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Genome Size, Ploidy, and Base Composition of Wild and Cultivated *Acer*

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Abstract. ***Acer*** **is a large and important genus of woody plants most commonly encountered as small to large trees in urban landscapes. Considerable investigation has been devoted to addressing the taxonomy of maples, but little is known about genome sizes across the genus. Relatively more work has been conducted to determine chromosome numbers and ploidy of more species, but much could be gained by expanding knowledge of genome sizes in combination with traditional cytology. Furthermore, base pair (bp) composition may have implications for a species' adaptability and also impacts nucleic acid stability at high temperatures. Our objectives were to determine the genome size of 195 accessions of maples, assign ploidy to each using inference as well as cytology, and determine base composition of a subset of 48 accessions. Most species had small genome sizes (1.4–3.5 pg) with the exception of section** ***Rubra*****, which contains many polyploids. Holoploid genome sizes ranged from 1.39 to 6.10 pg, with the latter being interpreted as 9*x*. The mean monoploid genome sizes (1C*x*) ranged from 0.43 pg in** ***A. carpinifolium*** **(section** ***Indivisa*****) to 1.66 pg in** ***A. caudatifolium*** **(section** ***Macrantha*****); mean monoploid genome sizes were significantly different among sections. Forty-four of the 48 accessions measured using both fluorochromes had greater estimates with 4′,6-diamidino-2-phenylindole (DAPI) than propidium iodide (PI). The proportion of the genome composed of guanosine and cytosine (GC%) among the taxa evaluated in this study ranged from just 38.61% to 43.96% and did not appear to be related to ecological adaptability or urban tolerance among these taxa.**

Acer is a diverse genus including shrubs, medium-size trees, and large shade trees, with species creating a continuum of these forms. Maples are highly diverse in habitat, habit, bark, leaf shape and size, vegetative buds, and inflorescence structure. Opposite leaves and characteristic schizocarps of joined samaras are the two unifying morphological characters. Maples are grown primarily for fall color, bark, and form. The importance of maples produced in cultivation as street trees, specimen trees, or shade trees is reflected in the 2014 U.S. Department of Agriculture Census of Horticultural Specialties (U.S. Department of Agriculture, 2016), which reported the overall sales of maples as $173.4 million nationwide. Oregon reported sales of maple in 2014 were more than $63 million, which accounted for greater than half the value of deciduous shade trees statewide.

There has been considerable taxonomic research in attempting to determine the classification of species within the genus and how these species relate to one another on the evolutionary timescale (Ackerly and Donoghue, 1998; Grimm et al., 2006; Li, 2011; Li et al., 2006; Pfosser et al., 2002; Renner et al., 2008; Suh et al., 2000; Tian et al., 2002; Zhang et al., 2010). There has also been much debate relative to these taxonomic relationships and phylogenetic order of the genus. This difference of opinion among taxonomists is illustrated by the disparity of species number, which varies from 129 to 200, depending on taxonomic treatment (Li, 2011; Suh et al., 2000; Zhang et al., 2010). Although many teams are working on clarifying taxonomy, relatively little has been reported on genome size and ploidy level within the genus. Three original papers (Loureiro et al., 2007; Olszewska and Osiecka, 1984; Siljak-Yakovlev et al., 2010) reported genome sizes of 11 species of maples as part of larger studies. Depending on taxonomic treatment in these reports, between 6% and 9% of species have been reported. Clearly, for such an ecologically and economically important species, this is a significant gap in our scientific knowledge.

Genome size data have been shown to reflect taxonomic relationships in Cornaceae (Shearer and Ranney, 2013) while also being reflective of genome evolution (Johnston et al., 2005; Yotoko et al., 2011). Genome size data can be used to determine ploidy in a genus when calibrated properly using chromosome counts, as demonstrated for the Ericaceae, Cornaceae, Magnoliaceae, Berberidaceae, and Lamiaceae (Contreras and Ruter, 2011; Jones et al., 2007; Parris et al., 2010; Rounsaville and Ranney, 2010; Shearer and Ranney, 2013). Genome size and ploidy data are useful tools in a breeding program because they can provide greater insight into a genus and thus aid in developing breeding strategies.

The base chromosome number of *Acer* is $x = 13$. Cytological reports for maples include a range of ploidy levels (Darlington and Wylie, 1956). The greatest occurrence of natural polyploidy has been reported in section *Rubra* including hexaploid ($2n = 6x = 78$) *A. pycnanthum*, hexaploid and octoploid ($2n = 8x = 104$) *A. rubrum*, and tetraploid ($2n = 4x = 52$), hexaploid, and aneuploid ($2n = 4x + 1 = 53$) *A. saccharinum* (Duffield, 1943; Foster, 1933; Santamour, 1965, 1971). Tetraploids have

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also been reported in section *Acer* (*A. heldreichii*, *A. pseudoplatanus*, *A. saccharum*, and *A. velutinum*), section *Indivisa* (*A. carpinifolium*), and section *Platanoidea* (*A. campestre*) (Foster, 1933; Santamour, 1965, 1971, 1988; Taylor, 1920). Most other maples investigated have been reported as diploid ($2n = 2x = 26$) (Table 1).

Polyploidy, or whole-genome duplication, can be used to facilitate wide hybrid crosses (Sanford, 1983) or to develop sterile ornamental cultivars with odd-ploidy levels such as triploids (Olsen et al., 2006; Trueblood et al., 2010). Variation in ploidy can also provide a barrier to successful hybridization in some cases (Sanford, 1983), and thus knowledge of ploidy in a group of taxa can be greatly beneficial in a developing breeding program.

Measuring genome size in plants can be accomplished quickly and effectively using flow cytometry, as demonstrated

Table 1. Previously reported chromosome numbers and holoploid (2C) genome sizes (measured in picograms) for *Acer* species evaluated in this study.

Taxon	Synonym	Previous findings	References
A. argutum		$2n = 2x = 26$	Takizawa (1952)
A. caesium		$2n = 2x = 26$	Santamour (1988)
A. campestre		$2n = 2x = 26$, 2C = 1.38 $2n = 4x = 52$, 2C = 2.70	Foster (1933), Siljak-Yakovlev et al. (2010)
A. carpinifolium		$2n = 4x = 52$, 2C = 0.75	Olszewska and Osiecka (1984), Taylor (1920)
A. circinatum		$2n = 2x = 26$	Foster (1933)
A. cissifolium		$2n = 2x = 26$	Takizawa (1952)
A. crataegifolium		$2n = 2x = 26$	Takizawa (1952)
A. diabolicum		$2n = 2x = 26$	Takizawa (1952)
A. griseum		$2n = 2x = 26$	Foster (1933)
A. heldreichii		$2n = 2x = 26$, $2n = 4x = 52$, 2C = 2.57	Santamour (1988), Siljak-Yakovlev et al. (2010)
A. heldreichii ssp. *trautvetteri*	*A. trautvetteri*	$2n = 2x = 26$	Santamour (1988)
A. hyrcanum		$2n = 2x = 26$	Santamour (1988)
A. japonicum		$2n = 2x = 26$	Takizawa (1952)
A. miyabei		$2n = 2x = 26$	Foster (1933)
A. monspessulanum		$2n = 2x = 26$, 2C = 1.46	Siljak-Yakovlev et al. (2010)
A. negundo		$2n = 2x = 26$, 2C = 1.08	Foster (1933), Loureiro et al. (2007), Takizawa (1952)
A. nikoense		$2n = 2x = 26$	Foster (1933)
A. opalus		$2n = 2x = 26$	Santamour (1988)
A. opalus ssp. *obtusatum*	*A. obtusatum*	$2n = 2x = 26$, 2C = 1.56	Siljak-Yakovlev et al. (2010)
A. palmatum		$2n = 2x = 26$	Foster (1933)
A. pictum	*A. mono* ssp. *eupictum*	$2n = 2x = 26$	Takizawa (1952)
A. platanoides		$2n = 2x = 26$, 2C = 1.42 $2n = 3x = 39$	Foster (1933), Santamour (1965), Siljak-Yakovlev et al. (2010), Taylor (1920)
A. pseudoplatanus		$2n = 4x = 52$, 2C = 2.7	Olszewska and Osiecka (1984), Santamour (1988), Siljak-Yakovlev et al. (2010), Taylor (1920)
A. pseudosieboldianum		$2n = 2x = 26$	Foster (1933)
A. rubrum		$2n = 6x = 78$ $2n = 8x = 104$ $n = 36$ $n = \pm 50$ $n = 52$ $n = 68–75$ $n = 72$	Duffield (1943), Foster (1933), Santamour (1965), Taylor (1920)
A. rufinerve		$2n = 2x = 26$	Foster (1933)
A. saccharum		$2n = 2x = 26$ $2n = 4x = 52$ $2n = 4x + 1 = 53$ $2n = 6x = 78$	Foster (1933), Santamour (1971), Takizawa (1952), van Gelderen et al. (1994)
A. saccharinum		$2n = 4x = 52$	Foster (1933), Santamour (1965), Taylor (1920)
A. tataricum		$2n = 2x = 26$, 2C = 1.19	Siljak-Yakovlev et al. (2010)
A. tataricum ssp. *ginnala*	*A. ginnala*	$2n = 2x = 26$	Takizawa (1952), Santamour (1971)
A. tschonoskii		$2n = 2x = 26$	Foster (1933)
A. velutinum		$2n = 4x = 52$	Santamour (1988)

by a number of genome size surveys of angiosperms (Jones et al., 2007; Lattier et al., 2014; Parris et al., 2010; Rounsaville and Ranney, 2010; Shearer and Ranney, 2013). Although these genome size and ploidy surveys are useful to ornamental plant breeders, they also answer the call put forth by Galbraith et al. (2011), who proposed a coordinated global census of genome size of angiosperms. According to them, genome size and ploidy data can aid in developing priorities for whole-genome sequencing. The Plant DNA C-values Database, an online repository for genome size data, has become an invaluable tool for accessing these data to allow breeders and other researchers easy access (Bennett and Leitch, 2012).

