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Identification, Nomenclature, Genome Sizes, and Ploidy Levels of *Liriope* and *Ophiopogon* Taxa

Jason D. Lattier[1] and Thomas G. Ranney[2,5]
Mountain Crop Improvement Laboratory, Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, 455 Research Drive, Mills River, NC 28759-3423

Paul R. Fantz[3]
Department of Horticultural Science, North Carolina State University Herbarium, Department of Plant Biology, North Carolina State University, Raleigh, NC 27518

Tony Avent[4]
Plant Delights Nursery, 9241 Sauls Road, Raleigh, NC 27603

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***Abstract. Liriope* Lour. and *Ophiopogon* Ker Gawl., collectively known as liriopogons, represent important evergreen groundcovers grown throughout the world for their ornamental features and medicinal qualities. As a result of the diversity of desirable traits and evidence of wide hybridization, there is considerable potential for breeding and improvement of liriopogons. However, confusion over taxonomy and proper identification and lack of information on ploidy levels and cytogenetics of individual clones and cultivars have constrained breeding efforts. Objectives of this study were to validate the identification and nomenclature and determine genome sizes and ploidy levels for an extensive reference collection of species and cultivars of liriopogons. Identification was accomplished using existing keys, nomenclature was corrected, and numerous accessions were reassigned based on morphology. Genome sizes were determined by flow cytometry. Ploidy levels for each species were confirmed by traditional cytology. Results confirmed a basic chromosome number of $x = 18$ for liriopogons with aneuploidy, polyploidy, and cytochimeras found in some cases. The *Liriope* examined included diploids (*L. graminifolia*, *L. longipedicellata*, *L. minor*, and some of the *L. platyphylla*), tetraploids (*L. muscari* and the remaining *L. platyphylla*), and hexaploids (*L. exiliflora* and *L. spicata*). The *Ophiopogon* studied included diploids (*O. intermedius*, *O. jaburan*, *O. planiscapus*, and *O. umbraticola*) and a tetraploid/hypotetraploid species (*O. japonicus*). Monoploid (1Cx) genome sizes varied by genus and species with 1Cx values ranging from 4.27 pg in *L. exiliflora* to 8.15 pg in *O. jaburan*. These results clarify nomenclature and taxonomy and provide specific information on genome sizes and ploidy levels of cultivated liriopogons. This information and associated reference collection will aid future taxonomic revisions and enhance efforts to develop new cultivars of liriopogons.**

Liriopogons (most recently Ruscaceae s.l. Hutch, formerly assigned to Convallariaceae Horan., Asparagaceae Juss., Haemodoraceae Arnot, Ophiopogonaceae Kunth, and Liliaceae Juss.) (Kim et al., 2010) comprise a class of valuable evergreen groundcovers (Skinner, 1971). Liriopogons are native to China, India, Japan, Korea, the Philippines, and Vietnam with *Liriope* consisting of approximately eight species (Chen and Tamura, 2000a) and *Ophiopogon* consisting of ≈65 species (Chen and Tamura, 2000b). Popularity of liriopogons is attributable, in part, to their adaptability (Li et al., 2011) and versatility in the landscape, easily filling the roles of groundcovers, foundation plants, edging and massing plants, and understory plants (Fantz, 1993).

The complex taxonomy of liriopogons has been developing since the initial designation of *Convallaria japonica* by Thunberg (1780). The following centuries resulted in many genera designations (*Anemarrhena* Bunge, *Chloopsis* Blume, *Convallaria* L., *Flueggea* Rich., *Liriope*, *Mondo* Adans., *Ophiopogon*, *Polygonastrum* Moench, and *Slateria* Desv.) and common names (aztec grass, bordergrass, lilyturf, liriope, mondo grass, monkeygrass, and snakesbeard) (Fantz, 1993; Nesom, 2010). Nevertheless, liriopogons' attractiveness, resistance to pests and diseases, hardiness, and utility in the landscape have made them important nursery crops. Wholesale values of liriopogons in North Carolina are estimated to be over $41 million for 2009 (Trueblood, 2009).

Much confusion surrounding liriopogons lies in morphological similarities between the two genera. Both *Liriope* and *Ophiopogon* are acaulescent, evergreen herbs that set summer/fall racemes of small pink to purple or white flowers. Floral whorls are found in multiples of three (dichasia to compound dichasia to small cymes) (Fantz, 2008a). The perianth has six indistinguishable sepals and petals and six stamens. Fruits of liriopogons are blue/black and berry-like or a three-celled capsule (Fantz, 2008a).

Anatomical studies by Cutler (1992) and Rudall (2000), as well as a molecular marker investigation by Mcharo et al. (2003), concluded that similarities between *Liriope* and *Ophiopogon* were too great to warrant separation into two genera. However, morphological studies by Bailey (1929), Conran and Tamura (1998), Hume (1961), and Skinner (1971), molecular phylogenetic studies by Kim et al. (2010), and a molecular marker study by Li et al. (2011) provided evidence supporting separation of *Liriope* and *Ophiopogon*.

A recent overview of *Liriope* and *Ophiopogon* cultivated in the United States by Nesom (2010) found floral characteristics the best method of distinguishing between *Liriope* and *Ophiopogon*, supporting Fantz (2008a). Flowers belonging to *Liriope* are erect with corollas cupulate to rotate and free anthers with apical poricidal openings and long filaments. In contrast, flowers of *Ophiopogon* are nodding with corollas campanulate and connate anthers in a column, which narrow apically, dehisce longitudinally, and have subsessile filaments (Fantz, 2008a; Nesom, 2010).

