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

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Significance to Industry: The sister genera, *Berberis* L. and *Mahonia* Nutt., represent the two largest groups within the family *Berberidaceae*, consisting of approximately 500 and 200 species respectively (6). This highly ornamental group of shrubs and small trees are valued for their evergreen or colored leaves, brilliant flowers, and often persistent fruit. Considering the tremendous diversity and the broad crossability found in these genera, the potential for breeding improved hybrids is considerable. 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 of progeny, morphology, and gene expression. Additionally, variation in genomic size can be used as an indicator of evolutionary history and taxonomic relationships (5, 10). This research provides an extensive survey of genome size and ploidy level of species, hybrids, and cultivars of *Berberis* and *Mahonia* that will serve as a valuable database for plant breeders, systematists, and evolutionary biologists.

Nature of Work: *Berberis* and *Mahonia* are each distributed into two well supported subgroups (6). Within *Berberis*, the Australes include all the species from Central and South America, the remaining species are placed in the Septentrionales, and occur entirely in the northern hemisphere except for two in East Africa and one in Java and Sumatra (1). Conversely, *Mahonia* are grouped longitudinally, with those of the eastern hemisphere in subgroup Orientales and all those of the western hemisphere (with the notable exception of *M. nervosa* (6)) in subgroup Occidentales. Although basic information on chromosome numbers, genome sizes and ploidy levels has been reported for some *Berberis* and *Mahonia*, sampling has been limited and little is known about ploidy levels of specific clones or cultivars. For *Mahonia,* most species have been reported to be diploid with $2n=2x=28$ (3,7), and in rare cases tetraploid with $2n=4x=56$ (8). Reports on *Berberis* species also favored $2n = 2x = 28$ diploids, though tetraploid species with $2n=4x=56$, were identified among both subfamilies (2,3). Flow cytometry is an efficient method for rapid determination of genome size in plants. For closely related taxa, where genome sizes are relatively conserved, flow cytometry can also be used for determination of ploidy level (4). The objectives of this study were to

determine genome sizes and ploidy levels of a diverse collection of species, hybrids, and cultivars of *Mahonia* and *Berberis* by using a combination of flow cytometry and traditional cytology.

A highly diverse collection of 52 *Berberis* taxa and 72 *Mahonia* taxa were obtained from various gardens and private collectors. Sampled taxa represented species from each of the four subgroups, many common cultivars, and a few purported artificial autopolyploids. Leaf tissue for each sample, as well as an internal standard (*Pisum sativum* 'Ctirad' 2C DNA = 8.76pg) was finely diced with a razor blade in a Petri dish containing 500 µL of nuclei extraction buffer. A solution containing 2 mL staining buffer, 12 µL propidium iodide (PI) stain, and 6µL RNAse was then added, and the samples were moved to a refrigerator at 4°C for one hour. A flow cytometer (Partec PA-II , Partec, Münster, Germany) was used to analyze the stained nuclei, with a minimum of 5,000 counts per sample, and two samples conducted for each taxon. Holoploid, 2C genome size was 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 = 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. Traditional cytology was conducted to verify previous work, and calibrate genome size with ploidy level. Actively growing root tips were immersed in a solution of (3: 1) 95% Ethanol : Propionic acid for 24 hours, then fixed in 70% Ethanol at 4°C for storage. Root tissue was removed from cold storage and hydrolyzed in a solution of (3:1) 95% Ethanol : 12N HCl for 5-10 seconds. Root tips were then placed into a drop of carbol fuschin stain on a glass microscope slide, and gently squashed with a coverslip. Chromosomes were counted using oil immersion at 1,500×.

Results and Discussion: The base, $1C_X$, genome size for *Mahonia* varied for each subgenus with a mean of 1.17 ρg for Occidentales and 1.27 ρg for Orientales (Table 1). There was no difference in base, $1C_X$ genome size, between the two subgenera of *Berberis*, but plants in the genus *Berberis* had a significantly higher mean (1.46 ρg) than either subgenus of *Mahonia*. There has been extensive debate among horticulturists and botanists as to whether *Mahonia* and *Berberis* are distinct genera, or should be collectively unified within *Berberis* (9). This data reveals a significant increase in genome size during the evolution of *Berberis*, apart from *Mahonia,* and provides support for maintaining these as separate genera.

Cytology performed on *Mahonia eurybracteata* 'Soft Caress' (2C genome size = 2.43 ρg) confirmed it to be $2n = 2x = 28$, allowing calibration of ploidy with genome size. Polyploid species were very infrequent in the taxa sampled for this project (Table 2). Within *Mahonia*, there were no tetraploid species found, nor were any accessions of *M*.

aquifolium and *M*. *nervosa* found to be tetraploid, as previously reported (8). The only polyploid *Mahonia* taxa found were *M*. *nervosa* MHCRS 2008-062 and *M*. *piperiana* x *nervosa* MHCRS 2006-136 which were estimated to be hexaploid (2*n*=6*x*=84), with respective 2C genome sizes of 7.45 ρg and 7.67 ρg. No natural polyploids were identified among the *Berberis* sampled in this study. However, artificially induced autopolyploids of *Berberis thunbergii* were confirmed with a mean 2C genome size of 5.9 ρg, and estimated to be tetraploid (2n=2x=56).

Overall, this study demonstrates that flow cytometry is an extremely useful tool for studying genome size and polyploidy in both *Berberis* and *Mahonia.* Data from this study provides valuable insights into evolutionary history, taxonomic treatment, and information on ploidy levels of specific taxa that will aid in the breeding and development of new hybrids.

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Table 1. Mean $1C_X$ Genome Size among the 4 subgenera of *Berberis* and *Mahonia*

Genus	Group	$1C_X$ Genome Size (ρg)	Taxa Sampled
Mahonia	Occidentales	1.17 ± 0.02 A[1]	24
	Orientales	1.27 ± 0.01 B	48
Berberis	Australes	1.45 ± 0.03 C	4
	Septentrionales	1.47 ± 0.02 C	48

1Values are means ± SEM. Values followed by different letters, within a column, are significantly different, P≤0.05.

Table 2. Mean 2C genome size and ploidy among key species of *Berberis* and *Mahonia*

Taxa	2C Genome Size (ρg)	Ploidy Level
Berberis thunbergii	2.93 ± 0.05	2x
Berberis thunbergii (oryzalin treated)	5.92 ± 0.07	4x
Mahonia eurybracteata 'Soft Caress'	2.43 ± 0.01	2x[1]
Mahonia nervosa	7.45 ± 0.04	6x
Mahonia piperiana x nervosa	7.67 ± 0.05	6x

[1]Chromosome number and ploidy level was confirmed via cytology.