HortScience 48(7):825–830. 2013.

Ploidy Levels and Relative Genome Sizes of Species, Hybrids, and Cultivars of Dogwood (*Cornus* spp.)

Kimberly Shearer[1,3] and Thomas G. Ranney[2]

Department of Horticultural Science, North Carolina State University, Mountain Crop Improvement Laboratory, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Mills River, NC 28759

Additional index words. cytology, DNA content, flow cytometry, interspecific hybridization, plant breeding, polyploidy

Abstract. **Dogwoods (*Cornus* spp.) are valuable nursery crops grown as landscape plants throughout much of the world. Although there has been considerable work on breeding and selecting dogwoods, there is little information available on genome sizes (DNA content) and ploidy levels within the genus, particularly for specific clones and cultivars. Our objective was to conduct a survey of relative genome sizes and ploidy levels of dogwood taxa representative of the big-bracted, cornelian cherry, and dwarf dogwood clades. Flow cytometry was used to determine relative genome sizes and ploidy levels of 94 accessions of various species, hybrids, and cultivars. Traditional cytology was performed on root cells of representative taxa to calibrate genome sizes with ploidy level. All dogwood accessions tested were diploid with the exception of *C. canadensis* that was tetraploid and the hybrid cultivar *C.* KN30-8 that was triploid. Relative genome sizes varied by clade, subgenus, and species with 1Cx values ranging from 1.07 pg for *C. canadensis* to 5.08 pg for *C. eydeana*. Relative genome sizes were also valuable for confirming hybridity of interspecific crosses in cases in which parents varied substantially in relative genome size and hybrids were intermediate. A broad range of interspecific hybrids was documented including *C. capitata* × *florida, C. capitata* × *kousa, C. elliptica* × *florida, C. hongkongensis* × *florida, C. kousa* × *elliptica,* and *C. kousa* × *florida*. These results provide further insights into the cytogenetics, reproductive biology, crossability, and relative genome sizes of dogwoods.**

Dogwoods include more than 50 species of shrubs, small trees, and a few herbaceous perennials with distribution that ranges across the northern hemisphere and rarely into the southern hemisphere (Eyde, 1988; Fan and Xiang, 2001; Reed, 2004; Xiang et al., 2006). Many of these species are valuable landscape plants and are frequently cultivated for their four-season attributes including attractive flowers, fruit, foliage, bark, and form (Cappiello and Shadow, 2005). According to the Census of Horticultural Specialties (USDA-NASS, 2010), 2009 sales of dogwoods totaled more than $30.9 million in the United States.

Considerable research has focused on determining the systematic relationships among dogwoods (Fan and Xiang, 2001, 2003; Xiang et al., 1993, 1996, 1998, 2002, 2006). A species-level phylogeny completed by Xiang et al. (2006), supported major clades within the genus including the big-bracted dogwoods (BB) comprising subgenus *Cynoxylon* and *Syncarpea*, the dwarf dogwoods (DW) comprising subgenus *Arctocrania*, and the cornelian cherries (CC) comprising subgenus *Cornus*.

Polyploidy, also referred to as whole genome duplication, has played a significant role in the evolution and diversification of angiosperms (Soltis and Burleigh, 2009; Soltis et al., 2004, 2009). Polyploidization may lead to reproductive isolation, rapid genomic rearrangements, novel patterns of gene expression, and adaptations, which can ultimately lead to divergence and speciation (Adams and Wendel, 2005; Comai, 2005; Hegarty and Hiscock, 2008; Soltis and Burleigh, 2009). Knowledge of ploidy levels is important for plant breeders because it can influence fertility, crossability, segregation, and gene expression (Chen and Ni, 2006; Soltis et al., 2004). Cytological studies on dogwoods have found the base chromosome number for BB to be $x = 11$, for CC to be $x =$ nine or 10, and for DW to be $x = 11$ with both diploids and tetraploids found in the DW (Dermen, 1932; Goldblatt, 1978; Xiang and Eyde, 1995; Xiang et al., 2006). Chromosome counts for *C. canadensis* (DW) vary and include diploid ($2n = 2x = 22$) (Murrell, 1994; Packer, 1964; Zhang et al., 2008) and tetraploid ($2n = 4x = 44$) (Dermen, 1932; Löve and Löve, 1982) assessments.