In addition to the historically complex taxonomy of liriopogons, nursery practices including sexual propagation of cultivars, plant substitution, mislabeling of cultivars, and seedling invasion of stock plants have resulted in cultivar degradation within the nursery industry (Fantz, 1994). Fantz (1994) investigated 22 named species and 88 labeled cultivars of *Liriope* and *Ophiopogon* collected from nurseries and found 17% of germplasm misidentified to genus and 36% misidentified to species.

A variety of ornamental features such as flower color, inflorescence height, inflorescence branching and fasciation, fruit color, foliar variegation, and medicinal qualities such as steroidal glycosides in tubers (Cheng, et al., 2006; Wang et al., 2012; Yu et al., 1996) indicate a high potential for breeding and improvement of liriopogons. A recent study by Zhou et al. (2009) also demonstrated that hybridization between tetraploid *L. spicata* and diploid *Ophiopogon* may be occurring naturally in the wild, suggesting new possibilities for breeding between genera in liriopogons. However, breeding systems and cytogenetics of liriopogons are complex. Previous karyological studies have demonstrated the basic chromosome number for liriopogons to be $x = 18$ (rarely $x = 17$) with high levels of polyploidy in many species (Table 1). Also, Fukai et al. (2008) investigated ploidy level and relative genome size through flow cytometry of six species (plus cultivars) of liriopogons (Table 1). Oinuma (1946) reported polyploid forms of liriopogons exhibited increased vigor and grew over a wider geographic

distribution than diploid forms. In addition to various ploidy levels, many studies have reported liriopogons to be uniquely tolerant of high amounts of aneuploidy (abnormal number of chromosomes) and cytochimerism (different chromosome numbers among cells in the same plant) (Table 1). Therefore, evaluating the cytogenetics of individual clones and cultivars is critical to developing a breeding strategy for liriopogons.

As a result of the wide range of ornamental traits found in liriopogons and evidence of interspecific and intergeneric hybridization, there is considerable potential for breeding and improvement of liriopogons. However, these efforts are constrained by confusion over proper taxonomy, lack of information on ploidy levels, and lack of information on cytogenetics of individual clones and cultivars. Objectives of this study were to 1) validate the identification and nomenclature; and 2) determine genome sizes and ploidy levels for an extensive reference collection of liriopogons.

Materials and Methods

Plant material. Accessions of diverse species and cultivars of liriopogons were collected from nurseries, arboreta, and various individuals (Table 2). Containerized and field specimens of liriopogons were examined in this study including *L. exiliflora* (L.H. Bailey) H. H. Hume, *L. gigantea* H. H. Hume, *L. graminifolia* (L.) Baker, *L. longipedicellata* F.T. Wang and T. Tang, *L. minor* (Maxim.) Makino, *L. muscari* (Decne.) L. H. Bailey, *L. platyphylla* F. T. Wang and T. Tang, *L. spicata* (Thunb.) Lour., *O. intermedius* D. Don, *O. jaburan* (Siebold) Lodd., *O. japonicus* (L. f.) Ker Gawl., and *O. umbraticola* Hance. Multiple herbarium vouchers were collected for nearly all taxa and identified based on previous descriptions and available keys for liriopogons (Broussard, 2007; Chen and Tamura, 2000a, 2000b; Cutler, 1992; Fantz, 2008a, 2008b, 2009; Hasegawa, 1968; Liu et al., 2007; Nesom, 2010; Tamura, 1990; Tanaka, 2000, 2001a, 2001b, 2001c; Zhang, 1998). The primary collection will be deposited at the North Carolina State University Herbarium, Department of Plant and Microbial Biology, Raleigh, and the Herbarium of the U.S. National Arboretum.

Survey of genome sizes and ploidy levels. Genome sizes and ploidy levels were determined by traditional cytology in combination with flow cytometry. To prepare samples for flow cytometry, leaf tips ($\approx$1 cm^2) from expanded leaves of each taxa were placed in petri dishes containing 500 µL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer®; Partec, Münster, Germany) and chopped finely with a razor blade until completely incorporated into buffer. Resulting solutions were pipetted through CellTrics™ (Partec) disposable filters with a pore size of 50 µm. Then, 2 mL of a nucleotide staining buffer solution combined with 6 µL RNase A and 12 µL propidium iodide (CyStain PI absolute P; Partec) was added to the filtered solutions. Samples were refrigerated (4 °C) and incubated for over 30 min, and the resulting stained nuclei were analyzed with a flow cytometer (Partec PA II; Partec) with counts exceeding a minimum of 3000 cells per analysis.

Mean fluorescence for each sample was compared with an internal standard of known genome size (*Pisum sativum* L. 'Ctirad', 2C DNA = 8.76 pg), and holoploid, 2C genome size (i.e., DNA content of entire non-replicated chromosome compliment irrespective of ploidy) was calculated as 2C = DNA content of standard × (mean fluorescence of sample/mean fluorescence of standard). Monoploid genome sizes (1Cx = DNA content of the non-replicated base set of chromosomes, 1*x*) were calculated for each sample as (2C genome size/ploidy level). Monoploid genome sizes were subjected to analysis of variance by genus and species, and means were separated using Fisher's least significant difference (Proc GLM; SAS Version 9.2; SAS Inst., Cary, NC).

Chromosome counts were performed on selected species of liriopogons to confirm ploidy levels and to allow for calibration of ploidy level with genome size. A root squash technique was used that allowed for direct counting of chromosomes. Actively growing root tips were collected and placed in freshly made vials of pre-fixative solution (2 mM 8-hydroxyquinoline + 70 mg·L^{-1} cyclohexamide) at room temperature (22 °C). After remaining in the dark for 3 h, all vials were moved into a dark refrigerator at $\approx$4 °C for 3 h, yielding a total pre-fixative treatment of 6 h. Root tips were then rinsed with distilled water and transferred to a freshly made fixative of 1:3 propionic acid to 95% ethanol and left at room temperature overnight. The following day, a 1:3 hydrolysis solution of 12 M HCl to 95% ethanol was made for the root squash procedure.

