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Ploidy Levels and Genome Sizes of *Berberis* L. and *Mahonia* Nutt. Species, Hybrids, and Cultivars

Todd J. Rounsaville[1] and Thomas G. Ranney[2,3]

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

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Abstract. **An extensive survey of genome sizes and ploidy levels was conducted for a diverse collection of *Berberis* and *Mahonia* taxa (Berberidaceae). Propidium iodide flow cytometric analysis was conducted using *Pisum sativum* L. 'Ctirad' (2C DNA = 8.76 pg) as an internal standard to determine genome sizes. Mean $1C_X$ genome sizes varied between the two *Mahonia* subgenera (*Occidentales* = 1.17 ± 0.02, *Orientales* = 1.27 ± 0.01), whereas those of *Berberis* subgenera were similar (*Australes* = 1.45 ± 0.03, *Septentrionales* = 1.47 ± 0.02) and each significantly larger than those of *Mahonia*. Traditional cytology was performed on representative species to calibrate genome sizes with ploidy levels. Polyploidy among both wild and cultivated taxa was found to be rare. Although the majority of species were determined to be diploid with $2n = 2x = 28$, artificially induced autopolyploid *Berberis thunbergii* seedlings were confirmed to be tetraploid and an accession of *Mahonia nervosa* was confirmed to be hexaploid. Genome size and ploidy level reports for the majority of taxa sampled are presented for the first time and are intended to be of use to plant breeders, ecologists, and systematists.**

The sister genera *Berberis* L. and *Mahonia* Nutt. represent the two largest groups within the family *Berberidaceae*, consisting of ≈400 and 100 species, respectively (Ahrendt, 1961; Kim et al., 2004). This highly ornamental group of shrubs and small trees is valued for their evergreen or multicolored leaves, brilliant flowers, and often showy fruit. The two genera have also been recognized for their pharmaceutical and medicinal properties (Alvarez et al., 2009) as well as their use in the printing and dyeing industry (Yan-Jun et al., 2006). Distribution of the two genera is nearly worldwide with centers of diversity in southern Asia as well as Central and South America and with minor representation in North America, Europe, Africa, and the Pacific Islands (Ahrendt, 1961).

Taxonomic standing of *Mahonia* and *Berberis* as distinct genera has been the subject of much debate among botanists and horticulturists. Before the development of DNA-based phylogenetics, morphological characters such as leaf and stem complexity, inflorescence structure, and floral anatomy had served to distinctly separate *Mahonia* from *Berberis*. However, *Mahonia* section *Horridae* Fedde (approximately nine species), which includes *M. freemontii* (Torr.) Fedde, *M. haematocarpa* (Wooton) Fedde, *M. nevinii* (A. Gray) Fedde, and *M. trifoliolata* (Moric.) Fedde, exhibits a blend of taxonomic features intermediate between *Mahonia* and *Berberis* (Ahrendt, 1961; Whittemore, 1997). These morphological inconsistencies have led some (Laferriere, 1997; Marroquin, 1993; Whittemore, 1997) to adopt a unified treatment of all *Berberis* and *Mahonia* species within *Berberis*. Nevertheless, the obvious difference in physical appearance between the two genera, with compound leaves within *Mahonia* versus simple leaves within *Berberis*, makes a unified circumscription hard to reconcile, and consequently, the horticultural field generally maintains the two groups as separate genera (Ahrendt, 1961; Dirr, 2009; Hinkley, 2009; Huxley et al., 1992; Yan-Jun et al., 2006).

When the taxonomy is viewed *sensu* Ahrendt, *Berberis* and *Mahonia* are each broken down into two subgenera set forth by Ahrendt (1961) and Schneider (1905). Within *Berberis*, the *Australes* C.K. Schneid. includes all the species from Central and South America; the remaining species are placed in the *Septentrionales* C.K. Schneid. and occur entirely in the northern hemisphere save for two in East Africa and one in Java and Sumatra (Ahrendt, 1961). Conversely, *Mahonia* are grouped longitudinally with those of the Eastern hemisphere in subgenus *Orientales* Ahrendt and all those of the Western hemisphere [with the notable exception of *M. nervosa* (Pursh) Nutt.] in subgenus *Occidentales* Ahrendt.

Recent phylogenetic analysis based on internal transcribed spacer (ITS) sequencing (Kim et al., 2004) has yielded further insight into the taxonomic relationships and evolutionary history of *Mahonia* and *Berberis*. For example, the postulation of Ahrendt (1961) of *Mahonia* as the progenitor of *Berberis* was supported. Examining the contemporary dispersal of the two genera from South America northward reveals that although the distinctive compound-leaved *Mahonia* is first encountered in Central America, a number of *Berberis* characters persist within *Mahonia* much further north. These transitional species, representing the aforementioned *Mahonia* section *Horridae*, showed a closer relationship with *Berberis* and thus a paraphyletic subgenus *Occidentales* (Kim et al., 2004). Although ITS phylogeny supported the subgenera proposed by Ahrendt (1961) and Schneider (1905), groupings below the subgeneric levels were not supported (Kim et al., 2004). Furthermore, *M. nervosa* was retained within the *Orientales*, albeit with weak support.