There are a number of fluorochromes, or stains, that can be used for flow cytometry. For genome sizing, the most commonly used stains are DAPI and PI. These two fluorochromes have contrasting binding characters. DAPI binds to A-T-rich regions of the nuclear genome whereas PI is an intercalating agent that binds indiscriminately to all nucleic acids, including RNA. These contrasting binding characters can be used to infer the base composition of a genome (Meister and Barow, 2007; Parris et al., 2010; Rothleutner et al., 2016). Base composition can be expressed as either GC%, for proportion of genome composed of guanine and cytosine, or as AT%, for proportion of genome composed of adenine and thymine. Although our work regarding genome size and ploidy is applied in nature and geared to support our breeding program, there are various hypotheses regarding the impact of varying GC% in plant genomes. Šmarda et al. (2014) reported genome sizes of 239 species of monocots and found that increased GC content was associated with species adapted to cold and/or dry environments. A major component of many woody plant breeding programs today is the development of plants that can tolerate such harsh conditions in our changing climate, including extreme cold, heat, and drought. Knowledge of base composition in woody plants may provide additional insight into the relationship between GC% and climatic adaptability. However, caution should be used to draw correlations between observations in monocots to trees such as maples. Furthermore, it should be noted that there are inherent differences between monocots and dicots beyond obvious morphological differences. Although their study was limited in scope, Li and Du (2014) reported that dicots (34%) have a lower GC% compared with monocots (46%).

The objectives of the current study were to determine relative genome sizes and ploidy levels of a diverse and wide-ranging selection of taxa within *Acer*, providing a foundation to facilitate future breeding efforts; to determine the base composition of a subset of maples in this study; and to contribute to the growing body of knowledge of genome size in angiosperms.

Materials and Methods

Plant material. Relative genome size was determined for 195 accessions representing 88 species and 18 taxonomic sections (Table 2). Plant material from Heritage Seedlings (Salem, OR), Hoyt Arboretum (Portland, OR), J. Frank Schmidt (Boring, OR), and Whitman Farms (Salem, OR) was collected onsite. Other plant material was collected and shipped to Oregon State University (OSU) by staff at the following institutions: Arnold Arboretum (Harvard University, Boston, MA), Cornell Plantations (Cornell University, Ithaca, NY), Morris Arboretum (University of Pennsylvania, Philadelphia, PA), The Morton Arboretum (Lisle, IL), Quarry Hill Botanical Garden (Glen Ellen, CA), and the U.S National Arboretum (Washington, DC). Terminal stem cuttings from each accession were collected and placed in plastic bags. Cuttings sent from other institutions were shipped overnight with ice packs. All material was kept refrigerated at 4 °C.

Collection information associated with material was provided by institutions directly or through public databases; in some cases, collection information such as Chinese province information was inferred through publicly available literature reporting and summarizing plant collection expeditions (Aiello and Dosmann, 2010; The Arnold Arboretum of Harvard University, 2016). Taxonomic relationships were adapted from van Gelderen et al. (1994), eFlora (2016), and Li (2016). Documentation of analyzed material includes deposition of herbarium vouchers at the OSU Herbarium (Corvallis, OR) or field planting of live plants at our research farm (Corvallis, OR) (Table 2). We used identification provided by the source. To our knowledge, the only area of confusion was related to red maple (*A. rubrum*) and freeman maple (*A. ×freemanii*), which often are used interchangeably depending on the nursery.

Flow cytometry. The relative 2C genome sizes were determined using flow cytometry. *Pisum sativum* 'Ctirad' (2C = 8.76 pg) was used as an internal standard based on its common use as a reference standard (Bai et al., 2012; Greilhuber et al., 2007). Three samples were analyzed for each accession. For each sample, 1 to 2 cm^2 or 20 mg fresh expanding leaf and vegetative bud tissue were finely cochopped with *P. sativum* 'Ctirad' in a polystyrene petri dish with 400 µL nuclei extraction buffer (Cystain® Ultraviolet Precise P Nuclei Extraction Buffer; Partec, Görlitz, Germany) using a sharp double-sided razor blade. The nuclei suspension was then filtered into sample tubes through 30-µm gauze filters (Celltrics®, Partec) and stained with 1.6 mL DAPI staining buffer (Cystain Ultraviolet Precise P Staining Buffer, Partec). For base composition analysis, a subset of samples was measured following the same methods for chopping and filtering using 500 µL nuclei extraction buffer and 1.5 mL PI solution. PI solution contained PI, RNase, and staining buffer; and was prepared according to manufacturer instructions (Cystain® PI Absolute P, Partec). After staining, samples were incubated on ice in the dark for at least 20 min to allow RNase digestion of RNA. Relative genome size was determined using a flow cytometer (Cyflow® Ploidy Analyser, Partec), with excitation appropriate for each fluorochrome—488 nm for DAPI and 532 nm for PI. Relative genome size (2C) DNA contents was calculated as

$$2C = \text{DNA content of standard} \times \frac{\text{Mean fluorescence value of sample}}{\text{Mean fluorescence value of the standard}}.$$

The relationship between ploidy level and genome size was determined initially using cytogenetically documented data (Table 1). Mean 1C*x* genome size was calculated as (Mean 2C genome size/Inferred ploidy level) for each accession. For taxa with biological replicates, the mean 1C*x* genome size reported in Table 3 reflects the monoploid genome size across all accessions of that taxa that were measured; whereas, in cases when biological replicates were not available, mean 1C*x* genome size was calculated using three samples measured from a single accession.

Table 2. Mean relative holoploid (2C) genome sizes, putative ploidy, and provenance and/or native range of *Acer* species, cultivars, and hybrids using flow cytometry analysis of nuclei stained with 4′,6-diamidino-2-phenylindole with *Pisum sativum* 'Ctirad' as the internal standard (2C = 8.76 pg).

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
Section *Acer*					
A. caesium	QHBG1995-051	OSC-V-254643	2.06 ± 0.02	2	W; Tibet*
A. caesium	MRS1994-005	OSC-V-254672	2.06 ± 0.04	2	W; western Himalayas*
A. grandidentatum	MRT276-742		1.67 ± 0.01	2	W; Guadalupe Mountains, Eddy County, NM
A. heldreichii var. *macropterum*	ARN200-85A	OSC-V-254631	3.47 ± 0.04	4	U
A. heldreichii ssp. *trautvetteri*	MRS2004-172	OSC-V-254661	3.55 ± 0.06	4	W; Caucasus, northern Turkey*
A. hyrcanum	MRT67-2001*1		1.88 ± 0.03	2	W; Republic of Georgia*
A. hyrcanum	ARN31-73A	OSC-V-254627	2.00 ± 0.02	2	U
A. monspessulanum ssp. *ibericum*	MRS2008-189	OSC-V-254662	2.02 ± 0.03	2	W; western Asia*
A. opalus	COR03-233		2.09 ± 0.04	2	G
A. opalus obtusatum	MRT326-82*1		1.99 ± 0.03	2	G
A. pseudoplatanus	USNA2836		3.29 ± 0.08	4	G
A. pseudoplatanus	COR83-361		3.56 ± 0.05	4	G
A. saccharum	OSU14-0147		1.84 ± 0.09	2[w]	G
A. saccharum f. *conicum*	MRT354-51*1		1.70 ± 0.03	2	G
A. saccharum ssp. *floridanum*	USNA78004	OSC-V-254610	1.70 ± 0.02	2	W; Alabama*
A. saccharum ssp. *skutchii*	USNA79379	OSC-V-254612	1.71 ± 0.03	2	Z
A. saccharum ssp. *skutchii*	MRS2014-242	OSC-V-254650	1.87 ± 0.01	2	Z; Mexico, Guatemala
A. sempervirens	HOYT1993-116		1.91 ± 0.03	2	U
A. velutinum	USNA78548	OSC-V-254360	3.34 ± 0.08	4	W; Azerbaijan*
A. velutinum	ARN1329-77B	OSC-V-254637	3.75 ± 0.05	4	W; Armenia*
A. ×*coriaceum* (*A. monspessulanum* × *A. opalus*)	HOYT1989-047		1.86 ± 0.03	2	G
Section *Arguta*					
A. acuminatum	QHBG1993-076	OSC-V-254652	1.97 ± 0.003	2	W; Himachal Pradesh, India*
A. acuminatum	QHBG1993-039	OSC-V-254359	1.93 ± 0.04	2	W; Himachal Pradesh, India*
A. acuminatum	QHBG1993-139	OSC-V-254640	1.93 ± 0.02	2	W; Himachal Pradesh, India*
A. acuminatum	MRS1994-009	OSC-V-254660	1.92 ± 0.04	2	W; Himalayas
A. argutum	ARN640-77B	OSC-V-254633	1.91 ± 0.06	2	W
A. argutum	OSU14-0194		1.78 ± 0.01	2[w]	G
A. barbinerve	MRT258-2002*1		1.96 ± 0.02	2	W; Shaanxi Province, China*
A. barbinerve	USNA68777		1.77 ± 0.00	2	W; Jilin Province, China*
A. stachyophyllum ssp. *betulifolium*	MRT854-2005*2		1.94 ± 0.01	2	W; Gansu Province, China*
Section *ginnala*					
A. tataricum	OSU14-0202		1.65 ± 0.02	2[w]	U
A. tataricum ssp. *aidzuense*	ARN1852-77A	OSC-V-254635	1.65 ± 0.02	2	U
A. tataricum ssp. *ginnala*	OSU12-0011-01	75.15	3.04 ± 0.06	4	G; product of chromosome-doubling experiment
A. tataricum ssp. *ginnala*	OSU12-0011-03	75.10	1.66 ± 0.05	2	G
A. tataricum ssp. *ginnala*	OSU12-0011-04	74.19	3.12 ± 0.03	4	G; product of chromosome-doubling experiment
A. tataricum ssp. *ginnala*	OSU12-0011-05	75.16	1.58 ± 0.02	2	G
A. tataricum ssp. *ginnala*	OSU12-0011-07	75.09	3.16 ± 0.00	4	G; product of chromosome-doubling experiment

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Table 2. Continued.