For each root squash, a fresh root was removed from the fixative and hydrolyzed for 12 to 20 s before being moved to a clean slide. The root tip was excised using a dissecting microscope (StereoZoom 6 Photo; Leica Microsystems GmbH, Wetzlar, Germany) and placed on a separate, clean slide with a drop of modified carbol fuchsin stain (Carr and Walker, 1961; Kao, 1975). A coverslip was placed over the droplet of stain containing the excised root tip and a clean sheet of bibulous paper was placed over the slide while gently applying pressure with a pencil eraser. An average of 10 highly resolved cells per specimen were used to visualize the total number of chromosomes using a light microscope (Eclipse 80i, Nikon, Melville, NY). Extended depth of field was achieved by layered images containing multiple focal points using Photoshop CS4 (Adobe Systems, San Jose, CA).

Results and Discussion

Liriope exiliflora. The relatively large 2C genome size of *L. exiliflora* ranged from 24.89 to 26.18 pg (Table 2). This range of genome size fell between that of *L. gigantea* and *L. muscari*. Cytology determined *L. exiliflora* (MCI 2011-100) to be a hexaploid at 2*n* = 6*x* = 108 (Fig. 1A). Possibly, former cytological studies included *L. exiliflora* under a different synonym, although no former studies were found to compare with our findings. Our results lend evidence for the treatment of *L. exiliflora* as a separate species in agreement with Fantz (2008b) and in contrast to claims of synonymy with *L. muscari* by Nesom (2010). Cultivars Silver Dragon and Quail Garden were acquired as *L. spicata* and were reassigned *L. exiliflora* according to Fantz (2008b). However, further investigation into a close relationship between *L. exiliflora* and *L. spicata* may be warranted based on their similar morphology, ploidy, and monoploid genome size values (Table 3).

Liriope gigantea. Often confused with *L. muscari*, four cultivars of *L. gigantea* including 'Green Giant', 'Evergreen Giant', 'Lynn Lowrey', and 'Merton Jacobs' were found to have larger 2C genome sizes (26.23 to 28.86 pg) than any *L. muscari* (17.64 to 21.53 pg) included in this study (Table 2). Cytology determined *L. gigantea* (MCI 2011-099) to be a tetraploid at 2*n* = 4*x* = 72. Monoploid genome sizes were significantly larger in *L. gigantea* (6.92 pg) than *L. muscari* (4.76 pg). Former cytological studies possibly included *L. gigantea* under a different synonym, although no former studies were found to compare with our findings. Cultivars Green Giant, Evergreen Giant, and Lynn Lowrey were acquired as *L. muscari*. However, these cultivars were reassigned according to Fantz (2008b) and Nesom (2010) and based on similarity of genome size and ploidy level with known *L. gigantea*.

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From a thesis submitted by J.D.L. in partial fulfillment of the requirements for the MS degree.
[1]Graduate Research Assistant.
[2]Professor Emeritus and Research Taxonomist.
[3]Professor.
[4]Owner of Plant Delights Nursery and founder of Juniper Level Botanical Gardens.
[5]To whom reprint requests should be addressed; e-mail tom_ranney@ncsu.edu.

Table 1. Previous cytological and cytometric analyses of liriopogons.

Taxa	Synonyms[z]	Previous findings	References
L. graminifolia	*L. angutissima, L. crassiuscula*, *L. spicata*	$2n = 36$	Wang et al., 2013; Zhang, 1998
		$2n = 72$	Zhang, 1998
		$2n = 108$	Zhang, 1998
L. minor	*L. cernua, L. graminifolia* var. *minor*, *L. spicata* var. *minor*	$2n = 36$	Fukai et al., 2008; Ge et al., 1987; Hasegawa, 1968; Matsuura and Suto, 1935; Oinuma, 1946, 1949; Sato, 1942
L. muscari	*L. graminifolia* var. *densiflora*	$2n = 36$	Fu and Hong, 1989; Ge et al., 1987; Zhang, 1998
		$2n = 72$	Oinuma, 1946; Westfall 1950; Zhang, 1998
		$2n = 112$[y]	Zhang, 1998
L. platyphylla	*L. muscari*, *L. muscari* var. *communis*, *L. graminifolia* var. *communis*	$2n = 72$	Fukai et al., 2008; Hasegawa, 1968; Oinuma, 1946, 1949
		$2n = 108$	Oinuma, 1946, 1949
L. spicata	*L. koreana*	$2n = 36$	Hasegawa, 1968; Wang et al., 2013; Zhang, 1998
		$2n = 45$[y]	Kondo et al., 1992
		$2n = 72$	Kondo et al., 1992; Yang et al., 1990; Zhang, 1998, Zhou et al., 2009
		$2n = 88$[y]	Zhang, 1998
		$2n = 90$	Zhang, 1998
		$2n = 108$	Fukai et al., 2008; Hasegawa, 1968; Liu et al., 1985; Terasaka and Tanaka, 1974; Zhang, 1998
O. intermedius	*O. aciformis*, *O. wallichianus*	$2n = 36$	Larsen, 1963; Malik, 1961; Roy et al., 1988, Sarkar et al., 1974; Sharma and Chaudhuri, 1964; Wang et al., 2013; Yang et al., 1990; Zhang, 1998
		$2n = 68$[y]	Sen, 1973
		$2n = 72$	Zhang, 1998
		$2n = 108$	Malik, 1961; Sheriff and Singh, 1975; Wang et al., 2013; Zhang, 1998
		$2n = 112$[y]	Dudgeon, 1923
O. jaburan		$2n = 36$	Denda et al., 2006; Hasegawa, 1968; Ko et al., 1985; Matsuura and Suto, 1935; Sato, 1942; Yamashita and Tamura, 2001
O. japonicus	*O. stolonifer*, *O. argyi*, *O. checkiangensis*	$2n = 34$[y]	Wang et al., 2013; Zhang, 1998
		$2n = 36$	Sato, 1942; Wang and Xu, 1990; Zhang, 1998
		$2n = 67$[y]	Nagamatsu and Noda, 1964, 1971
		$2n = 68$[y]	Cao et al., 2002; Ge et al., 1987, Liang et al., 1998; Liu et al., 1985; Nagamatsu and Noda, 1964, 1971; Wang et al., 2013; Zhang, 1998
		$2n = 70$[y]	Yamashita and Tamura, 2001
		$2n = 72$	Fukai et al., 2008; Hasegawa, 1968; Hsu, 1971; Ko et al., 1985; Liu et al., 1985; Oinuma, 1944, 1946, 1949; Sato, 1942; Sharma and Chaudhuri, 1964; Wang et al., 2013; Yang et al., 1990; Zhang, 1998
		$2n = 108$	Zhang, 1998
O. planiscapus		$2n = 36$	Fukai et al., 2008; Hasegawa, 1968; Hsu, 1971; Oinuma, 1946, 1949; Yamashita and Tamura, 2001
		$2n = 72$	Sato, 1942; Oinuma, 1944, 1946, 1949
O. umbraticola	*O. japonicus* var. *umbraticola*, *O. chingii*	$2n = 68$[y]	Zhang, 1998