Along with the monotypic herb *Ranzania japonica* T. Ito, *Berberis* and *Mahonia* form a monophyletic clade within Berberidaceae in which base chromosome number is $x = 7$ (Kim and Jansen, 1998). As Dermen (1931) noted in his cytological studies, chromosomes among widespread species of both genera are of similar size. Furthermore, artificial intergeneric hybridization events between *Mahonia* and *Berberis* originated in Europe as early as 1854 (Dirr, 2009). Despite a number of successful intergeneric hybrids (×*Mahoberberis* C.K. Schneid.), the resulting progeny have been horticultural curiosities at best, typically regarded as inferior to both parent taxa (Phillips and Barber, 1981). ×*Mahoberberis* tend to exhibit numerous leaf-morphs among single plants, and in general flowering and fruiting of the hybrids is known to be rare or nonexistent (Dirr, 2009; Wyman, 1958). In addition, all ×*Mahoberberis* hybrids have been comprised of only one species of *Mahonia* [*M. aquifolium* (Pursh) Nutt.], and the cross appears uni-directional with *Mahonia* only functioning as the maternal parent (Dirr, 2009; personal observation), further suggesting that the two genera are largely incompatible. Conversely, hybrids among species of *Berberis* and among species of *Mahonia* are commonplace (Huxley et al., 1992; personal observation).

Polyploidization is a significant phenomenon in the plant kingdom that can play a role in rapid genomic rearrangement, development of novel traits and adaptations, reproductive isolation, and can ultimately lead to speciation (Adams and Wendel, 2005; Soltis and Burleigh, 2009). Furthermore, polyploidy is an important consideration in plant breeding because it can influence crossability, morphology, fertility, and gene expression (Chen and Ni, 2006; Soltis et al., 2004). Sampling of ploidy levels has been very limited for *Mahonia* taxa. *Mahonia aquifolium*, *M. napaulensis* DC., *M. repens* (Lindl.)

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[1]Graduate Research Assistant.

[2]Professor.

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

G. Don., and *M. japonica* (Thunb.) DC. have been reported to be diploid with $2n = 2x = 28$ (Dermen, 1931; Xu et al., 1992). In other cases, *M. aquifolium* and *M. nervosa* were reported to be tetraploid with $2n = 4x = 56$ (Taylor and Taylor, 1997). Reports on 45 *Berberis* species found diploids, $2n = 2x = 28$, including *B. koreana* Palib., *B. seiboldii* Miq., *B. thunbergii* DC., *B. vulgaris* L., and *B. yunnanensis* Franch. as well as tetraploids, $2n = 4x = 56$, including *B. buxifolia* Lam., *B. heterophylla* Juss. ex Poir., and *B. turcomanica* Kar. (Bottini et al., 2000; Dermen, 1931).

Independent of variations in ploidy level, information on base genome size (base DNA content) can be used as an indicator of genome evolution and taxonomic relationships (Greilhuber, 1998; Vinogradov, 1994; Zonneveld and Duncan, 2010; Zonneveld and Van Iren, 2001), lending insight into species evolution and potential breeding applications. As it relates to breeding, disparities in genome sizes can reflect differences in chromosome sizes and arrangement that may influence crossability and fertility of hybrid progeny (Zonneveld, 2009). There are no published reports of *Mahonia* genome size, and those of *Berberis* are extremely limited in both number of taxa and species diversity. Previous reports of genome size among *Berberis* were determined using Feulgen microspectrophotometry with diploid species constituting a range of 1.5 pg for *B. bidentata* Lechler to 3.6 pg for *B. empetrifolia* Lam. (Bottini et al., 2000). A desirable alternative to microspectrophotometry is flow cytometry, which allows for much greater ease in sample preparation, rapid determination of genome size, and can be accurately performed using a variety of plant tissues (Doležel and Bartos, 2005; Doležel et al., 1998). For closely related taxa, in which genome sizes are relatively conserved, flow cytometry can also be used for determination of ploidy level. Although a number of different fluorochromes may be used to stain DNA, many including 4′,6-diamidino-2-phenylindole, Hoechst 33258, and olivomycin are exclusive to either AT or CG bps, whereas propidium iodide (PI) is known to be largely non-specific with only a slight preference toward CG (Doležel et al., 1998; Vinogradov, 1994).

Considering the tremendous diversity and crossability found in *Berberis* and *Mahonia*, the potential for breeding improved hybrids is considerable. However, a greater understanding of genome sizes and ploidy levels within these genera would greatly enhance future breeding efforts. Although basic information on chromosome numbers, genome sizes, and ploidy levels have been reported for some *Berberis* and *Mahonia*, sampling has been limited and little is known about ploidy levels of specific clones or cultivars. The objectives of this research were to conduct an extensive survey of genome sizes and ploidy levels of species, hybrids, and cultivars of *Berberis* and *Mahonia* using a combination of flow cytometry and traditional cytology. Taxa included for this survey exhibit attributes of value for the ornamental plant breeder and are representative of each major phylogenetic clade. As a result of the unresolved nature of the generic classification, and for purposes of comparison, we accept the treatment of Ahrendt (1961), whom conducted the last thorough review of the genera.