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
A. tataricum ssp. *ginnala*	OSU12-0011-08	75.11	3.15 ± 0.04	4	G; product of chromosome-doubling experiment
A. tataricum ssp. *ginnala*	OSU12-0011-10	75.18	3.15 ± 0.06	4	G; product of chromosome-doubling experiment
A. tataricum ssp. *ginnala*	OSU12-0011-13		3.07 ± 0.03	4	G; product of chromosome-doubling experiment
Section *Glabra*					
A. glabrum	OSU-campus		1.58 ± 0.03	2	U
Section *Indivisa*					
A. carpinifolium	HOYT1993-118		1.66 ± 0.05	4	U
A. carpinifolium	OSU14-0051	96.23	1.81 ± 0.02	4	G
Section *Lithocarpa*					
A. diabolicum	MRT1276-55*1		2.38 ± 0.01	2	U
A. diabolicum	USNA562	OSC-V-254609	2.37 ± 0.01	2	G
A. sterculiaceum ssp. *franchetii*	MRT332-2000*3		2.27 ± 0.02	2	W; Gansu Province, China*
A. yangbiense	ARN637-2007		1.90 ± 0.04	2	W
Section *Macrantha*					
A. caudatifolium	QHBG2002-156		3.32 ± 0.07	2	W; Taiwan*
A. crataegifolium	MRT220-73*2		3.04 ± 0.01	2	G
A. davidii	OSU14-0162	93.08	2.54 ± 0.02	2[w]	
A. forrestii	QHBG2003-394	OSC-V-254640	2.78 ± 0.08	2	W; Sichuan Province, China*
A. laxiflorum	QHBG2001-292	OSC-V-254647	1.63 ± 0.03	2	W; Sichuan Province, China*
A. morrisonense	QHBG2004-176	OSC-V-254654	2.87 ± 0.02	2	W; Taiwan*
A. morrisonense	QHBG2004-185	OSC-V-254651	2.95 ± 0.09	2	W; Taiwan*
A. pectinatum	OSU14-0197		2.34 ± 0.03	2	W; Gansu Province, China*
A. pensylvanicum	USNA74456	OSC-V-254608	2.47 ± 0.02	2	W
A. pensylvanicum	COR96-175		2.42 ± 0.05	2	W
A. rubescens	HOYT2003-169		2.92 ± 0.10	2	W; Taiwan*
A. rufinerve	HOYT1963-4000		2.69 ± 0.07	2	U
A. rufinerve 'Albo-limbatum'	OSU14-0200		2.91 ± 0.04	2	G
A. tegmentosum	USNA64194	OSC-V-254604	2.45 ± 0.03	2	W; Heilongjiang Province, China*
A. tegmentosum	MRS1993-342	OSC-V-254659	2.61 ± 0.02	2	W; Heilongjiang Province, China*
A. tschonoskii	MRT329-2000*3		2.16 ± 0.05	2	W; Jilin Province, China*
A. davidii × *A. davidii* ssp. *grosseri*	MRT244-2014		2.59 ± 0.06	2	G
A. davidii × *A. tegmentosum*	USNA65062	OSC-V-254944	2.76 ± 0.05	2	G
Section *Macrophylla*					
A. macrophyllum	OSU-campus		1.63 ± 0.01	2	U
A. macrophyllum	OSU14-0059-01		1.68 ± 0.08	2[w]	G
Section *Negundo*					
A. cissifolium	HOYT1989-056		1.53 ± 0.02	2	U
A. henryi	USNA48987	OSC-V-254362	1.60 ± 0.02	2	W; Hubei Province, China*
A. henryi	USNA72459	OSC-V-254605	1.58 ± 0.01	2	W; Shanxi Province, China*
A. negundo	OSU14-0089-01	92.20 & 94.24	1.53 ± 0.01	2	G; two plants are sister seedlings from accession listed
A. negundo var. *interius*	MRT227-86*3		1.39 ± 0.01	2	W; Alberta, Canada*
A. negundo var. *texanum*	MRT533-96*2		1.40 ± 0.01	2	W; Red Rock Canyon State Park, OK*

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Table 2. Continued.

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
Section *Oblonga*					
A. albopurpurascens	QHBG2003-088	OSC-V-254645	2.30 ± 0.01	2[w]	U
A. oblongum	QHBG2003-204	OSC-V-254649	2.36 ± 0.01	2	W; Taiwan*
Section *Palmata*					
A. amoenum	QHBG2001-087		2.00 ± 0.01	2	W; Honshu, Japan*
A. campbellii ssp. *flabellatum*	QHBG1994-182	OSC-V-254642	1.96 ±0.04	2	W; Sichuan, China*
A. ceriferum	USNA64942	OSC-V-254363	2.02 ± 0.01	2	W; Beijing, China*
A. circinatum	OSU14-0153	OSC-V-254673	1.91 ± 0.06	2	G
A. circinatum 'Hoyt's Witches Broom'	HOYT2006-073		1.78 ± 0.07	2	W; Hoyt Arboretum, OR*
A. elegantulum	HOYT2014-047		3.01 ± 0.08	3	U
A. erianthum	USNA67795	OSC-V-254606	2.18 ± 0.04	2	W; Qinling Mountains, China*
A. fabri	QHBG2003-087		2.24 ± 0.02	2	U
A. fabri	OSU14-0195		2.34 ± 0.02	2	W; Vietnam* (D. Hinkley, Hoyt Arboretum, Portland, OR)
A. japonicum	USNA62344		1.89 ± 0.00	2	W; southern Japan*
A. japonicum 'Aconitifolium'	HOYT1989-038		1.77 ± 0.05	2	G
A. olivaceum (*A. elegantulum*)	ARN249-95A	OSC-V-254630	2.08 ± 0.06	2	U
A. oliverianum	HOYT2014-271		2.14 ± 0.02	2	U
A. palmatum 'Ara kawa'	OSU14-0179		1.89 ± 0.09	2	G
A. palmatum 'Butterfly'	OSU14-0168		1.95 ± 0.04	2	G
A. palmatum 'Fireglow'	OSU14-0180		1.92 ± 0.04	2	G
A. palmatum 'Uki gomo'	OSU14-0170		2.02 ± 0.06	2	G
A. palmatum 'Wolff' (Emperor I®)	OSU14-0166		1.88 ± 0.04	2	G
A. palmatum ssp. *Matsumurae*	USNA44905		1.93 ± 0.00	2	W; Japan*
A. pauciflorum	OSU14-0057		1.92 ± 0.08	2	G
A. pseudosieboldianum	OSU14-0156	96.01	1.90 ± 0.05	2	U
A. pseudosieboldianum var. *koreanum*	ARN486-83A	OSC-V-254626	1.88 ± 0.03	2	W; Korea*
A. pubinerve	ARN50-90A	OSC-V-254625	2.11 ± 0.03	2	W
A. pubipalmatum	USNA61153	OSC-V-254607	1.91 ± 0.01	2	U
A. pubipalmatum	ARN320-2004A	OSC-V-254636	2.08 ± 0.05	2	W; China*
A. pubipalmatum	MRS2009-106	OSC-V-254656	1.94 ± 0.01	2	W; China*
A. serrulatum	QHBG2004-171	OSC-V-254653	2.14 ± 0.02	2	W; Taiwan*
A. shirasawanum	COR01262		2.09 ± 0.01	2	W
A. shirasawanum 'Aureum'	OSU14-0169		1.90 ± 0.02	2	G
A. sieboldianum	HOYT1974-3957		1.85 ± 0.09	2	U
A. sinense	QHBG2003-388	OSC-V-254646	3.12 ± 0.08	3	W; Sichuan Province, China*
A. wuyuanense	MRS2009-031	OSC-V-254663	2.03 ± 0.04	2	W
A. wuyuanense	USNA60719	OSC-V-254942	2.03 ± 0.01	2	W; China*
A. pseudosieboldianum × *A. palmatum* 'Koshimino'	MRT323-2003*1		1.95 ± 0.01	2	G
Section *Parviflora*					
A. distylum	MRS1995-163	OSC-V-254666	2.03 ± 0.05		G
A. distylum	WHT		2.08 ± 0.03		G

Continued next page

Table 2. Continued.