[z]Synonyms for taxa obtained from cited studies, from Tropicos®, Missouri Botanic Garden, or from common misidentifications in the U.S. nursery industry.
[y]Possible hypoploid or hyperploid specimen based on basic chromosome number of $x = 18$.

Liriope graminifolia. This grass-like species of *Liriope* is characterized by thin, soft leaves with heavy flowering inflorescences hidden among or just topping the leaves (Chen and Tamura, 2000a). *Liriope* matching this description in the present study included 'Porcupine', a clone (MCI 2010-063) early-blooming in April in North Carolina, and a wild-collected clone (MCI 2012-098) from Sichuan, China (D. Probst, personal communication). Although the leaf form was similar for all specimens in this group, inflorescence length varied. Some specimens exhibited flowers blooming well above the foliage (initially thought to be a narrow leaf form of *L. platyphylla*) and some specimens exhibited flowers blooming among the foliage. The 2C genome size for *L. graminifolia* ranged from 10.44 to 11.08 pg (Table 2) and cytology determined *L. graminifolia* Clone B (MCI 2012-098) and *L. graminifolia* 'Porcupine' (MCI 2010-056) to be a diploids with $2n = 2x = 36$. Monoploid genome sizes for *L. graminifolia* (5.41 pg) did not significantly differ from the morphologically similar diploid, *L. minor* (5.60 pg) (Table 3). In addition to diploids, previous research has reported *L. graminifolia* to be tetraploid ($2n = 72$) and hexaploid ($2n = 108$) (Table 1). *Liriope graminifolia* Clone B was acquired as *O. intermedius* and reassigned according to Chen and Tamura (2000a) and Nesom (2010).

Liriope longipedicellata. As the name suggests, the most identifiable feature of *L. longipedicellata* is its extended pedicels (Chen and Tamura, 2000a), giving the inflorescence a bottle brush appearance. Otherwise, the species resembles the narrow-leaved *L. graminifolia*, and similar genome sizes and ploidy levels further suggest a close relationship. *Liriope longipedicellata* 'Grape Fizz' was found to have a 2C genome size of 11.10 pg and the wild-collected *L. longipedicellata* (MCI 2012-092) was found to have a 2C genome size 12.31 pg, just outside the observed range for *L. graminifolia* (Table 2). Cytology demonstrated *L. longipedicellata* (MCI 2012-092) to be a diploid with $2n = 2x = 36$. Monoploid genome sizes for *L. longipedicellata* (5.86 pg) were found to be similar to *L. minor* (5.60 pg) but larger than *L. graminifolia* (5.41 pg), all being narrow leaf liriopes (Table 3). Although it is possible that former cytological studies included *L. longipedicellata* under a different synonym, no former studies were found to compare with our findings. *Liriope longipedicellata* 'Grape Fizz' was acquired as *O. intermedius* 'Grape Fizz' and was reassigned according to Chen and Tamura (2000a).

Liriope minor. This species represents a spreading, dwarf *Liriope* with narrow leaves occasionally blotched yellow with leaf and inflorescence length less than 20 cm (Chen and Tamura, 2000a; Fantz, 2008b). Both specimens in this study exhibited yellow blotched variegation. Cytometry revealed 2C genome sizes from 11.08 to 11.31 pg (Table 2), placing it in a similar range as *L. graminifolia* and *L. longipedicellata*. Further cytological examination of both specimens revealed *L. minor* to be a diploid $2n = 2x = 36$ (Fig. 1B), concurring with previous studies reporting *L. minor* also to be a diploid (Table 1). The similar appearance, genome sizes, and ploidy levels revealed in this study suggest a close relationship among *L. graminifolia*, *L. longipedicellata*, and *L. minor* with *L. minor*

Table 2. Genome sizes and estimated ploidy levels of cultivated *Liriope* and *Ophiopogon*.