Materials and Methods

A diverse collection of *Berberis* and *Mahonia* taxa were obtained from various institutions, gardens, and private collectors. All sampled plants from the Mountain Horticulture Crops Research Station (MHCRS) were container-grown, whereas additional sample material shared with the authors was collected from field-grown plants (Table 1). Sampled taxa represented species from each of the four subgenera (*Australes*, *Septentrionales*, *Occidentales*, and *Orientales*), many common cultivars, including interspecific hybrids, and a few purported artificially induced autopolyploids.

Flow cytometry was conducted on tissue (0.5 cm^2) taken from recently expanded leaves using a hole punch. Leaf tissue for each sample as well as an internal standard (*Pisum sativum* 'Ctirad' 2C DNA = 8.76 pg) was finely diced with a razor blade in a petri dish containing 500 µL of nuclei extraction buffer. On being filtered into a small test tube using a 50-µm filter, a solution containing 2 mL staining buffer, 6µL RNase A, and 12 µL PI (CyStain PI absolute P; Partec, Münster, Germany) was added, and the samples were moved to a refrigerator at 4 °C for 1 h. A flow cytometer (Partec PA-II; Partec) was used to analyze the stained nuclei with a minimum of 5000 counts per sample and two subsamples conducted for each taxon. Flow cytometry was conducted during the spring and summer of 2009 when fresh leaf material was available for sample. Holoploid, 2C genome size was calculated as: 2C = genome size of standard × (mean fluorescence value of sample/mean fluorescence value of standard). Genome size values presented within Table 1 represent the mean value of two subsamples conducted for each taxon. The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers (Bottini et al., 2000; Xu et al., 1992). Mean $1C_X$ monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 14) was calculated as (2C genome size/ploidy level) to assess variability in base genome size. Data were subjected to analysis of variance and means separation using the Waller procedure to compare means of genera and subgenera (SAS Institute Inc., Cary, NC).

Traditional cytology was conducted to verify previous work and calibrate genome size with ploidy level. Between 0800 and 0900 HR, actively growing root tips ≈5 mm in length were removed from container-grown plants and placed into small vials of 2 mM 8-hydroxyquinoline. The vials were left in the dark for 2 h at room temperature followed by 2 h in darkness at 4 °C. Roots tips were then thoroughly rinsed in cold distilled H_2O, blotted dry, and transferred to a fixative of (3:1) 95% ethanol:propionic acid overnight at room temperature. After 16 to 24 h in fixative, the root tissue was rinsed with 70% ethanol and transferred to storage in 70% ethanol at 4 °C. When time permitted, root tissue was removed from cold storage and hydrolyzed in a solution of (3:1) 95% ethanol:12N HCl for 5 to 10 s. Root tips were then placed into a drop of modified carbol fuschin stain (Kao, 1975) on a glass microscope slide and gently squashed with a coverslip. Chromosomes were counted using oil immersion at 1500×.

Results and Discussion

Cytology performed on *Mahonia eurybracteata* Fedde 'Soft Caress' (MHCRS 2008-267) and *B. thunbergii* var. *atropurpurea* 'Concorde' confirmed them to both be diploid, providing an additional confirmation and calibration of ploidy level with genome size. Flow cytometry was subsequently found to be an effective method for determining genome sizes and ploidy levels of *Mahonia* and *Berberis* (Table 1). The base, $1C_X$, genome size for *Mahonia* varied for each subgenus with a mean of 1.17 pg for *Occidentales* and 1.27 pg for *Orientales* (Table 2). There was no difference in $1C_X$ genome size between the two subgenera of *Berberis*, but plants in the genus *Berberis* had a significantly higher mean (1.45 pg) than either subgenus of *Mahonia*. These data indicate that a significantly greater (≈18%) expansion in genome size occurred during the evolution of *Berberis* compared with *Mahonia*. The substantial difference in genome size between *Mahonia* and *Berberis* could compromise fertility of intergeneric hybrids as a result of chromosomal sterility and may explain why these hybrids are typically sterile. Of additional interest are the genome sizes for species in *Mahonia* section *Horridae* (*Occidentales*). Although ITS phylogeny (Kim et al., 2004) indicated plants in this section were more closely allied with *Berberis* than to *Mahonia*, the mean $1C_X$ value for *M. haematocarpa*, *M. nevinii*, and *M. trifoliolata* (all in section *Horridae*) was 1.19 pg, significantly lower ($P < 0.05$) than either subgenus of *Berberis* or *Mahonia* subgenus *Orientales* but consistent with *Mahonia* subgenus *Occidentales*.

