	1.00	ghi
C. alba	0.98	ghi
C. alternifolia	0.97	ghij
C. alternifolia 'Bachone' Gold Bullion™ dogwood	0.96	hij
C. amomum (Easy Branch B)	0.94	hij
C. amomum subsp. *obliqua*	0.93	ij
C. alternifolia 'W. Stackman' Golden Shadows® dogwood	0.92	ij
C. controversa 'June Snow-JFS' June Snow™ dogwood	0.91	ij
C. controversa	0.85	j

Literature Cited

Cappiello, P. and D. Shadow.2005. Dogwoods: The genus *Cornus*. Timber Press Portland Oregon.

Darlington, C.D. and Wylie, A.P. 1956. Chromosome atlas of flowering plants. MacMillan New York, New York.

de Mendiburu, F. 2019. Agricolae: Statistical procedures for agricultural research. R package version 1.3-1. https://CRAN.R-project.org/package=agricolae

Shearer, K., and Ranney, T.G. (2013). Ploidy levels and relative genome sizes of species, hybrids, and cultivars of dogwood (Cornus spp.). HortScience *48*(7):825–830.

United States Department of Agriculture. 2014. Retrieved from https://www.nass.usda.gov/Publications/Ag Census/2012/Online_Resources/Census_of_ Horticulture_Specialties/

Xiang, Q.Y.J., Thomas, D.T., Zhang, W., Manchester, S.R., and Murrell, Z. (2006). Species level phylogeny of the genus *Cornus* (Cornaceae) based on molecular and morphological evidence — implications for taxonomy and Tertiary intercontinental migration. Taxon *55*(1):9–30.