Genome size data reflect fundamental biodiversity characters and can be reflective of genome evolution and taxonomic relationships (Greilhuber, 1998; Rounsaville and Ranney, 2010; Zonneveld and Duncan, 2010; Zonneveld et al., 2005). Genome size data can also be used to determine ploidy levels among closely allied species when properly calibrated (Jones et al., 2007; Palmer et al., 2009; Parris et al., 2010; Rounsaville and Ranney, 2010). In some cases, when parents vary considerably in genome sizes, hybrids can be verified based on intermediate values (Galbraith et al., 2005; Keller et al., 1996; Parris et al., 2010).

Despite the considerable breeding, selection, and widespread cultivation of dogwoods, sampling for genome size and ploidy level of species, cultivars, and hybrids has been limited and little is known concerning ploidy level and genome size of specific cultivars and hybrids. The objectives of this study were to determine relative genome sizes and ploidy levels of diverse taxa within *Cornus*, specifically for the BB, CC, and DW clades.

Materials and Methods

Flow cytometry. Relative 2C genome sizes were determined using flow cytometry (Greilhuber et al., 2007). Plant tissue, including expanding leaf tissue, vegetative buds, and floral buds, was collected from 94 accessions (Table 1) at the Mountain Horticultural Crop Research and Extension Center of North Carolina State University located in Mills River, NC. These included nine species and seven groups of hybrids representing BB, CC, and DW dogwoods that were sampled over time (May 2012 through Mar. 2013) in a completely randomized design. In addition, leaf tissue from the original *Cornus* 'KN30-8' (Venus™) was provided by Dr. Thomas Molnar at Rutgers University. Approximately 1 cm^2 or 20 mg of tissue was finely chopped in a polystyrene petri dish with 400 µL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany) using a sharp razor blade. The nuclei suspension was then filtered through 50-µm nylon filters and stained using 1.6 mL 4′, 6-diamidino-2-phenylindole (DAPI) staining buffer (Cystain ultraviolet Precise P Staining Buffer; Partec). Relative genome size was determined using a flow cytometer with fluorescence excitation provided by a mercury arc lamp (Partec PA-I; Partec). A minimum of 3000 nuclei was analyzed for each sample with a maximum CV of 4%, though it was typically below 3%. *Pisum sativum* 'Ctirad' (absolute 2C = 8.75 pg) was used as an internal standard for most taxa based on its common use as a reference standard (Bai et al., 2012; Greilhuber et al., 2007) and because its genome size was similar but distinct from most *Cornus* spp. As a result of the large

Received for publication 5 Apr. 2013. Accepted for publication 30 Apr. 2013.

This research was funded, in part, by the North Carolina Agricultural Research Service (NCARS), Raleigh, NC, the North Carolina Biotechnology Center, Research Triangle Park, NC, the Kenan Institute, Chapel Hill, NC, and the North Carolina Nursery and Landscape Association, Raleigh, NC. Technical assistance of Nathan Lynch, Joel Mowrey, Tom Eaker, Jeremy Smith, and Jason Lattier is gratefully acknowledged. We also thank Dr. Thomas Molnar at Rutgers University for providing samples of *Cornus* 'KN30-8' (Venus™).

[1]Undergraduate Research Intern.

[2]Professor.

[3]To whom reprint requests should be addressed; e-mail kmsheare@ncsu.edu.