Within *Berberis* subgenus *Septentrionales*, diploid 2C genome sizes ranged from 2.48 pg for *B. wilsonii* var. *stapfiana* (C.K. Schneid.) C.K. Schneid. (JCRA, accession #E41) to 3.36 pg for *B. sieboldii* (MHCRS 2005-179) with a mean of 2.94 pg for the subgenus. Only four taxa were available for sampling from *Berberis* subgenus *Australes* and ranged in 2C genome size from 2.77 pg for *B.* ×*stenophylla* Hort. (MHCRS 2000-210) to 3.02 pg for *Berberis trigona* Kunze ex Poepp. & Endl. 'Orange King' (MHCRS 2003-053) with a mean of 2.90 pg for the subgenus, well within the range found for *Berberis* subgenus *Septentrionales*. No natural polyploids were identified among the *Berberis* sampled in this study. However,

Table 1. Mean 2C genome sizes and ploidy levels of *Berberis* and *Mahonia* species, hybrids, and cultivars.

Subgenus	Taxa[z]	2C genome size (pg)[y]	Ploidy level (*x*)
Australes	*Berberis darwinii* Hook. (MHCRS 2007-018)	2.92 ± 0.01	2
Australes	*Berberis* ×*lologensis* Sandwith 'Apricot Queen' (PDN 31368)	2.87 ± 0.03	2
Australes	*Berberis* ×*stenophylla* Hort. (MHCRS 2000-210)	2.77 ± 0.03	2
Australes	*Berberis trigona* Kunze ex Poepp. & Endl. 'Orange King' (MHCRS 2003-053)	3.02 ± 0.06	2
Septentrionales	*Berberis aggregata* C.K. Schneid. (JCRA 001745)	2.62 ± 0.01	2
Septentrionales	*Berberis aristata* Sims (JCRA 001752)	2.83 ± 0.10	2
Septentrionales	*Berberis calliantha* Mulligan (MHCRS 2003-066)	2.63 ± 0.06	2
Septentrionales	*Berberis calliantha* Mulligan (PDN 21861)	2.85 ± 0.04	2
Septentrionales	*Berberis* ×*carminea* Ahrendt 'Pirate King' (MHCRS 2004-033)	2.62 ± 0.03	2
Septentrionales	*Berberis dasystachya* Maxim. (MHCRS 2004-034)	2.91 ± 0.01	2
Septentrionales	*Berberis fendleri* A. Gray (MHCRS 2006-222)	3.07 ± 0.02	2
Septentrionales	*Berberis francisci-ferdinandii* C.K. Schneid. (PDN 31140)	2.91 ± 0.01	2
Septentrionales	*Berberis* ×*frikartii* C.K.Schneid. ex H.J.van deLaar 'Telstar' (MHCRS 2000-155)	3.01 ± 0.04	2
Septentrionales	*Berberis gilgiana* Fedde (JCRA 001680)	2.74 ± 0.08	2
Septentrionales	*Berberis* ×*gladwynensis* E. Anders. 'William Penn' (MHCRS 2000-178)	3.04 ± 0.01	2
Septentrionales	*Berberis henryana* C.K. Schneid (MHCRS 2005-196)	2.87 ± 0.03	2
Septentrionales	*Berberis* ×*interposita* Ahrendt 'Wallich's Purple' (MHCRS 2000-160)	2.66 ± 0.01	2
Septentrionales	*Berberis jamesiana* Forrest & W.W. Sm. (MHCRS 2005-198)	2.97 ± 0.03	2
Septentrionales	*Berberis jamesiana* Forrest & W.W. Sm. (PDN 26519)	3.00 ± 0.04	2
Septentrionales	*Berberis koreana* Palib. (JCRA 971069)	3.03 ± 0.01	2
Septentrionales	*Berberis koreana* Palib. 'Red Tears' (PDN 14990)	3.02 ± 0.02	2
Septentrionales	*Berberis koreana* Palib. 'Red Tears' (MHCRS 200-133)	2.97 ± 0.00	2
Septentrionales	*Berberis lempergiana* Ahrendt (MHCRS 2005-199)	3.06 ± 0.01	2
Septentrionales	*Berberis lycium* Royle (MHCRS 2005-197)	2.89 ± 0.07	2
Septentrionales	*Berberis* ×*media* Groot. ex Boom 'Red Jewel' (MHCRS 2002-162)	3.01 ± 0.10	2
Septentrionales	*Berberis* ×*ottawensis* C.K. Schneid. 'Superba' (MHCRS 2003-063)	3.01 ± 0.01	2
Septentrionales	*Berberis prattii* C.K. Schneid. (MHCRS 2007-207)	3.06 ± 0.04	2
Septentrionales	*Berberis replicata* W.W. Sm. (MHCRS 2007-204)	3.03 ± 0.08	2
Septentrionales	*Berberis sieboldii* Miq. (MHCRS 2005-179)	3.36 ± 0.02	2
Septentrionales	*Berberis soulieana* C.K. Schneid. 'Claret Cascade' (MHCRS 2005-283)	2.93 ± 0.00	2
Septentrionales	*Berberis* sp. (MHCRS China collection, purple flowers)	2.96 ± 0.05	2
Septentrionales	*Berberis temolaica* Ahrendt (MHCRS 2007-199)	2.59 ± 0.04	2