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
Section *Pentaphylla*					
A. buergerianum	OSU12-008-003	72.19	4.38 ± 0.08	4	G; from ploidy manipulation experiment
A. buergerianum	OSU12-008-004	72.17	3.29 ± 0.11	3	G; from ploidy manipulation experiment
A. buergerianum	OSU12-008-005	72.16	2.07 ± 0.03	2	G; from ploidy manipulation experiment
A. buergerianum	OSU12-008-008	73.17	4.04 ± 0.03	4	G; from ploidy manipulation experiment
A. buergerianum	OSU14-0158		2.11 ± 0.02	2[w]	G
A. buergerianum	OSU14-0198	94.19	2.05 ± 0.06	2	G
A. pentaphyllum	HOYT2011-12420		1.76 ± 0.01	2	W; Sichuan Province, China*
A. yui	MRS2005-204	OSC-V-254667	3.11 ± 0.02	4	W; Gansu Province, China*
Section *Platanoidea*					
A. amplum ssp. *catalpifolium*	QHBG2009-235	OSC-V-254644	2.22 ± 0.06	2[w]	W; Sichuan Province, China*
A. campestre	OSU14-0196	94.23	2.01 ± 0.02	2[w]	W
A. campestre ssp. *leiocarpum*	ARN1053-76A	OSC-V-254629	2.09 ± 0.08	2	G
A. campestre ssp. *leiocarpum*	ARN1053-76B	OSC-V-254628	2.01 ± 0.01	2	G
A. cappadocicum ssp. *sinicum*	QHBG1991-129	OSC-V-254657	2.05 ± 0.01	2	W; Sichuan, China*
A. cappadocicum ssp. *sinicum*	QHBG1998-054	OSC-V-254650	1.92 ± 0.04	2	W; Sichuan, China*
A. cappadocicum ssp. *sinicum*	QHBG2001-404	OSC-V-254655	2.11 ± 0.02	2	W; Sichuan, China*
A. mayrii (*A. mono* var. *mayrii*)	ARN12505A	OSC-V-254366	2.00 ± 0.02	2	W; Sapporo, Hokkaido, Japan*
A. miyabei	QHBG1997-131	OSC-V-254638	1.92 ± 0.02	2	W; Hokkaido, Japan*
A. miyabei ssp. *miaotaiense*	MRS1996-395	OSC-V-254670	2.09 ±0.05	2	W; Qinling Mountains, China*
A. mono f. *subtrifidum*	MRT183-76*1		1.83 ± 0.02	2	G
A. okamotoanum (*A. mono* var. *okamotoanum*)	ARN1620-81A	OSC-V-254632	1.95 ± 0.01	2	U
A. okamotoanum (*A. mono* var. *okamotoanum*)	MRS1991-080	OSC-V-254665	1.78 ± 0.04	2	W; Korea*
A. pictum	COR84-161		1.99 ± 0.004	2	W
A. pictum ssp. *macropterum*	QHBG1996-115	OSC-V-254639	1.90 ± 0.02	2	W; Sichuan Province, China*
A. pictum ssp. *Macropterum*	QHBG1999-087		1.90 ± 0.01	2	W; Sichuan Province, China*
A. platanoides	OSU11-0154-079		1.84 ± 0.01	2	G
A. platanoides	OSU11-0153-139	71.17	3.68 ± 0.10	4	G; from ploidy manipulation experiment
A. platanoides 'Columnare'	OSU-FL76.03	76.03	1.89 ± 0.002	2	G
A. platanoides 'Deborah'	OSU-FL75.04	75.04	1.84 ± 0.01	2	G
A. platanoides 'Emerald Queen'	OSU10-0033		1.84 ± 0.02	2	G
A. platanoides 'Emerald Queen'	OSU-FL76.09	76.09	1.87 ± 0.02	2	G
A. platanoides 'Royal Red'	OSU-FL75.06	75.06	1.79 ± 0.01	2	G
A. shenkanense	ARN635-2010B		2.05 ± 0.01	2	W; Shaanxi, China*
A. shenkanense	MRS2010-158	OSC-V-254660	1.89 ± 0.03	2	W; China
A. truncatum	USNA45011		1.96 ± 0.02	2	W; Japan
A. truncatum	USNA44904		1.85 ± 0.03	2	W; Japan
A. ×*dieckii* (*A. platanoides* × *A. cappadocicum*)	ARN181-86A	OSC-V-254365	1.97 ± 0.01	2	U

Continued next page

Table 2. Continued.

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
A. ×*hillieri* (*A. miyabei* × *A. cappadocicum*)	ARN245-39B	OSC-V-254674	1.90 ± 0.03	2	G
A. ×*zoeschense*	USNA15648	OSC-V-254611	1.89 ± 0.01	2	U
A. ×*zoeschense* 'Annae'	USNA17443	OSC-V-254943	1.88 ± 0.04	2	G
A. campestre × *A. miyabei*	MRT65-2007*1		1.90 ± 0.02	2	G
A. platanoides × *A. truncatum*	MRT761-50*4		1.82 ± 0.03	2	G
Section *Pubescentia*					
A. pentapomicum	MRT560-2001*2		1.80 ± 0.01	2	Z; Hissar Mountains, Tajikistan*
A. pilosum	ARN287-2008A		3.21 ± 0.06	4	W; Gansu Province, China*
A. pilosum var. *stenolobum*	MRS2007-056	OSC-V-254664	3.13 ± 0.06	4	W
Section *Rubra*					
A. pycnanthum	USNA67194		5.94 ± 0.13	6	U
A. pycnanthum	QHBG1987-466		6.00 ± 0.10	6	G
A. rubrum	OSU14-0193		5.01 ± 0.04	8[w]	U
A. rubrum (Halka selection)	JFS	OSC-V-254618	4.00 ± 0.11	6	G
A. rubrum 'Autumn Flame'	JFS	OSC-V-254616	5.26 ± 0.06	8	G
A. rubrum 'Autumn Spire'	JFS	OSC-V-254624	4.09 ± 0.02	6	G
A. rubrum 'Bowhall'	JFS	OSC-V-254623	4.15 ± 0.04	6	G
A. rubrum 'Brandywine'	OSU14-0186		5.17 ± 0.02	8	G
A. rubrum 'Celebration'	OSU14-0183	95.13	3.94 ± 0.02	6	G
A. rubrum 'Columnare'	JFS		4.12 ± 0.04	6	G
A. rubrum 'Morgan'	JFS	OSC-V-254620	4.13 ± 0.04	6	G
A. rubrum 'October Glory'	JFS	OSC-V-254615	5.20 ± 0.09	8	G
A. rubrum 'Red Rocket'	JFS	OSC-V-254619	4.17 ± 0.05	6	G
A. rubrum 'Scarsen' (Scarlet Sentinel®)	JFS	OSC-V-254361	4.00 ± 0.14	6	G
A. rubrum 'Somerset'	JFS		5.29 ± 0.08	8	G
A. rubrum var. *trilobum*	USNA31022		5.89 ± 0.10	9	W
A. rubrum var. *trilobum*	MRS1961-382	OSC-V-254669	6.10 ± 0.00	9	W; coastal southeastern United States
A. rubrum 'Vanity'	OSU14-0131	92.21	5.02 ± 0.09	8	Dancing Oaks Nursery, Monmouth, OR
A. saccharinum	HOYT1981-028		2.42 ± 0.05	4	U
A. saccharinum	OSU-campus		2.71 ± 0.01	4	U
A. saccharinum var. *laciniatum*	ARN201-55A		2.78 ± 0.01	4	U
A. ×*freemanii* 'Autumn Blaze'	OSU14-0181	93.13	3.80 ± 0.03	6	G
A. ×*freemanii* 'Celzam' (Celebration®)	JFS	OSC-V-254621	4.08 ± 0.09	6	G
A. ×*freemanii* 'DTR 102' (Autumn Fantasy®)	JFS	OSC-V-254617	3.43 ± 0.03	5	G
A. ×*freemanii* 'Firefall'	COR08-228		3.49 ± 0.01	5	G
A. ×*freemanii* 'Jenner'	MRT349-2005*1		3.84 ± 0.04	6	G
A. ×*freemanii* 'Sienna' (Sienna Glen®)	JFS	OSC-V-254613	4.10 ± 0.04	6	G
Section *Spicata*					
A. caudatum ssp. *multiserratum*	MRT878-2005*1		2.02 ± 0.03	2	W; Gansu Province, China*
A. caudatum ssp. *ukurunduense*	HOYT2014-041		1.88 ± 0.03	2	U
A. spicatum	HOYT1989-057		2.06 ± 0.06	2	U

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Table 2. Continued.