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

Taxa	Accession no.[z]	Relative 2C genome size [mean ± SEM (pg)]	Ploidy level (x)
Big-bracted dogwoods (BB)			
Subgenus *Cynoxylon*			
Cornus florida			
'Appalachian Joy'	NCSU 2012-009	3.14 ± 0.03	2
'Appalachian Spring'	NCSU 2001-051	3.18 ± 0.03	2
'Comco No. 1' Cherokee Brave™	NCSU 2001-050	3.07 ± 0.07	2
'Dixie Colonnade'	NCSU 2006-064	3.12 ± 0.07	2
'Eternal Dogwood'	NCSU 2012-010	3.11 ± 0.05	2
'Little Princess'	NCSU 2003-034	3.22 ± 0.05	2
'Rutnam' Red Beauty®	NCSU 2003-025	3.16 ± 0.06	2
'Spartanburg'	NCSU 2012-011	3.20 ± 0.01	2
Unnamed seedling	NCSU H2012-045	3.12 ± 0.10	2[Y]
Unnamed fragrant selection	NCSU 2012-103	3.15 ± 0.12	2
Var. *urbiniana*	NCSU 2012-111	3.32 ± 0.05	2
'World's Fair'	NCSU 2006-055	3.05 ± 0.05	2
Cornus nuttallii			
'Barrick'	NCSU 2011-077	3.32 ± 0.00	2
'Colrigo Giant'	NCSU 2011-078	3.45 ± 0.14	2[y]
'Colrigo Giant'	NCSU 2012-006	3.51 ± 0.01	2
Subgenus *Syncarpea*			
Cornus capitata			
'Mountain Moon'	NCSU 2008-322	4.57 ± 0.08	2[y]
'Yoko'	NCSU 2002-009	4.47 ± 0.08	2
Unnamed seedling	NCSU H2012-056	4.58 ± 0.02	2
Cornus elliptica (*Cornus angustata*)			
'Elsbry' (Empress of China™)	NCSU 2006-030	4.23 ± 0.12	2
'First Choice'	NCSU 2003-005	4.31 ± 0.06	2
Unnamed seedling	NCSU H2007-025	4.29 ± 0.02	2
Cornus hongkongensis			
'Gekkou'	NCSU 2012-004	4.20 ± 0.01	2[y]
'Snowcap'	NCSU 2008-253	4.20 ± 0.01	2
Unnamed seedling	NCSU 2002-057	4.02 ± 0.10	2
Cornus kousa			
'Akabana'	NCSU 1998-108	3.72 ± 0.03	2
'Akatsuki'	NCSU 2003-042	3.91 ± 0.09	2
'Beni Fuji'	NCSU 2008-167	3.76 ± 0.01	2
'Blue Shadow'	NCSU 1998-113	4.02 ± 0.20	2
Bodnant form	NCSU 1998-114	3.72 ± 0.20	2
'Bonfire'	NCSU 1998-115	3.89 ± 0.05	2
Cedar Farm G selection	NCSU 1998-117	3.82 ± 0.05	2
'Cherokee'	NCSU 1998-118	3.81 ± 0.03	2
'Dwarf Pink'	NCSU 1998-120	3.64 ± 0.14	2
'Girard's Nana'	NCSU 1998-124	3.85 ± 0.00	2
'Greensleeves'	NCSU 2007-136	3.95 ± 0.07	2
'Little Beauty'	NCSU 1998-130	3.83 ± 0.12	2
'Lustgarten Weeping'	NCSU 1998-131	3.92 ± 0.01	2
'Madison' Crown Jewel™	NCSU 2011-020	3.86 ± 0.05	2
'Milky Way'	NCSU 2007-137	3.86 ± 0.02	2
'Milky Way Select'	NCSU 1998-133	3.79 ± 0.12	2
'National'	NCSU 1998-135	3.85 ± 0.01	2
'Radiant Rose'	NCSU 1998-137	3.79 ± 0.04	2
'Rochester'	NCSU 1998-138	3.80 ± 0.00	2
'Satomi'	NCSU 2007-135	3.94 ± 0.03	2
'Snowbird'	NCSU 1998-140	3.89 ± 0.04	2
'Speciosa'	NCSU 1998-141	3.87 ± 0.02	2
'Spinners'	NCSU 1998-142	3.82 ± 0.07	2
'Square Dance'	NCSU 1998-143	3.76 ± 0.13	2
'Summer Majesty'	NCSU 1998-145	3.84 ± 0.05	2
'Temple Jewel'	NCSU 1998-147	3.87 ± 0.08	2
'Wolf Eyes'	NCSU 1998-152	3.80 ± 0.06	2
Interspecific BB hybrids			
Cornus capitata × *C. florida*			
Unnamed seedling	NCSU H2007-020-065	3.88 ± 0.01	2
Unnamed seedling	NCSU H2008-006-299	3.87 ± 0.03	2
Unnamed seedling	NCSU H2011-058-009	4.15 ± 0.11	2

(*Continued on next page*)

Table 1. (*Continued*) Relative genome sizes and ploidy levels, determined using flow cytometry, for species, hybrids, and cultivars of *Cornus*.