Septentrionales	*Berberis thunbergii* DC. (MHCRS h2002-030-008)[x]	5.86 ± 0.04	4
Septentrionales	*Berberis thunbergii* DC. (MHCRS h2002-030-009)[x]	5.82 ± 0.06	4
Septentrionales	*Berberis thunbergii* DC. (MHCRS h2002-030-021)[x]	6.05 ± 0.04	4
Septentrionales	*Berberis thunbergii* DC. (MHCRS h2002-030-024)[x]	5.82 ± 0.02	4
Septentrionales	*Berberis thunbergii* DC. (MHCRS h2002-030-025)[x]	6.03 ± 0.02	4
Septentrionales	*Berberis thunbergii* DC. 'Aurea' (MHCRS 2002-168)	3.04 ± 0.04	2
Septentrionales	*Berberis thunbergii* DC. 'Bogozam' (MHCRS 2006-168)	3.21 ± 0.05	2
Septentrionales	*Berberis thunbergii* DC. 'Rose Glow' (MHCRS 2004-105)	3.02 ± 0.01	2
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault (MHCRS h2002-029-006)[x]	6.15 ± 0.06	4
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault (MHCRS h2002-029-013)[x]	5.79 ± 0.03	4
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault (MHCRS h2002-029-022)[x]	6.00 ± 0.06	4
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault (MHCRS h2002-029-024)[x]	5.96 ± 0.11	4
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault (MHCRS h2002-029-028)[x]	5.80 ± 0.01	4
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault 'Concorde' (MHCRS 2000-138)	2.93 ± 0.08	2[w]
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault 'Helmond Pillar' (MHCRS 2000-139)	3.09 ± 0.03	2
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault 'Royal Cloak' (PDN 5070)	3.10 ± 0.02	2
Septentrionales	*Berberis thunbergii* var. *atropurpurea* Chenault 'Royal Cloak' (MHCRS 2005-076)	3.10 ± 0.09	2
Septentrionales	*Berberis vernae* C.K. Schneid. (MHCRS 2003-088)	2.91 ± 0.03	2
Septentrionales	*Berberis wilsoniae* Hemsl. (PDN 96CSC134)	2.68 ± 0.07	2
Septentrionales	*Berberis wilsonii* var. *stapfiana* (C.K. Schneid.) C.K. Schneid. (JCRA bed E41)	2.48 ± 0.06	2
Occidentales	*Mahonia aquifolium* (Pursh) Nutt. (PDN 32862 blue leaves)	2.40 ± 0.02	2
Occidentales	*Mahonia aquifolium* (Pursh) Nutt. 'Apollo' (MHCRS 2008-315)	2.28 ± 0.03	2
Occidentales	*Mahonia aquifolium* (Pursh) Nutt. 'Apollo' (MHCRS 2009-006)	2.28 ± 0.02	2
Occidentales	*Mahonia aquifolium* (Pursh) Nutt. 'Compacta' (MHCRS 2007-021)	2.31 ± 0.01	2
Occidentales	*Mahonia aquifolium* (Pursh) Nutt. 'Orange Flame' (MHCRS 2008-163)	2.26 ± 0.02	2
Occidentales	*Mahonia gracilis* Benth. (Fedde) (MHCRS 2005-184)	2.42 ± 0.00	2
Occidentales	*Mahonia gracilis* Benth. (Fedde) (MHCRS 2006-105)	2.36 ± 0.00	2
Occidentales	*Mahonia haematocarpa* (Wooton) Fedde (JCRA 090501)	2.32 ± 0.01	2
Occidentales	*Mahonia hartwegii* (Benth.) Fedde (MHCRS 2005-201)	2.41 ± 0.01	2
Occidentales	*Mahonia ilicina* Schltdl. (MHCRS 2005-195)	2.57 ± 0.03	2
Occidentales	*Mahonia lanceolata* (Benth.) Fedde (MHCRS 2005-207)	2.45 ± 0.06	2
Occidentales	*Mahonia lanceolata* (Benth.) Fedde 'El Cielo' (MHCRS 2005-208)	2.53 ± 0.01	2
Occidentales	*Mahonia nevinii* (A. Gray) Fedde (MHCRS 2006-137)	2.47 ± 0.02	2
Occidentales	*Mahonia pallida* (Benth.) Fedde (MHCRS 2005-252)	1.90 ± 0.04	2
Occidentales	*Mahonia pallida* (Benth.) Fedde (PDN 28953)	2.21 ± 0.10	2
Occidentales	*Mahonia pallida* (Benth.) Fedde (PDN 40338)	2.66 ± 0.01	2
Occidentales	*Mahonia paniculata* Oerst. (MHCRS 2006–144)	2.60 ± 0.01	2
Occidentales	*Mahonia pumila* (Greene) Fedde (MHCRS 2006-138)	2.32 ± 0.02	2
Occidentales	*Mahonia repens* (Lindl.) G. Don (MHCRS 2008-063)	2.31 ± 0.01	2
Occidentales	*Mahonia repens* (Lindl.) G. Don (MHCRS 2009-007)	2.29 ± 0.02	2
Occidentales	*Mahonia* sp. (MHCRS 2009-010)	1.71 ± 0.01	2

(*Continued on next page*)

Table 1. (*Continued*)