Taxon	Source/ accession[z]	Voucher no./field location[y]	2C [mean ± SE (pg)]	Putative ploidy	Provenance/native distribution[x]
Section *Trifoliata*					
A. griseum	HOYT1998-023		1.93 ± 0.06	2	G
A. maximowiczianum	OSU14-0148		2.30 ± 0.22	2	Heritage Seedlings, Salem, OR
A. maximowiczianum	OSU14-0149	95.17	2.15 ± 0.02	2	Heritage Seedlings, Salem, OR
A. maximowiczianum	OSU14-0150		2.22 ± 0.10	2[w]	Heritage Seedlings, Salem, OR
A. nikoense	HOYT1969-3958		1.90 ± 0.06	2	U
A. triflorum	OSU14-0143	95.21	2.05 ± 0.01	2[w]	U
A. triflorum	USNA58016	OSC-V-254945	1.80 ± 0.03	2	W; Korea*
A. griseum × *A. nikoense* (Rochester Group)	MRT243-94*1		1.96 ± 0.02	2	G
A. griseum × *A. triflorum*	MRT70-2011*1		2.85 ± 0.04	3	G
A. maximowiczianum × *A. griseum*	ARN641-91A		2.14 ± 0.01	2	G

[z]ARN = Arnold Arboretum, Boston, MA; COR, Cornell Plantations, Ithaca, NY; HOYT = Hoyt Arboretum, Portland, OR; JFS = J. Frank Schmidt Arboretum, Boring, OR; MRS = Morris Arboretum, Philadelphia, PA; MRT = Morton Arboretum, Lisle, IL; OSU = Oregon State University Ornamental Plant Breeding Collection, Corvallis, OR; OSU-camp = OSU campus landscape plants, Corvallis, OR; OSU-FL = field-planted material located at the Lewis-Brown Farm, Corvallis, OR; QHBG, Quarry Hill Botanical Garden, Glen Ellen, CA; USNA = U.S. National Arboretum, Washington, DC; WHT = Whitman Farms, Salem, OR.

[y]Either voucher number at the OSU Herbarium formatted as OSC-V-254XXX or current field location formatted as "row.plant" (e.g., 74.19 is the 74th row and 19th plant within that row).

[x]W = collected in the wild; Z = from cultivated plant of known wild origin, G = cultivated plant of garden origin, U = unknown. If an asterisk is included, then area listed is known to be the area from which the seed or propagule was collected. Native distribution indicates general distribution of the species.

[w]Ploidy was determined with cytology.

The bp composition of 48 accessions representing 17 taxonomic sections was evaluated. Base pair composition was estimated according to the equation: AT% = AT% for internal standard × [(mean fluorescence standard DAPI / mean fluorescence sample DAPI) / (mean fluorescence standard PI / mean fluorescence sample PI)]$^{(1/\text{binding length})}$ (Godelle et al., 1993), where AT% of internal standard is 61.5 and the binding length of DAPI is 3.5 bp (Meister and Barow, 2007) (Table 4). Values reported in Table 4 were calculated as GC% = 100 – AT%.

Cytology. Cytology was completed on 12 species representing nine taxonomic sections (Fig. 1), including diploids and one octoploid. For cytological analysis, cuttings were rooted or plants were grown from seed. Actively growing root tips were collected before 1100 HR on mornings following two sunny days. Roots tips were suspended in a prefixative solution of 2 mM 8-hydroxyquinoline + 0.24 mM cycloheximide in glass vials and incubated in the dark on ice for 3 h. After the prefixative treatment, root tips were rinsed three to four times in distilled water and placed in Carnoy's solution (1 glacial acetic acid : 3 chloroform : 6 100% ethanol) and incubated in the dark at room temperature overnight. The following morning, root tips were rinsed four times using 70% aqueous ethanol and then stored at 4 °C in vials of 70% ethanol until observation.

In preparation for enzyme digestion, tissue was excised from root apical meristems using a scalpel. A 0.5% enzyme solution including cellulase, pectolyase, and cytohelicase dissolved in 50 mM citrate buffer was used to break down cell walls. Root tips were placed in an Eppendorf tube containing enzyme solution, the tube was floated in a water-filled beaker, and was incubated for ≈3 h at ≈32 °C. After incubation, excised tissue was removed from the tube using a glass pipette and was placed on a microscope slide. Excess liquid was wicked away using single-ply low-lint tissue. A drop of modified carbol fuchsin (Kao, 1975) was placed on the excised tissue, then a coverslip was placed on top. After 3 min, the material was gently squashed. Slides were scanned and micrographs were captured using a light microscope at 630× and 1000× [Axio imager.A1 (Zeiss, Thornwood, NY), AxioCam MRm (Zeiss)]. A minimum of three to five cells were counted for each accession.

Statistical analyses. Analysis of variance was performed on monoploid genome sizes using PROC GLM (SAS version 9.4; SAS Institute, Cary, NC), and Tukey's honestly significant difference test at $\alpha = 0.05$ was used to separate means of each section. A paired *t* test was used to compare holoploid genome sizes for 48 taxa calculated using both DAPI and PI to determine whether differences were significant using these two fluorochromes.

Results and Discussion

With few exceptions, *Acer* had genome sizes in the range considered "small" (1.4–3.5 pg) using the definition used by Soltis et al. (2003). The 2C relative genome size ranged from 1.39 pg in *A. negundo* var. *interius* to 6.10 pg in *A. rubrum* var. *trilobum* (Table 2). The mean monoploid genome size (1C*x*) of taxonomic sections, species, and grex ranged from 0.43 pg in *A. carpinifolium* (section *Indivisa*) to 1.66 pg in *A. caudatifolium* (section *Macrantha*) (Table 3).

Cytology confirmed accessions of *A. campestre* (section *Platanoidea*) observed in this study are diploids (Fig. 1) with a mean relative 2C genome size of 2.01 pg using DAPI and 1.50 pg using PI (Table 4) compared with the reported 2C value of

Table 3. Average monoploid (1C*x*) genome size for taxonomic sections, species, and grex of *Acer* using flow cytometry analysis of nuclei stained with 4′,6-diamidino-2-phenylindole with *Pisum sativum* 'Ctirad' as the internal standard (2C = 8.76 pg).

Section	1C*x* (pg)	Species/grex	1C*x* [mean ± SE (pg)]
Acer	0.93 efg[z]	*A. caesium*	1.03 ± 0.02
		A. heldreichii	0.88 ± 0.01
		A. grandidentatum[y]	0.84 ± 0.00
		A. hyrcanum	0.97 ± 0.03
		A. monspessulanum[y]	1.01 ± 0.01
		A. opalus	1.02 ± 0.03
		A. pseudoplatanus	0.86 ± 0.03
		A. saccharum	0.89 ± 0.02
		A. sempervirens[y]	0.95 ± 0.02
		A. velutinum	0.89 ± 0.05
		A. ×coriaceum[y]	0.93 ± 0.02
Arguta	0.95 def	*A. acuminatum*	0.97 ± 0.01
		A. argutum	0.92 ± 0.03
		A. barbinerve	0.93 ± 0.05
		A. stachyophyllum[y]	0.97 ± 0.01
Ginnala	0.80 ghi	*A. tataricum*[y]	0.83 ± 0.01
		A. tataricum ssp. *aidzuense*	0.82 ± 0.01
		A. tataricum ssp. ginnala	0.79 ± 0.01
Glabra	0.79 ghi	*A. glabrum*[y]	0.79 ± 0.03
Indivisa	0.43 j	*A. carpinifolium*	0.43 ± 0.02
Lithocarpa	1.11 bc	*A. diabolicum*	1.19 ± 0.00
		A. sterculiaceum[y]	1.14 ± 0.01
		A. yangbiense[y]	0.95 ± 0.02
Macrantha	1.32 a	*A. caudatifolium*[y]	1.66 ± 0.04
		A. crataegifolium[y]	1.52 ± 0.01
		A. davidii	1.28 ± 0.01
		A. forrestii[y]	1.39 ± 0.04
		A. laxiflorum[y]	0.81 ± 0.01
		A. morrisonense	1.46 ± 0.02
		A. pectinatum[y]	1.17 ± 0.02
		A. pensylvanicum	1.22 ± 0.01
		A. rubescens[y]	1.46 ± 0.05
		A. rufinerve	1.40 ± 0.06
		A. tegmentosum	1.26 ± 0.04
		A. tschonoskii[y]	1.08 ± 0.02
		A. davidii ×tegmentosum	1.38 ± 0.03
Macrophylla	0.83 fgh	*A. macrophyllum*	0.83 ± 0.01
Negundo	0.75 hi	*A. cissifolium*[y]	0.76 ± 0.01
		A. henryi	0.79 ± 0.00
		A. negundo	0.72 ± 0.02
Oblonga	1.16 b	*A. albopurpurascens*[y]	1.15 ± 0.00
		A. oblongum[y]	1.18 ± 0.00
Palmata	0.99 cde	*A. amoenum*[y]	1.00 ± 0.01
		A. campbellii[y]	0.98 ± 0.02
		A. ceriferum[y]	1.01 ± 0.01
		A. circinatum	0.92 ± 0.03
		A. elegantulum[y]	1.00 ± 0.03
		A. erianthum[y]	1.09 ± 0.02
		A. fabri	1.15 ± 0.02
		A. japonicum[y]	0.94 ± 0.00
		A. olivaceum[y]	1.04 ± 0.01
		A. oliverianum[y]	1.07 ± 0.01
		A. palmatum	0.96 ± 0.01
		A. pauciflorum	0.96 ± 0.04
		A. pseudosieboldianum	0.95 ± 0.01

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1.38 pg for diploids (Table 1). We did not observe any tetraploid *A. campestre* or variation in holoploid genome size among accessions, which is in contrast with Siljak-Yakovlev et al. (2010), who identified diploid (2C = 1.38 pg) and tetraploid (2C = 2.70 pg) cytotypes.