Taxa	Accession no.[z]	Relative 2C genome size [mean ± SEM (pg)]	Ploidy level (x)
Cornus capitata × *C. kousa*			
Unnamed seedling	NCSU H2012-056-143	4.28 ± 0.11	2
Unnamed seedling	NCSU H2010-040-008	4.21 ± 0.01	2
Unnamed seedling	NCSU H2010-040-036	4.22 ± 0.02	2
Unnamed seedling	NCSU H2010-005-010	4.19 ± 0.01	2
Cornus elliptica × *C. florida*			
Unnamed seedling	NCSU H2006-010-017	3.72 ± 0.01	2
Unnamed seedling	NCSU H2008-004-015	4.36 ± 0.04	2
Unnamed seedling	NCSU H2008-004-016	3.58 ± 0.05	2
Cornus hongkongensis × *C. florida* -			
Unnamed seedling	NCSU H2007-018-065	3.73 ± 0.09	2
Unnamed seedling	NCSU H2007-018-179	3.73 ± 0.02	2
Unnamed seedling	NCSU H2007-018-181	3.70 ± 0.01	2
Cornus hongkongensis × *C. kousa*			
Unnamed seedling	NCSU H2003-013-019	4.11 ± 0.02	2
Unnamed seedling	NCSU H2003-013-020	4.09 ± 0.03	2
Unnamed seedling	NCSU H2003-013-014	4.00 ± 0.01	2
Cornus hongkongensis × *C. kousa*, F_2			
Unnamed seedling	NCSU H2007-017-154	4.13 ± 0.01	2
Unnamed seedling	NCSU H2007-017-088	4.14 ± 0.01	2
Unnamed seedling	NCSU H2007-017-100	4.18 ± 0.02	2
Cornus kousa × *C. elliptica* –			
Unnamed seedling	NCSU H2009-016-001	4.02 ± 0.07	2
Unnamed seedling	NCSU H2009-016-060	4.00 ± 0.03	2
Unnamed seedling	NCSU H2009-016-247	4.04 ± 0.00	2
(*Cornus kousa* × *C. nuttallii*) × *C. kousa*			
'KN30-8' (Venus™)	NCSU 2012-008	5.52 ± 0.06	3[y]
Cornus ×*rutgersensis* (*C. kousa* × *C. florida*)			
'KF1-1' (Saturn®)	NCSU 2003-022	3.51 ± 0.03	2
'Rutban' (Aurora®)	NCSU 1998-153	3.44 ± 0.00	2
'Rutcan' (Constellation®)	NCSU 1998-154	3.53 ± 0.04	2
'Rutdan' (Celestial®)	NCSU 1998-155	3.39 ± 0.05	2
'Rutfan' (Stardust®)	NCSU 1998-157	3.36 ± 0.06	2
'Rutgan' (Stellar Pink®)	NCSU 2012-112	3.62 ± 0.08	2
'Rutlan' (Ruth Ellen®)	NCSU 1998-156	3.41 ± 0.06	2
Unnamed seedling	NCSU H2010-021	3.38 ± 0.02	2
Dwarf dogwoods (DW)			
Subgenus *Arctocrania*			
Cornus canadensis—unnamed seedling	NCSU 2012-102-001	4.27 ± 0.05	4[y]
Cornus canadensis—unnamed seedling	NCSU 2012-102-002	4.29 ± 0.02	4
Cornus canadensis—unnamed seedling	NCSU 2012-102-003	4.22 ± 0.03	4
Cornelian cherries (CC)			
Subgenus *Cornus*			
Cornus eydeana—unnamed seedling	NCSU 2008-214-001	10.31 ± 0.07	2[y]
Cornus eydeana—unnamed seedling	NCSU 2008-214-002	10.04 ± 0.07	2
Cornus eydeana—unnamed seedling	NCSU 2008-214-003	10.10 ± 0.07	2
Cornus mas			
'Aurea'	NCSU 1996-087	6.50 ± 0.02	2
'Elegantissima'	NCSU 1996-144	6.69 ± 0.07	2
'Golden Glory'	NCSU 1996-088	6.67 ± 0.05	2
Cornus officinalis			
'Kintoki'	NCSU 2008-264	6.44 ± 0.09	2
'Spring Glow'	NCSU 1998-430	6.64 ± 0.08	2[y]
Unnamed seedling	NCSU 1998-305	6.57 ± 0.03	2