Subgenus	Taxa[z]	2C genome size (pg)[y]	Ploidy level (*x*)
Occidentales	*Mahonia* sp. (MHCRS 2009-011)	2.46 ± 0.05	2
Occidentales	*Mahonia trifoliolata* (Moric.) Fedde (JCRA 970613)	2.33 ± 0.01	2
Occidentales	*Mahonia* ×*wagneri* (Jouin) Rehder 'Kings Ransom' (MHCRS 1996-169)	2.31 ± 0.03	2
Orientales	*Mahonia bealei* (Fortune) Carrière (BA 2005-0391a)	2.49 ± 0.03	2
Orientales	*Mahonia bealei* (Fortune) Carrière (JCRA bed E19d)	2.50 ± 0.05	2
Orientales	*Mahonia bodinieri* Gagnep. (OJ Yamaguchi/Ogisu collection)	2.75 ± 0.06	2
Orientales	*Mahonia duclouxiana* Gagnep. (MHCRS 2009-130)	2.52 ± 0.04	2
Orientales	*Mahonia duclouxiana* Gagnep. (PDN 03856)	2.58 ± 0.04	2
Orientales	*Mahonia eurybracteata* Fedde Heronswood form (PDN 31413)	2.49 ± 0.03	2
Orientales	*Mahonia eurybracteata* Fedde (MHCRS 2005-180)	2.43 ± 0.03	2
Orientales	*Mahonia eurybracteata* Fedde (MHCRS 2005-193)	2.46 ± 0.00	2
Orientales	*Mahonia eurybracteata* Fedde 'Nari Hira' (MHCRS 2005-182)	2.54 ± 0.01	2
Orientales	*Mahonia eurybracteata* Fedde 'Soft Caress' (MHCRS 2008-267)	2.43 ± 0.01	2*
Orientales	*Mahonia* cf. *fargesii* (PDN CPC 3.5.01.3A)	2.49 ± 0.04	2
Orientales	*Mahonia* cf. *fargesii* (PDN CPC 5.5.01.1B)	2.53 ± 0.01	2
Orientales	*Mahonia* cf. *fargesii* (OJ 04052)	2.34 ± 0.02	2
Orientales	*Mahonia fortunei* (Lindl.) Fedde (BA 2005-0392a)	2.52 ± 0.01	2
Orientales	*Mahonia fortunei* (Lindl.) Fedde 'Curlyque' (MHCRS 2006-143)	2.57 ± 0.00	2
Orientales	*Mahonia fortunei* (Lindl.) Fedde 'Dan Hinkley' (PDN 39659)	2.62 ± 0.01	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (PDN DJHC-755)	2.66 ± 0.01	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (MHCRS 2005-181)	2.63 ± 0.00	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (MHCRS 2006-142)	2.67 ± 0.05	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (MHCRS 2008-005)	2.57 ± 0.04	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (OJ 94008)	2.67 ± 0.00	2
Orientales	*Mahonia gracilipes* (Oliv.) Fedde (OJ 94058)	2.59 ± 0.07	2
Orientales	*Mahonia japonica* (Thunb.) DC. (OJ 97001)	2.55 ± 0.03	2
Orientales	*Mahonia leptodonta* Gagnep. (PDN 34396)	2.68 ± 0.02	2
Orientales	*Mahonia leptodonta* Gagnep. (OJ Yamaguchi/Ogisu collection)	2.50 ± 0.01	2
Orientales	*Mahonia* ×*lindsayae* Yeo 'Cantab' (MHCRS 2005-189)	2.54 ± 0.00	2
Orientales	*Mahonia longibracteata* Takeda (PDN 26555)	2.74 ± 0.02	2
Orientales	*Mahonia longibracteata* Takeda (OJ Yamaguchi/Ogisu collection)	2.38 ± 0.02	2
Orientales	*Mahonia* ×*media* C.D.Brickell 'Lionel Fortescue' (MHCRS 2005-190)	2.52 ± 0.01	2
Orientales	*Mahonia napaulensis* DC. (MHCRS 2006-139)	2.52 ± 0.01	2
Orientales	*Mahonia napaulensis* DC. (MHCRS 2008-300)	2.61 ± 0.02	2
Orientales	*Mahonia napaulensis* DC. Grayswood Hybrid (MHCRS 2005-203)	2.52 ± 0.01	2
Orientales	*Mahonia napaulensis* DC. 'Maharajah' (MHCRS)	2.53 ± 0.02	2
Orientales	*Mahonia nervosa* (Pursh) Nutt. (MHCRS 2008-062)	7.45 ± 0.04	6
Orientales	*Mahonia* cf. *nitens* (MHCRS 2005-205)	2.59 ± 0.01	2
Orientales	*Mahonia nitens* C.K. Schneid. (JCRA 041803)	2.69 ± 0.01	2
Orientales	*Mahonia nitens* C.K. Schneid. (MHCRS 2005-204)	2.59 ± 0.02	2
Orientales	*Mahonia nitens* C.K. Schneid. (OJ 94010)	2.55 ± 0.00	2
Orientales	*Mahonia nitens* C.K. Schneid. (OJ 94044)	2.57 ± 0.06	2
Orientales	*Mahonia nitens* × *eurybracteata* (PDN#12191)	2.57 ± 0.04	2
Orientales	*Mahonia oiwakensis* Hayata (JCRA MWT-112)	2.46 ± 0.04	2
Orientales	*Mahonia oiwakensis* Hayata (PDN 33792)	2.55 ± 0.01	2
Orientales	*Mahonia* cf. *pallida* (MHCRS 2005-191)	2.56 ± 0.02	2
Orientales	*Mahonia polyodonta* Fedde (MHCRS 2005-200)	2.52 ± 0.02	2
Orientales	*Mahonia polyodonta* Fedde (PDN OJ04CH123)	2.53 ± 0.01	2
Orientales	*Mahonia* ×*savilliana* (PDN 29590)	2.51 ± 0.01	2
Orientales	*Mahonia* sp. (PDN CPC 6.5.01.1)	2.76 ± 0.00	2
Orientales	*Mahonia* sp. (OJ 04052)	2.28 ± 0.09	2