The monoploid genome size of section *Acer* was calibrated using root squashes of *A. saccharum* (Fig. 1). The tetraploid *A. pseudoplatanus* was also confirmed through genome sizing. The 2C genome size for *A. pseudoplatanus* using PI was reported as 2.70 pg (Siljak-Yakovlev et al., 2010), which agrees with our findings for *A. pseudoplatanus* (U.S. National Arboretum 2836) using PI (2C = 2.86 pg), but is less than our estimates using DAPI for these two accessions (2C = 3.29 pg and 3.56 pg). Another species in section *Acer* reported to be tetraploid is *A. heldreichii*. Although we did not confirm this directly through cytology, the 2C genome size provides strong evidence (2C = 3.51 pg) that it is a tetraploid. The 2C value measured in the current study did not align precisely with reported values (2C = 2.57 pg) (Siljak-Yakovlev et al., 2010). Our estimate was produced using DAPI, which regularly yields a greater genome size than PI (Table 4), as observed in other taxa, such as *Cotoneaster* (Rothleutner et al., 2016).

Cytology confirmed *A. rubrum* (section *Rubra*) OSU14-0193 is an octoploid (Fig. 1). This chromosome count provided the calibration necessary to determine the ploidy levels of other accessions of section *Rubra*. Genome size calibrated with cytology confirmed a natural ploidy series in *A. rubrum*, with hexaploids ($2n = 6x = 72$) and octoploids observed in this study. There was no evidence of tetraploid *A. rubrum* among accessions analyzed. The tetraploid *A. saccharinum* was confirmed through cytological analysis (Fig. 1). Given that the monoploid genome size of species within section *Rubra* appears to be consistent (1C*x* = 0.69 pg) among many of the accessions, *A. pycnanthum* was an outlier based on our interpretation of the data (1C*x* = 0.99 pg). This is an uncommon species, endemic to the island of Honshu,

Table 3. Continued.

Section	1C*x* (pg)	Species/grex	1C*x* [mean ± SE (pg)]
		A. pubinerve	1.05 ± 0.02
		A. pubipalmatum	0.99 ± 0.03
		A. serrulatum[y]	1.07 ± 0.01
		A. shirasawanum	1.00 ± 0.05
		A. sieboldianum	0.93 ± 0.04
		A. sinense	1.04 ± 0.03
		A. wuyuanense	1.01 ± 0.00
		A. pseudosieboldianum × *A. palmatum*	1.01 ± 0.03
Parviflora	1.03 bcde	*A. distylum*	1.03 ± 0.01
Pentaphylla	1.00 cde	*A. buergerianum*	1.05 ± 0.01
		A. pentaphyllum[y]	0.88 ± 0.00
		A. yui[y]	0.78 ± 0.01
Platanoidea	0.97 de	*A. amplum*[y]	1.11 ± 0.03
		A. campestre	1.02 ± 0.01
		A. cappadocicum	1.01 ± 0.03
		A. mayrii[y]	1.00 ± 0.01
		A. miyabei	1.00 ± 0.04
		A. mono[y]	0.92 ± 0.01
		A. okamotoanum	0.93 ± 0.04
		A. pictum	0.96 ± 0.02
		A. platanoides	0.92 ± 0.01
		A. shenkanense	0.99 ± 0.04
		A. truncatum	0.95 ± 0.03
		A. ×*dieckii*[y]	0.99 ± 0.00
		A. ×*hillieri*[y]	0.95 ± 0.02
		A. ×*zoeschense*	0.94 ± 0.00
		A. campestre × *A. miyabei*	0.95 ± 0.01
		A. platanoides × *A. truncatum*	0.91 ± 0.01
Pubescentia	0.83 fghi	*A. pentapomicum*[y]	0.90 ± 0.01
		A. pilosum	0.79 ± 0.01
Rubra	0.69 i	*A. pycnanthum*	0.99 ± 0.01
		A. rubrum	0.66 ± 0.01
		A. saccharinum	0.66 ± 0.03
		A. ×*freemanii*	0.67 ± 0.01
Spicata	0.99 cde	*A. caudatum*	0.97 ± 0.04
		A. spicatum[y]	1.03 ± 0.03
Trifoliata	1.02 bcd	*A. griseum*[y]	0.96 ± 0.03
		A. maximowiczianum	1.11 ± 0.02
		A. nikoense[y]	0.95 ± 0.03
		A. triflorum[y]	0.90 ± 0.06
		A. griseum × *A. nikoense*[y]	0.98 ± 0.01
		A. griseum × *A. triflorum*[y]	0.95 ± 0.01
		A. maximowiczianum × *A. griseum*[y]	1.07 ± 0.01
Tukey's HSD	0.1378		

[z]Values within column followed by different letters are significantly different based on Tukey's honestly significant difference (HSD; $\alpha = 0.05$).
[y]Three samples of one accession were used to calculate the average 1C*x* value as a result of the lack of biological replicates.

Japan, that has been reported as a hexaploid ($2n = 6x = 78$). Given the monoploid value of section *Rubra* is consistent, the measured genome size indicated it was an octoploid (van Gelderen et al., 1994). Cytological investigation is needed to confirm ploidy for this accession.

Acer carpinifolium (section *Indivisa*) was reported to be a tetraploid (Taylor, 1920) with a holoploid genome size of 0.75 pg (Olszewska and Osiecka, 1984). Our 2C estimate using PI was 1.36 pg. Based on available reports, there has not been a study that simultaneously investigated this species with flow cytometry and cytology. As a result of the prevalence of ploidy variation in the genus, it appears there are diploid and tetraploid cytotypes of *A. carpinifolium*. Olszewska and Osiecka (1984) included a diploid cytotype whereas Taylor (1920) and the current study included tetraploids. However, both the accession included in the current study and that used by Olszewska and Osiecka (1984) were received from Rogów Arboretum, Poland. Because plants of garden origin may hybridize freely, it is possible that hybrid seed was received by one or both groups. We attempted to use cytology to confirm our findings but were unable to as a result of difficulty in breaking down the cells walls sufficiently to allow for adequate spreading of metaphase cells. Although the cell walls of all other species in the cytological study were broken down effectively with enzyme digestion, *A. carpinifolium* proved to be recalcitrant. It may be necessary to attempt another method of cell wall digestion, such as long-term enzyme digestion on slides (Lattier et al., 2017), which yielded excellent results in identifying triploid *A. ginnala* in addition to five other diverse species.

Often, genome size data among diverse taxa of a given family are compared using the monoploid genome size because of ploidy variation. There were some significant differences among monoploid genome sizes of the 18 sections ($P < 0.0001$). The greatest mean monoploid genome size was of section *Macrantha* (1.32 pg), which was significantly greater than all other sections. Section *Indivisa* had the smallest monoploid genome size based on reported ploidy and cytometric analysis (1C*x* = 0.43 pg). Monoploid genome size was wide ranging and somewhat regularly distributed from low to high, with a noticeable gap between sections *Indivisa* and *Rubra* (Fig. 2). Based on personal observations of successful, but accidental or ''naturally'' occurring intersectional hybrids that arose when nursery crops were grown in proximity to native species [e.g., *A. griseum* (section *Trifoliata*) × *A. macrophyllum* (section *Macrophylla*)], monoploid genome

Table 4. Base pair composition of 48 *Acer* taxa determined by comparing holoploid (2C) genome size determined using flow cytometry analysis of nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) using *Pisum sativum* 'Ctirad' as the internal standard (2C = 8.76 pg).