[z]Accession number from the North Carolina State University, Mountain Crop Improvement Laboratory, Mills River, NC.
[y]Indicates ploidy was confirmed with cytology.

relative genome size of *C. eydeana, Magnolia virginiana* 'Jim Wilson' (Moonglow™) was used as an internal standard (absolute 2C = 3.92 pg) for those analyses following Parris et al. (2010). Internal standards were cochopped with samples during nuclei extraction. Holoploid, 2C DNA contents were calculated as: 2C = DNA content of standard × (mean fluorescence value of sample ÷ mean fluorescence value of the standard). Analyses were completed on two subsamples for each replicate. The relationship between ploidy level and genome sizes was initially determined for species with documented chromosome numbers (Harrington et al., 1985; Li and Shang, 2002; Sandhu and Mann, 1988). Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated

Table 2. Monoploid genome sizes (1Cx), determined using flow cytometry, for *Cornus* spp., grouped by clade, subgenus, and species/grex.

Clade	1Cx (pg)	Subgenus	1Cx (pg)	Species/grex	1Cx (pg)
Cornelian cherries (CC)	3.88 ± 0.30A[z]	*Cornus*	3.89 ± 0.30 A	*C. eydeana*	5.08 ± 0.04 A
				C. mas	3.31 ± 0.03 B
				C. officinalis	3.28 ± 0.03 B
Big-bracted dogwoods (BB)	1.89 ± 0.02 B	*Syncarpea*	2.00 ± 0.02 B	*C. capitata*	2.27 ± 0.05 C
				C. elliptica	2.14 ± 0.01 D
				C. hongkongensis	2.07 ± 0.03 DE
				C. kousa	1.92 ± 0.01 HI
				C. capitata × *C. kousa*	2.11 ± 0.01 D
				C. hongkongensis × *C. kousa*	2.03 ± 0.02 EF
				C. hongkongensis × *C. kousa,* F_2	2.08 ± 0.01 DE
				C. kousa × *C. elliptica*	2.01 ± 0.01 EFG
		Syncarpea × *Cynoxylon*	1.83 ± 0.03 BC	*C. capitata* × *C. florida*	1.98 ± 0.05 FGH
				C. hongkongensis × *C. florida*	1.86 ± 0.01 I
				C. elliptica × *C. florida*	1.94 ± 0.12 GH
				C. ×*rutgersensis*	1.73 ± 0.02 J
		Cynoxylon	1.60 ± 0.02 C	*C. florida*	1.58 ± 0.01 K
				C. nuttallii	1.71 ± 0.03 J
Dwarf dogwoods (DW)	1.07 ± 0.01C	*Arctocrania*	1.07 ± 0.01 D	*C. canadensis*	1.07 ± 0.01 L

[z]Values followed by different letters within a column are significantly different, least significant difference, $P \leq 0.05$.

base set of chromosomes) was calculated as 2C genome size ÷ ploidy level.