Taxa	Accession[z]	2C genome size[y]	Ploidy[x]	1Cx genome size (pg)[w]
L. exiliflora	MCI 2011-100	24.89 ± 0.87	6*x*[t]	4.15
L. exiliflora 'Silver Dragon'	MCI 2010-039	25.81 ± 0.38	6*x*	4.30
L. exiliflora 'Quail Garden'	MCI 2010-043	26.18 ± 0.48	6*x*	4.36
L. gigantea 'Green Giant'	MCI 2011-099	27.90 ± 0.20	4*x*[t]	6.89
L. gigantea 'Evergreen Giant'	MCI 2010-041	28.86 ± 0.40	4*x*	7.22
L. gigantea 'Lynn Lowrey'	JCRA 042084	28.05 ± 0.64	4*x*	7.01
L. gigantea 'Merton Jacobs'	PDN JL12001	26.23 ± 0.04	4*x*	6.56
L. graminifolia (Early Blooming)	MCI 2010-063	11.08 ± 0.19	2*x*[t]	5.54
L. graminifolia 'Porcupine'	MCI 2010-056	10.73 ± 0.13	2*x*[t]	5.37
L. graminifolia 'Porcupine'	PDN JL12002	11.04 ± 0.27	2*x*	5.52
L. graminifolia (Clone B)	MCI 2012-098	10.44 ± 0.14	2*x*[t]	5.22
L. longipedicellata	MCI 2012-092	12.31 ± 0.10	2*x*[t]	6.16
L. longipedicellata 'Grape Fizz'	MCI 2012-100	11.10 ± 0.19	2*x*	5.55
L. minor	MCI 2011-101	11.08 ± 0.05	2*x*[t]	5.54
L. minor 'Torafu'	MCI 2010-023	11.31 ± 0.14	2*x*[t]	5.65
L. muscari 'Big Blue'	MCI 2010-033	21.40 ± 0.10	4*x*	5.35
L. muscari 'Bigun' Cleopatra™	MCI 2007-183	20.38 ± 0.09	4*x*	5.09
L. muscari 'Blue Giant'	JCRA 020548	20.66 ± 0.02	4*x*	5.17
L. muscari 'Hawk's Feather'	JCRA xx0048	18.27 ± 0.17	4*x*	4.57
L. muscari 'John Burch'	PDN JL12003	17.64 ± 0.10	4*x*	4.41
L. muscari 'Marant' Marc Anthony®	MCI 2007-182	18.45 ± 0.28	4*x*	4.61
L. muscari 'Monroe White'	MCI 2010-040	18.16 ± 0.11	4*x*	4.54
L. muscari 'Okina'	JCRA 950596	17.94 ± 0.44	4*x*	4.49
L. muscari 'Pee Dee Ingot'	MCI 2010-042	19.98 ± 0.16	4*x*	5.00
L. muscari 'Samona'	MCI 2011-143	18.17 ± 0.29	4*x*	4.54
L. muscari 'Sideswiped'	MCI 2012-097	18.36 ± 0.18	4*x*	4.59
L. muscari 'Silvery Sunproof'	MCI 2010-046	18.56 ± 0.35	4*x*[t]	4.64
L. muscari 'Sno Cone'	JCRA 080869	18.49 ± 0.07	4*x*	4.62
L. muscari 'Snow Dragon'	PDN JL12004	18.16 ± 0.09	4*x*	4.54
L. muscari 'Superba'	JCRA 020549	21.53 ± 0.62	4*x*	5.38
L. muscari 'Tokai Waname'	PDN JL12005	18.50 ± 0.11	4*x*	4.63
L. muscari (Yellow Splash)	JCRA 031562	19.01 ± 0.42	4*x*	4.75
L. platyphylla	MCI 2012-124	10.02 ± 0.12	2*x*	5.01
L. platyphylla	MCI 2010-019	10.23 ± 0.14	2*x*[t]	5.11
L. platyphylla	MCI 2010-048	10.36 ± 0.01	2*x*	5.18
L. platyphylla (branched infl.)	MCI 2012-095	10.71 ± 0.21	2*x*	5.35
L. platyphylla	MCI 2010-153	19.55 ± 0.25	4*x*	4.89
L. platyphylla	MCI 2010-051	19.90 ± 0.27	4*x*[t]	4.97
L. platyphylla (ABG Form)	MCI 2012-125	19.04 ± 0.24	4*x*	4.76
L. platyphylla 'Korean Giant'	MCI 2011-127	19.95 ± 0.26	4*x*	4.99
L.spicata	JCRA (S07)	23.85 ± 0.02	6*x*[t]	3.98
L. spicata	Gardner Arb.	23.91 ± 0.36	6*x*	3.99
O. intermedius 'Aztec'	MCI 2011-098	11.10 ± 0.06	2*x*[t]	5.55
O. intermedius 'Twisted Variegated'	MCI 2010-236	11.21 ± 0.17	2*x*	5.60
O. intermedius 'Variegata'	PDN JL12006	11.16 ± 0.01	2*x*	5.58
O. jaburan 'HOCF' Crystal Falls®	MCI 2007-146	16.08 ± 0.06	2*x*	8.04
O. jaburan 'Crystal Fan'	MCI 2010-047	16.30 ± 0.18	2*x*[t]	8.15
O. jaburan 'Vittatus'	MCI 2010-045	16.45 ± 0.45	2*x*	8.22
O. jaburan 'Ursala's Blue Fruit'	PDN JL12007	16.21 ± 0.12	2*x*	8.11
O. jaburan 'Wuhan Variegated'	MCI 2010-045	16.49 ± 0.42	2*x*	8.25
O. japonicus	MCI 2010-054	20.64 ± 0.50	4*x*[u]	5.16
O. japonicus 'Aritake'	PDN JL12008	20.39 ± 0.22	4*x*[u]	5.10
O. japonicus 'Bluebird'	MCI 2010-055	20.95 ± 0.25	4*x*[u]	5.24
O. japonicus 'Comet'	MCI 2010-061	21.21 ± 0.01	4*x*[u]	5.30
O. japonicus 'Fiuri Gyoku Ryu'	MCI 2010-058	14.90 ± 0.20	3*x*[u,v]	4.97
		21.00 ± 0.00	4*x*[u,v]	5.25
O. japonicus 'Gyoku Ryu'	JCRA 981407	22.04 ± 0.36	4*x*[u]	5.51
O. japonicus 'Shiroshima Ryu'	MCI 2012-099	20.75 ± 0.25	4*x*[u]	5.19
O. japonicus 'Tama Ryu Nishiki'	MCI 2010-111	15.65 ± 0.35	3*x*[u,v]	5.22
		21.20 ± 0.10	4*x*[u,v]	5.30
O. japonicus 'Variegatus'	MCI 2010-053	18.20 ± 0.10	3*x*[u,v]	6.07
		21.55 ± 0.05	4*x*[u,v]	5.39
O. japonicus var. *caespitosa* 'Seoulitary Man'	MCI 2010-057	25.44 ± 0.13	4*x*[t]	6.36
O. japonicus var. *gracilis* 'Tears of Gold'	MCI 2010-064	25.24 ± 0.09	4*x*	6.31
O. planiscapus 'Arabicus'	MCI 2010-024	12.66 ± 0.15	2*x*	6.33
O. planiscapus 'Black Dragon'	MCI 2010-037	12.63 ± 0.18	2*x*	6.31
O. planiscapus 'Ebknizam'	MCI 2010-038	12.46 ± 0.06	2*x*	6.23
O. planiscapus 'Edge of Night'	PDN JL12009	12.15 ± 0.32	2*x*	6.07
O. planiscapus 'Haku Ryo Ko'	MCI 2010-110	12.54 ± 0.07	2*x*	6.27
O. planiscapus 'Little Tabby'	MCI 2010-062	12.36 ± 0.02	2*x*[t]	6.18
O. planiscapus 'Nigrescens'	MCI 2010-043	12.45 ± 0.06	2*x*[t]	6.23