Taxon	Source/accession[z]	2C genome size [mean ± SE (pg)] DAPI	PI	DAPI-PI	*P* value[y]	GC%[x]
Section *Acer*						
A. grandidentatum	MRT276-742	1.67 ± 0.01	1.45 ± 0.02	0.22	0.0007	41.01
A. hyrcanum	MRT67-2001*1	1.88 ± 0.03	1.63 ± 0.01	0.25	0.0014	40.94
A. pseudoplatanus	USNA2836	3.29 ± 0.08	2.86 ± 0.09	0.43	0.0201	41.01
A. sempervirens	HOYT1993-116	1.91 ± 0.03	1.55 ± 0.02	0.36	0.0006	42.05
Section *Arguta*						
A. argutum	OSU14-0194	1.78 ± 0.01	1.57 ± 0.01	0.21	0.0043	41.30
A. barbinerve	MRT258-2002*1	1.96 ± 0.02	1.64 ± 0.02	0.32	0.0004	41.54
A. barbinerve	USNA68777	1.77 ± 0.00	1.50 ± 0.01	0.27	<0.0001	41.36
A. stachyophyllum ssp. *betulifolium*	MRT854-2005*2	1.94 ± 0.01	1.62 ± 0.02	0.32	0.0002	41.64
Section *ginnala*						
A. tataricum	OSU14-0202	1.65 ± 0.02	1.36 ± 0.01	0.29	<0.0001	41.10
Section *Glabra*						
A. glabrum	OSU-camp	1.58 ± 0.03	1.31 ± 0.01	0.27	0.0007	41.71
Section *Indivisa*						
A. carpinifolium	OSU14-0051	1.81 ± 0.02	1.36 ± 0.02	0.45	<0.0001	43.29
Section *Lithocarpa*						
A. diabolicum	MRT1276-55*1	2.38 ± 0.01	1.96 ± 0.01	0.42	<0.0001	41.84
A. sterculiaceum ssp. *franchetii*	MRT332-2000*3	2.27 ± 0.02	1.96 ± 0.03	0.31	0.0015	41.03
Section *Macrantha*						
A. crataegifolium	MRT220-73*2	3.04 ± 0.01	2.87 ± 0.02	0.17	0.0010	39.50
A. tegmentosum	USNA64194	2.61 ± 0.02	2.44 ± 0.12	0.17	0.9144	38.61
A. davidii × *A. davidii* ssp. *grosser*	MRT244-2014	2.59 ± 0.06	2.49 ± 0.02	0.10	0.1711	39.28
A. davidii × *A. tegmentosum*	USNA65062	2.76 ± 0.05	2.60 ± 0.04	0.16	0.0715	39.55
Section *Macrophylla*						
A. macrophyllum	OSU-camp	1.63 ± 0.01	1.27 ± 0.01	0.36	<0.0001	42.74
Section *Negundo*						
A. negundo	OSU14-0089-01	1.53 ± 0.01	1.10 ± 0.01	0.43	<0.0001	43.96
A. negundo var. *interius*	MRT227-86*3	1.39 ± 0.01	1.05 ± 0.01	0.34	<0.0001	43.28
A. negundo var. *texanum*	MRT533-96*2	1.40 ± 0.01	1.03 ± 0.02	0.37	<0.0001	43.57
Section *Palmata*						
A. japonicum	USNA62344	1.89 ± 0.00	1.64 ± 0.02	0.25	0.0002	40.88
A. pubipalmatum	USNA61153	1.91 ± 0.01	1.64 ± 0.01	0.27	0.0001	41.09
A. wuyuanense	USNA60719	2.03 ± 0.01	1.72 ± 0.01	0.31	<0.0001	41.28
A. pseudosieboldianum × *A. palmatum* 'Koshimino'	MRT323-2003*1	1.95 ± 0.01	1.65 ± 0.02	0.30	0.0001	41.39
Section *Parviflora*						
A. distylum	WHT	2.08 ± 0.05	1.98 ± 0.03	0.10	0.1975	39.39
Section *Pentaphylla*						
A. buergerianum	OSU12-008-003	4.38 ± 0.08	3.48 ± 0.04	0.90	0.0006	42.39
A. buergerianum	OSU12-008-004	3.29 ± 0.11	2.56 ± 0.03	0.73	0.0031	42.74
A. buergerianum	OSU12-008-005	2.07 ± 0.03	1.74 ± 0.02	0.33	0.0005	41.55
A. buergerianum	OSU12-008-008	4.04 ± 0.03	3.31 ± 0.01	0.73	<0.0001	41.90
A. buergerianum	OSU14-0198	2.05 ± 0.06	1.72 ± 0.03	0.33	0.0094	41.56
Section *Platanoidea*						
A. campestre	OSU14-0196	2.01 ± 0.02	1.50 ± 0.01	0.51	<0.0001	43.46
A. platanoides 'Deborah'	OSUFL 75.04	1.84 ± 0.01	1.56 ± 0.02	0.28	0.0005	41.30
A. platanoides 'Emerald Queen'	OSU10-0033	1.87 ± 0.02	1.58 ± 0.02	0.29	0.0004	41.17
A. ×*zoeschense*	USNA15648	1.89 ± 0.01	1.54 ± 0.02	0.35	<0.0001	42.00
A. campestre × *A. miyabei*	MRT65-2007*1	1.90 ± 0.02	1.48 ± 0.02	0.42	<0.0001	42.81

Continued next page

Table 4. Continued.

Taxon	Source/accession[z]	2C genome size [mean ± SE (pg)] DAPI	PI	DAPI-PI	*P* value[y]	GC%[x]
Section *Pubescentia*						
A. pentapomicum	MRT560-2001*2	1.80 ± 0.01	1.43 ± 0.01	0.37	<0.0001	42.36
Section *Rubra*						
A. rubrum	OSU14-0193	5.01 ± 0.04	4.37 ± 0.03	0.64	0.0002	40.90
A. rubrum 'Celebration'	OSU14-0183	3.94 ± 0.02	3.27 ± 0.12	0.67	0.0046	41.71
A. rubrum 'Vanity'	OSU14-0131	5.02 ± 0.09	4.29 ± 0.11	0.73	0.0066	41.27
A. saccharinum	OSU-camp	2.42 ± 0.05	2.34 ± 0.05	0.08	0.0013	41.01
A. ×*freemanii* 'Autumn Blaze'	OSU14-0181	3.80 ± 0.03	3.30 ± 0.06	0.50	0.0018	40.92
A. ×*freemanii* 'Jenner'	MRT349-2005*1	3.84 ± 0.04	3.37 ± 0.01	0.47	0.0004	40.74
Section *Spicata*						
A. caudatum ssp. *ukurunduense*	HOYT2014-041	1.88 ± 0.03	1.68 ± 0.01	0.20	0.0053	40.52
A. spicatum	HOYT1989-057	2.06 ± 0.06	1.80 ± 0.01	0.26	0.0122	40.87
Section *Trifoliata*						
A. triflorum	USNA58016	1.80 ± 0.03	1.56 ± 0.05	0.24	0.0170	40.93
A. triflorum	OSU14-0143	2.05 ± 0.01	1.70 ± 0.02	0.35	<0.0001	41.73
A. griseum × *A. nikoense*	MRT243-94*1	1.96 ± 0.02	1.59 ± 0.03	0.37	0.0007	42.08

[z]ARN = Arnold Arboretum, Boston, MA; COR = Cornell Plantations, Ithaca, NY; HOYT, Hoyt Arboretum, Portland, OR; JFS = J. Frank Schmidt Arboretum, Boring, OR; MRS = Morris Arboretum, Philadelphia, PA; MRT = Morton Arboretum, Lisle, IL; OSU = Oregon State University Ornamental Plant Breeding Collection, Corvallis, OR; OSU-camp = OSU campus landscape plants, Corvallis, OR; OSU-FL = field-planted material located at the Lewis-Brown Farm, Corvallis, OR; QHBG = Quarry Hill Botanical Garden, Glen Ellen, CA; USNA = U.S. National Arboretum, Washington, DC; WHT = Whitman Farms, Salem, OR. Use of * within MRT accessions is a convention used within that organization's accessioning method.

[y]*P* value based on paired *t* test comparing mean holoploid genome sizes determined using DAPI with PI.

[x]GC% = 100 – {AT% for internal standard × [(mean fluorescence standard DAPI / mean fluorescence sample DAPI) / (mean fluorescence standard PI / mean fluorescence sample PI)]$^{(1/\text{binding length})}$} (Godelle et al., 1993), where AT% of internal standard = 61.5 and binding length of DAPI = 3.5 bp (Meister and Barow, 2007).

size could be used as a tool for assessing hybridization that is more rapid and easier than molecular markers. Monoploid genome size within a section does not appear to be significantly different, with the exception of section *Pentaphylla* and potentially section *Rubra* if *A. pycnanthum* is proved to be a hexaploid.

Based on average monoploid genome sizes calibrated by intrasectional cytological analysis, three potential natural triploids ($2n = 3x = 39$) have been identified: *A. elegantulum* and *A. sinsense* of section *Palmata*, and a hybrid accession from the Morton Arboretum identified as a cross between *A. griseum* and *A. triflorum* (section *Trifoliata*). Confirmation of triploidy through cytological assessment would be ideal. Although *A. elegantulum* is the accepted name of the species, another accession in this study carries the synonym *A. olivaceum*. The relative genome sizes of these two plants is significantly different: 2C = 3.01 pg and 2C = 2.08 pg, respectively. If the two can be grouped together taxonomically, then this difference in genome size would further support the putative triploidy of *A. elegantulum*. If the *A. elegantulum* accession is indeed a diploid, then this genome size data would indicate further examples of multiple cytotypes within a species ($2x$ and $3x$) or could support taxonomic separation of these species.

In the fluorochrome comparison, there was a significant difference in mean relative genome size measurements in 44 of the 48 accessions measured, with samples measured using DAPI being consistently larger (Table 4). It is interesting that within section *Macrantha*, three of the four taxa used to compare DAPI and PI were not statistically different (Table 4). The lack of difference in genome size estimation between fluorochromes in section *Macrantha* (39.2% GC) is a result of its similarity in base composition to the internal standard (38.5% GC). There was no difference in genome size estimate between fluorochromes for *A. distylum* (section *Parviflora*; 39.4% GC) and this was the only other section that had a GC% less than 40%. Other interesting points noted in section *Macrantha* were that it had the lowest GC% among sections and, as discussed previously, had the largest monoploid genome size. Doležel et al. (1992) noted an overestimation of genome size using DAPI when they compared PI, DAPI, and mithramycin. Factors contributing to this overestimation include differences in base composition or sequence between the internal standard and measured sample, and differences in binding properties of the fluorochromes. Thus, there is the potential for overestimation of genome size when using base-specific fluorochromes such as DAPI; however, it is still a useful, effective, efficient, and inexpensive means to estimate a relative genome size. In addition, it can provide a tool for estimating base composition when used in conjunction with an intercalating dye such as PI.