Cytology. Cytology was used to verify chromosome numbers and ploidy levels of eight taxa representing different taxonomic groups and to further calibrate genome sizes with ploidy levels (Table 1). Actively growing root tips, ≈10 mm in length, were collected from seedlings and rooted stem cuttings in the mornings before 1000 HR. Root tips were suspended in a pre-fixative solution of 2 mM 8-hydroxyquinoline + 0.24 mM cycloheximide in glass vials and stored in the dark at room temperature for 3 h. Root tips were then refrigerated at 6 °C for 3 h in the dark. After pre-fixative treatment, root tips were rinsed four times using refrigerated distilled H_2O and then placed into a fixative solution of three parts 95% ethanol:one part propionic acid. Samples were then stored at room temperature overnight. The next morning, root tips were rinsed four times using 70% ethanol and stored in vials of 70% ethanol until needed.

Before squashing, root tip cell walls were hydrolyzed using three 95% ethanol:one 12 M hydrochloric acid for ≈3 min. After hydrolysis, root tips were then placed into a staining solution of modified carbol fuschin (Kao, 1975) for ≈5 min. The distal end of the stained root tip was excised under a dissecting microscope (Leica Stereozoom 6 Photo, Buffalo Grove, IL) and then placed on a glass microscope slide and gently squashed with a coverslip. Chromosomes were viewed using a light microscope (Nikon Eclipse 80i, Melville, NY).

Data for monoploid genome sizes (1Cx) were subjected to analysis of variance by clade, subgenus, and species/grex, and means were separated using Fisher's least significant difference (Proc GLM; SAS Version 9.2; SAS Inst., Cary, NC).

Results and Discussion

Flow cytometry was an effective, efficient, and consistent method for determining relative genome sizes and ploidy levels of *Cornus* (Table 1). Values for the two subsamples of each replicate were consistent with SEMS for 2C values ranging from less than 0.01 to 0.2 pg with a mean of 0.05 pg. Few studies have reported genome sizes in *Cornus*, although Bai et al. (2012) reported the 2C value of one sample of *C. canadensis* as 4.4 pg [using propidium iodide (PI) stain] that is close to our findings that ranged from 4.2 to 4.3 pg (using DAPI stain). Zonneveld et al. (2005) found the 2C value of one sample of *C. mas* as 6.8 pg (using PI stain) similar to our values that ranged from 6.5 to 6.7 pg (using DAPI stain). Although different fluorochrome stains may give slightly different estimates of genome size (Doležel and Bartoš, 2005), both PI and DAPI have been found to be effective and consistent for determining and comparing ploidy levels and relative genome size among closely related taxa (Parris et al., 2010). Furthermore, the DAPI procedure is faster, less expensive, uses less toxic compounds, and typically produces results with a lower CV for mean nuclei fluorescence. Our estimates of 2C values for *C. florida* ranged from 3.05 to 3.32 pg, which was considerably higher than estimates of 2.19 to 2.77 pg reported by Wang et al. (2009), although both studies used DAPI stain.

Monoploid genome sizes (1Cx) varied among clades, subgenera, and species and ranged from 1.07 pg for *C. canadensis* to 5.16 pg for *C. eydeana* and were very consistent within species/grex with SEMS ranging from 0.01 to 0.12 pg and a mean SEM of 0.03 pg (Table 2). Based on the taxa sampled, mean 1Cx values were smallest for the DW at 1.07 pg, the BB were intermediate at 1.89 pg, and the CC were considerably larger at 3.88 pg (Table 2).

There were also differences among subgenera within clades. For the BB dogwoods, mean 1Cx values for subgenus *Syncarpea* (2.00 pg) were significantly higher than for subgenus *Cynoxylon* (1.60 pg), whereas hybrids between these two subgenera were intermediate (1.83 pg). In some cases, there were also differences in 1Cx values among species within subgenera. For example, within subgenus *Cornus*, *C. eydeana* was significantly larger (5.08 pg) than *C. mas* (3.31 pg) and *C. officinalis* (3.28 pg). Also, within subgenus *Cynoxylon*, *C. florida* had a 1Cx mean of 1.58 pg, whereas *C. nuttallii* was significantly larger with a 1Cx mean of 1.71 pg. Monoploid genome sizes of evergreen species within *Syncarpea*