(*Continued on next page*)

Table 2. (*Continued*) Genome sizes and estimated ploidy levels of cultivated *Liriope* and *Ophiopogon*.

Taxa	Accession[z]	2C genome size[y]	Ploidy[x]	1Cx genome size (pg)[w]
O. planiscapus var. *leucanthus*	PDN JL12010	12.33 ± 0.00	2*x*	6.16
O. umbraticola	MCI 2010-059	14.41 ± 0.19	2*x*[t]	7.20
O. umbraticola	JCRA 990336	14.45 ± 0.09	2*x*	7.23

[z]JCRA = J.C. Raulston Arboretum, Raleigh, NC; PDN = Plant Delights Nursery, Raleigh, NC; MCI = Mountain Crop Improvement Laboratory, North Carolina State University, Mills River, NC.; Gardner Arb. = Gardner Arboretum, North Carolina State University, Raleigh, NC.
[y]Holoploid genome sizes were determined using propidium iodide as the flourochrome stain. Values are x ± SEM, n = 2–3.
[x]Ploidy levels, 2*x* = diploid; 3*x* = triploid; 4*x* = tetraploid; 5*x* = pentaploid; 6*x* = hexaploids.
[w]1Cx values were calculated as 2C value/ploidy level.
[v]Cytochimera of multiple ploidy levels (mixoploid) confirmed by flow cytometry.
[u]Possible hypoploid specimens based on high variability in attempted chromosome counts.
[t]Ploidy levels confirmed by cytology.

having the smallest stature. *Liriope minor* 'Torafu' was acquired as *L. muscari* 'Torafu' and reassigned according to Chen and Tamura (2000a) and Fantz (2008b).

Liriope muscari. Seventeen cultivars of *L. muscari* ranged in 2C genome size from 17.64 to 21.53 pg (Table 2). Cultivars with large genome sizes such as 'Superba' (21.53 pg) and 'Big Blue' (21.40 pg) were observed to have the most vigor (fastest growth and largest clumps) and heaviest fruit set of all *L. muscari* tested (J. Lattier, personal observation). A possible explanation for this is suggested in an earlier study by Westfall (1950) where many *L. muscari* studied were found to be sterile or only partially fertile as a result of high levels of aneuploidy. In addition, hypotetraploid lines were found to have high levels of variation in inflorescence morphology, leaf width, vigor, and fertility (Westfall, 1950). Our results indicate a similar trend with all green leaf forms (except for the white-flowering 'Monroe White') having a 2C genome size greater than 20 pg and all variegated forms or forms with abnormal inflorescences having a 2C genome size less than 20 pg (Table 2). Monoploid genome sizes for *L. muscari* (4.76 pg) indicated a unique genome size within *Liriope*, with the exception of *L. platyphylla* which had a similar genome size of (5.03 pg) (Table 3). Cytology of 'Silvery Sunproof' confirmed it to be a tetraploid with 2*n* = 4*x* = 72, contrasting with one report of a diploid at 2*n* = 36 and one report of a rare specimen at 2*n* = 112 for *L. muscari* (Table 1).