In a recent genomic characterization study, base composition for sugar maple (*A. saccharum*) was determined using whole-genome sequencing. Staton et al. (2015) determined that the GC% for the sugar maple sample was 38.1%. Although *A. saccharum* was not evaluated using PI, four accessions of section *Acer* had an average GC% of 41.2% (Table 4). Although monoploid genome size was wide

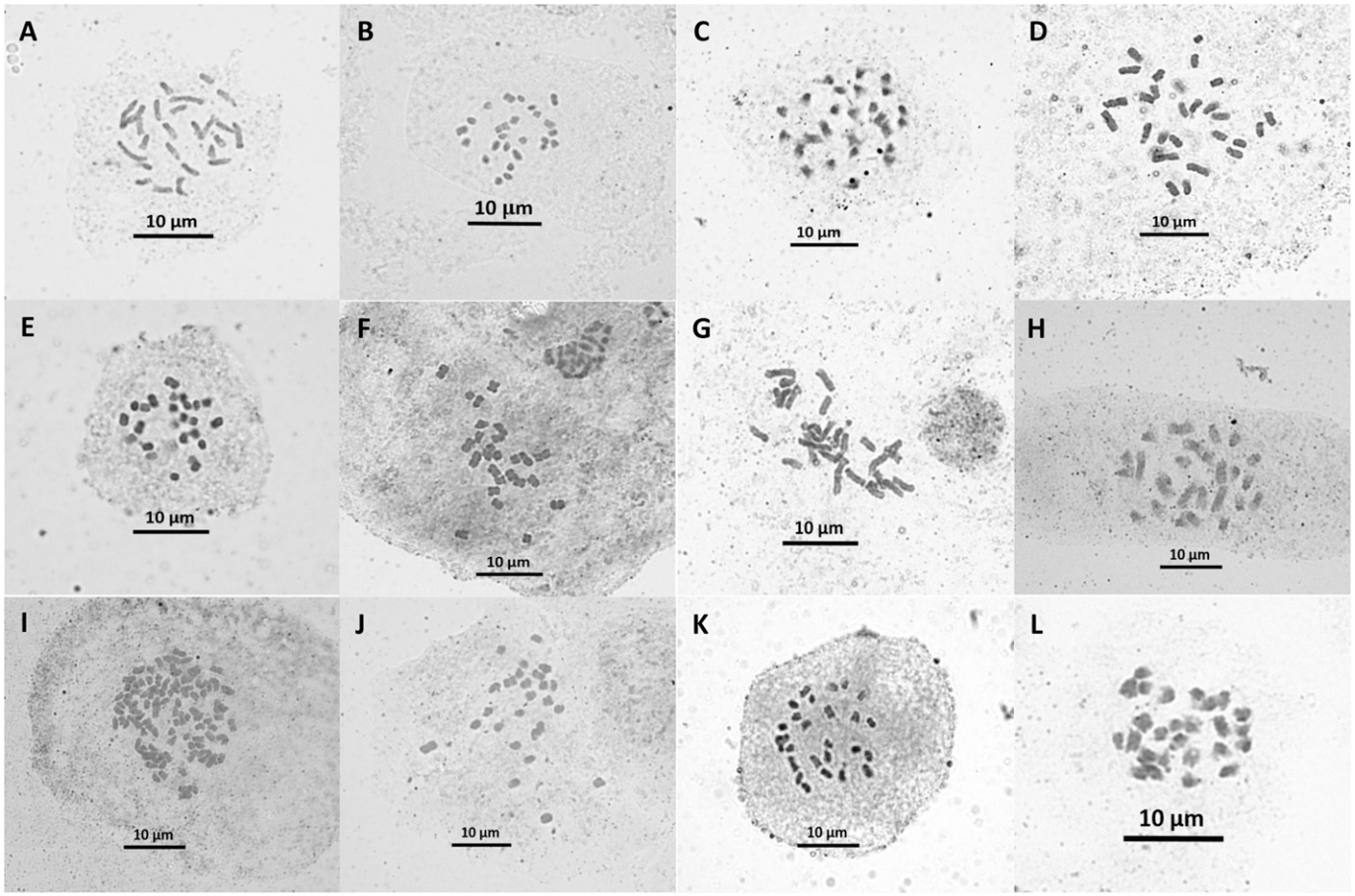

Fig. 1. Photomicrographs of root apical meristem cells from six diploid *Acer* species. Chromosomes stained using modified carbol-fuchsin. (**A**) *A. albopurpurascens* QHBG2003-088 ($2n = 2x = 26$). (**B**) *A. amplum* QHBG2009-235 ($2n = 2x = 26$). (**C**) *A. argutum* OSU14-0194 ($2n = 2x = 26$). (**D**) *A. buergerianum* OSU14-0158 ($2n = 2x = 26$). (**E**) *A. campestre* OSU14-0196 ($2n = 2x = 26$). (**F**) *A. davidii* OSU14-0162 ($2n = 2x = 26$). (**G**) *A. macrophyllum* OSU14-0059-01 ($2n = 2x = 26$). (**H**) *A. maximowiczianum* OSU14-0150 ($2n = 2x = 26$). (**I**) *A. rubrum* OSU14-0193 ($2n = 8x = 104$). (**J**) *A. saccharum* OSU14-0147 ($2n = 2x = 26$). (**K**) *A. tataricum* OSU14-0202 ($2n = 2x = 26$). (**L**) *A. triflorum* OSU14-0143 ($2n = 2x = 26$). Source of material used in cytological study includes Quarry Hill Botanical Garden (QHBG; Glen Ellen, CA) and Oregon State University (OSU; Corvallis, OR) accessions.

ranging, with significant differences present, there was little apparent variation in GC% among the taxa evaluated in this study, ranging from just 38.61% to 43.96%. It is unclear whether taxa in this study with relatively greater GC% have any ecological advantage. However, it should be noted that norway maple (*A. platanoides*) is extremely ecologically adaptable—to the point of invasiveness—and has a lower GC% (41.24%) than bigleaf maple [*A. macrophyllum* (42.74% GC)], which performs poorly in urban conditions and overall is less adaptable. Future studies may further investigate whether there is a correlation between GC% and climate at the site of evolution of maple species.

Conclusions

This study provides valuable information for maple breeding programs, contributes to the growing database of angiosperm genome size, and provides additional data for flow cytometry methods and material. Bennett and Leitch (2005) describe ''very small'' genome size ($2C \leq 1.4$ pg) as ancestral in angiosperms compared with ''small'' genome size (>1.4 to ≤3.5 pg). It follows that the small genome size of *Acer* represents a more evolved condition. Furthermore, small genome size is often associated with developmental characters that many maples exhibit, such as rapid seedling establishment, relatively short generation time (some of our triploid *A. ginnala* flower in 1 year from seed), and increased reproductive rate (Bennett and Leitch, 2005). These traits contribute to evolutionary and ecological adaptability, which maples have in large order. This ecological plasticity or broad adaptability has led to many species escaping cultivation, which has prompted breeding for reduced fertility in the genus through the development of plants with odd ploidy (e.g., triploid, pentaploid). Although overall the coverage of the genus in this study is broad, there are some taxonomic sections in which there is a considerable depth of coverage, including sections *Acer*, *Macrantha*, *Palmata*, and *Platanoidea*. The current depth of coverage reflects the availability of material in arboreta, botanical gardens, and nurseries. Future work can focus on expanding our knowledge of the genus in areas with less coverage, and can aim toward continued cytological studies to provide clarification regarding ploidy and genome size.

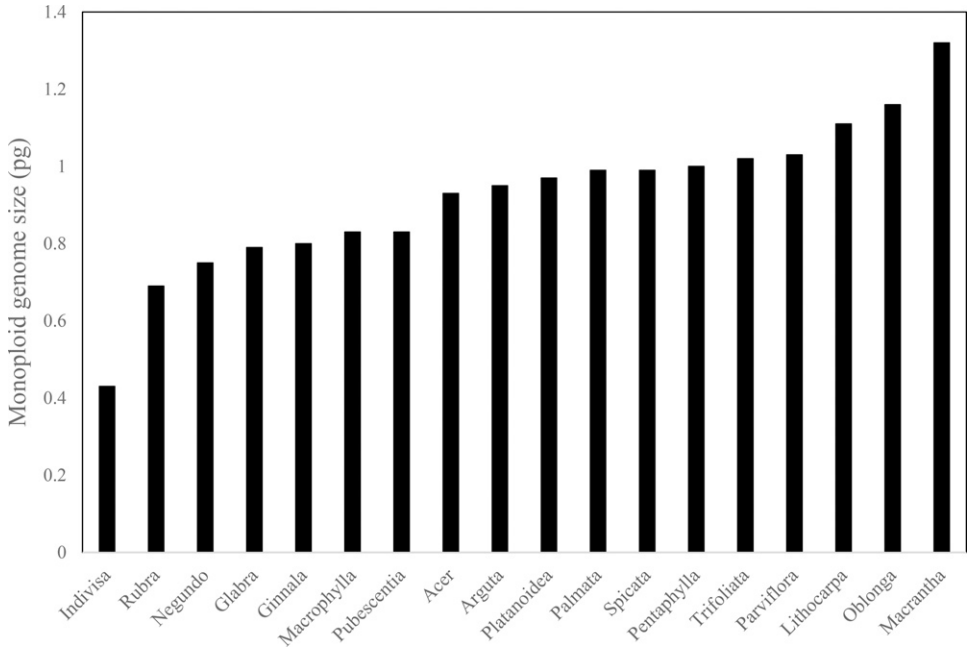

Fig. 2. Monoploid (1C*x*) genome size of the 18 sections of *Acer* determined using flow cytometry analysis of nuclei stained with 4′,6-diamidino-2-phenylindole with *Pisum sativum* 'Ctirad' as the internal standard (2C = 8.76 pg).

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