[z]Taxa (source and accession). BA = Bartlett Arboretum, Charlotte, NC; JCRA = JC Raulston Arboretum, Raleigh, NC; MHCRS = Mountain Horticultural Crops Research Station, Mills River, NC; OJ = Mr. Ozzie Johnson, Atlanta, GA; PDN = Plant Delights Nursery, Juniper Level Botanical Garden, Raleigh, NC.
[y]Values are means ± SEM.
[x]Taxa represent open-pollinated seedlings of *B. thunbergii* that were treated with oryzalin to induce polyploidy.
[w]Chromosome number and ploidy level was confirmed by cytology.

Table 2. Mean $1C_X$ genome size among the four subgenera of *Berberis* and *Mahonia*

Genus	Subgenus	$1C_X$ genome size (pg)	Taxa sampled (no.)
Berberis	*Australes*	1.45 ± 0.03 C[z]	4
	Septentrionales	1.47 ± 0.02 C	48
Mahonia	*Occidentales*	1.17 ± 0.02 A	24
	Orientales	1.27 ± 0.01 B	48

[z]Values are means ± SEM. Values followed by different letters, within a column, are significantly different ($P \leq 0.05$).

artificially induced autopolyploid seedlings from both *Berberis thunbergii* var. *atropurpurea* and *Berberis thunbergii* 'Rose Glow' yielded mean 2C genome sizes of 5.93 pg, effectively confirming them as tetraploids. Although the previous report on *Berberis* genome sizes by Bottini et al. (2000) was exclusive to the *Australes*, this subgenus was only modestly sampled in our study, and therefore there was very little overlap between the studies. Our 2C genome size of 2.92 pg for *B. darwinii* Hook. was congruent with the range of 2.88 to 3.22 pg reported by Bottini et al. (2000) in wild populations of the species. Additionally, *Berberis trigona* (syn. *Berberis linearifolia* Phil.) cultivar Orange King had a genome size slightly less (3.02 pg) than the reported range of 3.24 to 3.57 pg (Bottini et al., 2000).

Genome sizes of diploid *Mahonia* subgenus *Occidentales* ranged from 1.71 pg (*M.* sp., Mexican origin; MHCRS 2009-010) to 2.66 pg [*M. pallida* (Benth.) Fedde PDN 40338] with a mean of 2.35 pg. Subgenus *Orientales* had a higher mean of 2.55 pg for diploids, ranging from 2.28 pg (*M.* sp.; OJ

04052) to 2.76 pg (*M.* sp.; PDN CPC 6.5.01.1). We have not found other published reports of 2C genome sizes for *Mahonia*. No tetraploid *Mahonia* were identified among the taxa sampled for this project. However, prior reports (Taylor and Taylor, 1997) have documented tetraploid clones of *M. aquifolium* and *M. nervosa*, indicating that these species may include a polyploid series. The only polyploid *Mahonia* found in this survey was *M. nervosa* (MHCRS 2008-062), which was estimated to be hexaploid ($2n = 6x = 84$) with a genome size of 7.45 pg. Interestingly, *M. nervosa* is the only new world species placed within subgenus *Orientales* based on both morphology (Ahrendt, 1961) and DNA phylogeny (Kim et al., 2004).

Overall, this study demonstrates that PI flow cytometry is an extremely useful tool for studying genome sizes and polyploidy in both *Berberis* and *Mahonia.* Substantial differences in base $1C_X$ genome size between *Berberis* and *Mahonia* demonstrate considerable variation in genome evolution between these groups. However, genome sizes were strongly conserved within *Berberis* and *Mahonia* subgenera, which allowed for rapid and consistent calibration with ploidy levels. Although polyploidy appears to be uncommon among species of both *Berberis* and *Mahonia*, one accession of *M. nervosa* was found to be hexaploid. Furthermore, artificially induced plants of *B. thunbergii* were confirmed to be tetraploids. Data from this study provide insight into evolutionary history, taxonomic treatment, and information on ploidy levels of specific taxa that will aid in the breeding and development of new hybrids and serve as a valuable database for plant breeders, systematists, and evolutionary biologists.

Literature Cited

Adams, K.L. and J.F. Wendel. 2005. Novel patterns of gene expression in polyploidy plants. Trends Genet. 21:539–543.