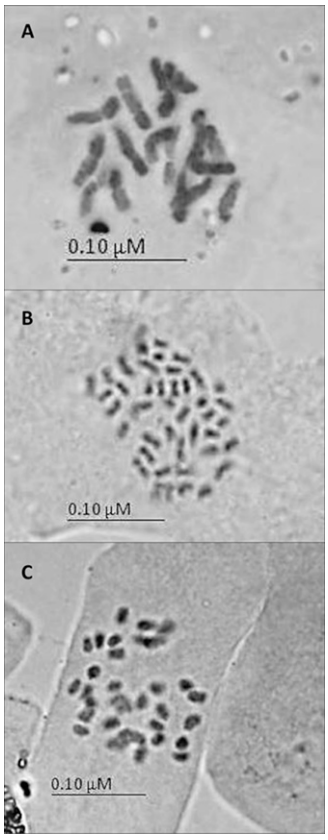

Fig. 1. Photomicrographs of somatic chromosomes of (**A**) *Cornus eydeana* ($2n = 2x = 18$), (**B**) *C. canadensis* ($2n = 4x = 44$), and (**C**) *C.* 'KN30-8' Venus™ ($2n = 3x = 33$).

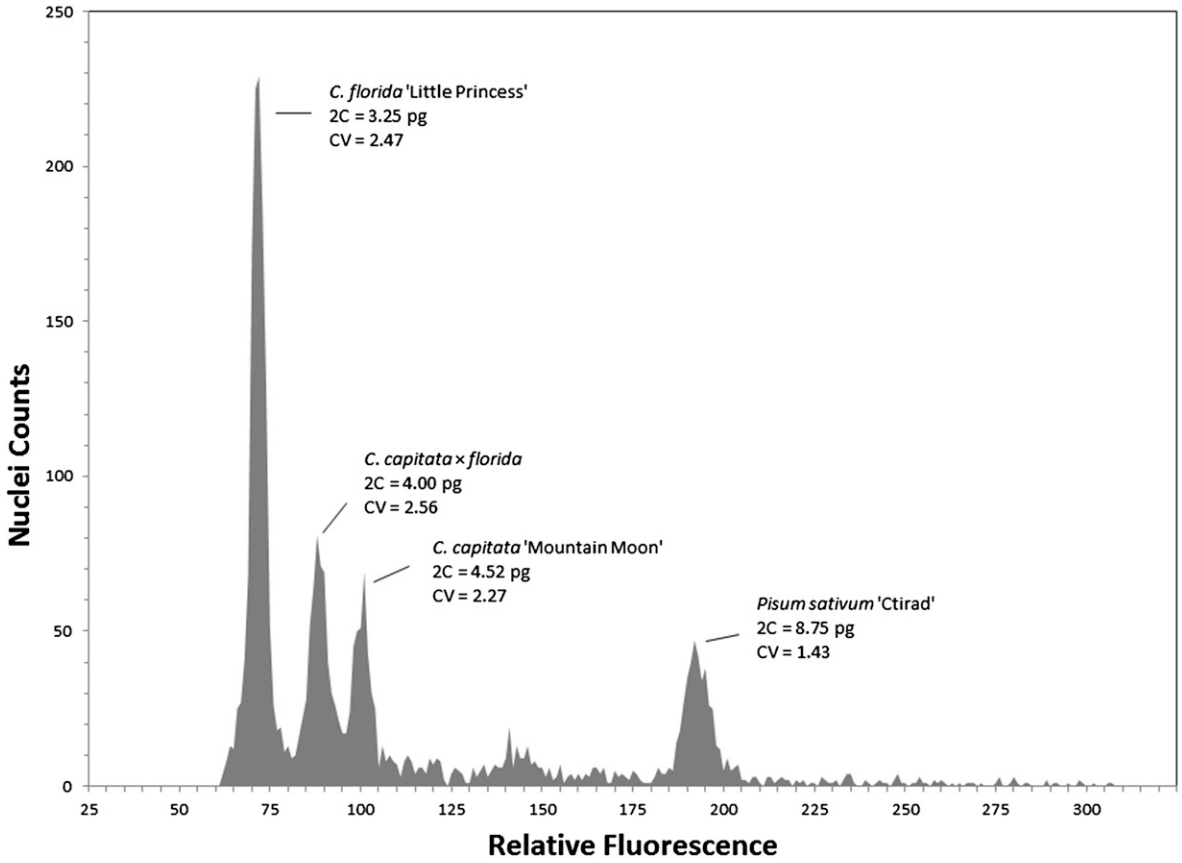

Fig. 2. Cytometer histogram showing relative fluorescence peaks of 4′, 6-diamidino-2-phenylindole-stained nuclei and calculated relative 2C genome sizes and CV for *Cornus florida, C. capitata*, their interspecific hybrid, and *Pisum sativum* 'Ctirad' as an internal standard.