Liriope platyphylla. Recent taxonomic studies including Nesom (2010) have treated the broad-leaved *L. platyphylla* as synonymous with *L. muscari*, or a variety of *L. muscari*, based on the merger of these two species by Hara (1984) and Hsu and Li (1981). Also, molecular studies have indicated a close relationship between *L. muscari* and *L. platyphylla* (Wu et al., 1998). However, the combination of the two species has led to much confusion in interpretation of previous literature and identification of this distinctive *Liriope* (Fantz, 2008b). Samples in the present study were distinguishable easily from *L. muscari* in agreement with Fantz (2008b) based on their wide, leathery leaves and elongated inflorescence extending well above the foliage. Eight specimens were tested including one with a branching rachis (MCI 2012-095), one particularly large form from the Atlanta Botanical Garden with a nearly 4-foot tall inflorescence (MCI 2012-125) and one cultivar (Korean Giant). Flow cytometry revealed a ploidy series including four specimens with genome sizes from 10.02 to 10.71 pg and four specimens with 2C genome sizes from 19.04 to 19.95 pg (Table 2). Although morphologically distinct, 1Cx values of *L. platyphylla* (5.03 pg) were similar to *L. muscari* (4.76 pg) (Table 3). There was a general trend of the tetraploid specimens exhibiting more vigorous growth and larger overall sizes. A further cytological study was conducted finding MCI 2010-019 to be a diploid with 2*n* = 2*x* = 36, whereas MCI 2010-051 was found to be a tetraploid with 2*n* = 4*x* = 72. The only other reported ploidy level for *L. platyphylla* was found to be hexaploid with 2*n* = 108, although it is likely that cytological studies have been conducted under different synonyms (Table 1).

Liriope spicata. The relatively large 2C genome size of *L. spicata* ranged from 23.85 to 23.91 pg but was slightly lower than *L. exiliflora*. Monoploid genome sizes for *L. spicata* (3.99 pg) and *L. exiliflora* (4.27 pg) were not significantly different (Table 3). Cytology determined *L. spicata* (JCRA S07) to be a hexaploid at 2*n* = 6*x* = 108. In addition to hexaploids, previous research has reported many different ploidy levels for *L. spicata* including (2*n* = 45, 72, 88, 90) (Table 1). This species represents a diminutive *Liriope*, which spreads aggressively by rhizomes (Fantz, 2008b). This species is often confused in the trade with the larger *L. exiliflora*, which clumps for several years before spreading, although less aggressively than *L. spicata* (Fantz, 2008b).

Ophiopogon intermedius. Cytometric analysis of 'Aztec', 'Twisted Variegated', and two samples of 'Variegatus' showed a range of 2C genome sizes from 11.10 to 11.21 pg (Table 2). Cytology determined 'Aztec' to be a diploid with 2*n* = 2*x* = 36. However, previous studies have reported wide ranges of ploidy levels for *O. intermedius* at 2*n* = 36, 68, 72, 108, and 112 (Table 1). *Ophiopogon intermedius* 'Aztec' was acquired as *L. spicata* 'Aztec' and was reassigned in agreement with Fantz (2009) and Nesom (2010). *Ophiopogon intermedius* 'Aztec' is misidentified commonly in the trade as *L. muscari* or *O. jaburan* (Fantz, 2009).

Ophiopogon jaburan. Cytometry of *O. jaburan* 'HOCF', 'Crystal Fan', 'Vittatus', 'Ursala's Blue Fruit', and 'Wuhan Variegated' found a range of 2C genome sizes from 16.08 to 16.49 pg (Table 2). Cytology of 'Crystal Fan' documented it to be a diploid with 2*n* = 2*x* = 36. Interestingly, *O. jaburan* had a significantly larger monoploid genome size (8.15 pg) than all other liriopogons (Table 3). Compared with other liriopogons in this study, *O. jaburan* had a surprisingly consistent cytological record of existing

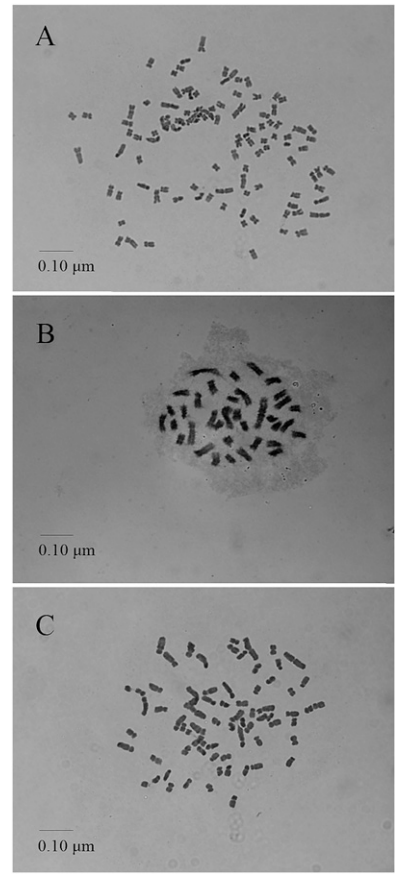

Fig. 1. Representative photomicrographs of metaphase chromosomes from root tip cell of liriopogons viewed at ×1000: (**A**) *Liriope exiliflora* (2*n* = 6*x* = 108); (**B**) *L. minor* (2*n* = 2*x* = 36); (**C**) *Ophiopogon japonicus* var. *caespitosa* 'Seoulitary Man' (2*n* = 4*x* = 72).

Table 3. Monoploid (1Cx) genome sizes of cultivated *Liriope* and *Ophiopogon* determined using flow cytometry and grouped by genus and species.