Ahrendt, L.W.A. 1961. *Berberis* and *Mahonia*, a taxonomic revision. J. Linnean Soc. London. 57:1–410.

Alvarez, M.A., N.F. Eraso, S.I. Pitta-Alvarez, and P.L. Marconi. 2009. Two-stage culture for producing berberine by cell suspension and shoot cultures of *Berberis buxifolia* Lam. Biotechnol. Lett. 31:457–463.

Bottini, M.C.J., E.J. Greizerstein, M.B. Aulicino, and L. Poggio. 2000. Relationships among genome size, environmental conditions and geographical distribution in natural populations of NW Patagonian species of *Berberis* L. Ann. Bot. (Lond.) 86:565–573.

Chen, Z.J. and Z. Ni. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays 28:240–252.

Dermen, H. 1931. A study of chromosome number in two genera of Berberidaceae: *Mahonia* and *Berberis*. J. Arnold Arbor. 12:281–287.

Dirr, M. 2009. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 6th Ed. Stipes, Champaign, IL.

Doležel, J. and J. Bartos. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Ann. Bot. (Lond.) 95:99–110.

Doležel, J., J. Greilhuber, S. Lucretti, A. Meister, M.A. Lysa'k, L. Nardi, and R. Obermayer. 1998. Plant genome size estimation by flow cytometry: Inter-laboratory comparison. Ann. Bot. (Lond.) 82:17–26.

Greilhuber, J. 1998. Intraspecific variation in genome size: A critical reassessment. Ann. Bot. 82:27–35.

Hinkley, D.J. 2009. The explorer's garden shrubs and vines from the four corners of the world. Timber, Portland, OR.

Huxley, A., M. Griffiths, and M. Levy. 1992. The New Royal Horticultural Society dictionary of gardening. Macmillian Press Ltd, London, UK.

Kao, K.N. 1975. A chromosomal staining method for cultured cells, p. 63–64. In: Gambourg, O.L. and L.R. Wetter (eds.). Plant tissue culture methods. NRC Canada, Saskatoon, Canada.

Kim, Y.-D. and R.K. Jansen. 1998. Chloroplast DNA restriction site variation and phylogeny of the Berberidaceae. Amer. J. Bot. 85:1766–1778.

Kim, Y.-D., S.-H. Kim, and L.R. Landrum. 2004. Taxonomic and phytogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). J. Plant Res. 117:175–182.

Laferriere, J.E. 1997. Transfer of specifc and infraspecific taxa from *Mahonia* to *Berberis* (Berberidaceae). Bot. Zhurn. 82:95–99.

Marroquin, J.S. 1993. Berberidaceae. Flora de Veracruz. Fasciculo 75:1–16.

Phillips, C.E. and P.N. Barber. 1981. Ornamental shrubs. Van Nostrand Reinhold, New York, NY.

Schneider, C.K. 1905. Die Gattung *Berberis* (Euberberis) Vorarbeiten fur eine monographie. Bull Herb Boissier. Ser. 2:33–48.

Soltis, D.E. and J.G. Burleigh. 2009. Surviving the K-T mass extinction: New perspectives of polyploidization in angiosperms. Proc. Natl. Acad. Sci. USA 106:5455–5456.

Soltis, D.E., P.S. Soltis, and J.A. Tate. 2004. Advances in the study of polyploidy since plant speciation. New Phytol. 161:173–191.

Taylor, R.L. and S. Taylor. 1997. Chromosome numbers of vascular plants of British Columbia. Syesis 10:125–138.

Vinogradov, A.E. 1994. Measurement by flow cytometry of genomic AT/CG ratio and genome size. Cytometry 16:34–40.

Whittemore, A.T. 1997. *Berberis*, p. 276–286. In: Morin, N.R. (ed.). Flora of North America. Vol. 3. Oxford University Press, New York, NY.

Wyman, D. 1958. Two new *Mahoberberis* hybrids. Arnoldia 18:9–12.

Xu, B.S., R.F. Weng, and M.Z. Zhang. 1992. Chromosome numbers of Shanghai plants. I. Investigatio et Studium. Naturae 12:48–65.

Yan-Jun, Z., A.-P. Meng, J.-Q. Li, H.-S. Dang, and X.-W. Li. 2006. Observation on meiotic behavior in three Mahonia species, with special reference to the intergeneric relationship of *Mahonia* and *Berberis*. Caryologia 59:305–311.

Zonneveld, B.J.M. 2009. The systematic value of nuclear genome size for 'all' species of *Tulipa* L. (*Liliaceae*). Plant Syst. Evol. 281:217–245.

Zonneveld, B.J.M. and G.D. Duncan. 2010. Genome sizes of *Eucomis* L'Hér.(Hyacinthaceae) and a description of the new species *Eucomis grimshawii* G.D. Duncan & Zonneveld. Plant Syst. Evol. 284:99–109.

Zonneveld, B.J.M. and F. Van Iren. 2001. Genome size and pollen viability as taxonomic criteria: Application to the genus *Hosta*. Plant Biol. 3:176–185.