(e.g., *C. capitata*, *elliptica*, and *hongkongensis*) varied some with *C. capitata* having the largest mean of 2.27 pg, and all of these species had significantly larger 1Cx values (2.07 to 2.27 pg) than that of *C. kousa* (1.92 pg). These results support the taxonomic groupings developed by Xiang et al. (2006) because the range of genome sizes for each of the subgenera and clades are distinct and discontinuous.

Cytological and cytometrical analyses confirmed that the majority of taxa tested were diploid (Table 1). Even *C. eydeana*, with a particularly large 2C genome size of 10.31 pg, was $2n = 2x = 18$ (Fig. 1A). The only tetraploid found was *C. canadensis* with $2n = 4x = 44$ (Fig. 1B). There are conflicting reports on the ploidy level of *C. canadensis*. Dermen (1932) and Löve and Löve (1982) also found *C. canadensis* to be a tetraploid; however, other studies have suggested that the only tetraploid species of the genus is *C. unalaschkensis* (Zhang et al., 2008), a putative allopolyploid hybrid between *C. canadensis* and *C. suecica* (Murrell, 1994). Considering the widespread distribution of *C. canadensis* (Xiang and Boufford, 2005), there may be a ploidy series within the species. Ploidy series are commonly found within species, some of which exhibit ploidy varying with distribution (Baack, 2004; Burton and Husband, 1999; Hardy and Vekemans, 2001; Li et al., 2010; Nakagawa, 2006; Whittemore and Olsen, 2011), whereas others exhibit ploidy heterogeneity within populations (Halverson et al., 2008; Trávníček et al., 2011).

The only triploid identified in this study was the interspecific hybrid *C.* 'KN30-8' (NCSU 2012-008), [(*C. kousa* × *C. nuttallii*) × *C. kousa*], with $2n = 3x = 33$ (Fig. 1C). It has been noted that hybrids often produce unreduced gametes at a particularly high frequency (Ramsey and Schemske, 1998), which may have contributed to the formation of this rare polyploidy in the BB clade. There has previously been some speculation that *C. officinalis* 'Spring Glow' may be a triploid based on its extremely low fruit set (personal observation), though its relative genome size is consistent with other diploids. It is also interesting to note that F_1 hybrids of *C. kousa* × *C. elliptica* retain fertility (personal observation) though the parents vary in genome size by over 11%.

Relative genome sizes were also valuable for confirming hybridity of interspecific crosses in cases in which parents varied substantially in genome size (Fig. 2). Hybrids between species in the subgenera *Syncarpea* and *Cynoxylon* were readily apparent based on intermediate genome sizes including: *C. capitata* × *C. florida*, *C. hongkongensis* × *C. florida*, *C. elliptica* × *C. florida*, and *C.* × *rutgersensis* (Table 2). In some cases, interspecific hybrids within subgenus *Syncarpea* could also be confirmed, including *C. capitata* × *C. kousa* and *C. kousa* × *C. elliptica*, though relative genome sizes were too similar between *C. hongkongensis* and C. *kousa* to verify hybrids between those species. Confirmation of these hybrids further suggests that there is considerable potential for the development of new hybrids among diverse species of BB dogwoods.

This study provides new and pertinent information on genome sizes and ploidy levels for species, cultivars, and hybrids of dogwoods. Additionally, it was found that genome size data can be an efficient and effective means of confirming hybridization among many BB dogwood species. These results provide further insights into the cytogenetics, systematics, reproductive biology, and crossability of dogwoods and contribute to the larger census of genome sizes of angiosperms.

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