Genus	1Cx (pg)	Species	1Cx (pg)
Liriope	5.10 ± 0.12 a[z]	*L. exiliflora*	4.27 ± 0.06 a[z]
Ophiopogon	6.27 ± 0.19 b	*L. gigantea*	6.92 ± 0.14 g
		L. graminifolia	5.41 ± 0.07 d
		L. longipedicellata	5.86 ± 0.04 e
		L. minor	5.60 ± 0.06 de
		L. muscari	4.76 ± 0.08 b
		L. platyphylla	5.03 ± 0.06 bc
		L. spicata	3.99 ± 0.01 a
		O. intermedius	5.58 ± 0.01 de
		O. jaburan	8.15 ± 0.04 h
		O. japonicus	5.27 ± 0.04 cd
		O. japonicus var.[y]	6.34 ± 0.03 f
		O. planiscapus	6.22 ± 0.03 f
		O. umbraticola	7.22 ± 0.02 g

[z]Values represent means ± SEM. Means separated within columns using Fisher's least significant difference with $P < 0.05$.

[y]Includes the morphologically unique varieties *O. japonicus* var. *caespitosa* and *O. japonicus* var. *gracilis*.

primarily as a diploid at $2n = 36$ (Table 1) in agreement with our findings.

Ophiopogon japonicus. Cytometry of 11 taxa of *O. japonicus* showed two distinct ranges of 2C genome sizes. The majority of *O. japonicus* samples ranged from 20.39 to 22.04 pg, whereas 'Tears of Gold' and 'Seoulitary Man' ranged from 25.24 to 25.44 pg (Table 2). These two clones represented varieties with larger overall form and were found to have a significantly different 1Cx value (6.34 pg) than the other *O. japonicus* (5.27 pg) (Table 3). Originally thought to be a hyperploid specimen, cytology confirmed 'Seoulitary Man' to be a tetraploid with $2n = 4x = 72$ (Fig. 1C). Ploidy analysis remains inconclusive for other *O. japonicus*, likely as a result of high levels of hypotetraploidy as reported in previous studies (Table 1). A wide range of ploidy levels has been previously reported for *O. japonicus* including $2n = 36$, 67, 68, 70, and 72 (Table 1). In the present study, three samples examined using flow cytometry ('Fiuri Gyoku Ryu', 'Tama Ryu Nishiki', and 'Variegatus') yielded multiple fluorescence peaks with hypotetraploid peaks being associated with the variegated tissue. This phenomenon was only observed in variegated cultivars of *O. japonicus*. Wang and Xu (1990) also found cytochimeras in *O. japonicus* with diploid, triploid, and tetraploid cells existing in the same plant.

Ophiopogon planiscapus. Of the eight taxa of *O. planiscapus* tested, 2C genome sizes ranged from 12.15 to 12.66 pg (Table 2). With the exception of the few large varieties of *O. japonicus* included in this study, *O. planiscapus* had a significantly different 1Cx value (6.22 pg) among the liriopogons. Cytology of the cultivar Nigrescens showed it to be $2n = 2x = 36$. The majority of former cytological studies of *O. planiscapus* agrees with the present study; however, tetraploid forms ($2n = 72$) have been reported (Table 1).

Ophiopogon umbraticola. Often mislabeled as *O. chingii* in the nursery industry (J. Lattier, personal observation), two samples of *O. umbraticola* (MCI 2010-059, JCRA 990336) had 2C genome sizes ranging from 14.41 to 14.45 pg, which represents a unique range of genome size compared with all liriopogons tested in the present study (Table 2). Further cytological study of *O. umbraticola* (MCI 2010-059) revealed it to be a diploid at $2n = 2x = 36$ in contrast to one previous report of hypotetraploidy, $2n = 68$ (Table 1). Monoploid genome sizes for *O. umbraticola* (7.22 pg) were significantly different from the rest of the *Ophiopogon* (Table 3). *Ophiopogon umbraticola* (MCI 2010-059) was acquired as *O. chingii* and reassigned according to Chen and Tamura (2000b) and Tanaka (2001a).

Information on ploidy levels and genome sizes can have important implications for plant breeding (Ranney, 2006). Intraploid hybridizations are often more productive than interploid hybridizations, although interploid hybridizations can often provide an avenue for developing seedless cultivars. Also, compatibility of genomes/chromosomes is necessary for meiosis to function properly, and similarity in genome sizes can be indicative of close phylogenetic relationships and genome compatibility within taxonomic groups. Development of fertile hybrids may be improved when breeding among plants with similar genome sizes and ploidy levels (Parris et al., 2010).

This study details the development and documentation of an extensive collection of both living specimens and herbarium vouchers for liriopogons. Several source names were misidentified to genus, and many were misidentified to species like in a previous report by Fantz (1994). Genome sizes and ploidy levels were determined for all taxa in this study. Results confirm the basic chromosome number of $x = 18$ for liriopogons with aneuploidy, polyploidy, and cytochimeras found in some cases. Based on our sampling, *Liriope* examined fit into three ploidy groups with one exception of a ploidy series of *L. platyphylla*. The diploid group consisted of *L. graminifolia*, *L. longipedicellata*, *L. minor*, and some *L. platyphylla*. The tetraploid group consisted of *L. muscari* and the remaining *L. platyphylla*. The hexaploid group consisted of *L. exiliflora* and *L. spicata*. Although controversy surrounds the maintenance of *L. gigantea*, *L. graminifolia*, and *L. exiliflora* as separate species, differences in 1Cx genome size lends evidence for maintaining *L. gigantea* as a distinct species separate from *L. muscari* and as suggested by Fantz (2008b) and Nesom (2010), *L. muscari* as a distinct species separate from *L. exiliflora* or *L. spicata* as suggested by Fantz (2008b), and *L. graminifolia* as a distinct species separate from *L. spicata* or *L. exiliflora* as suggested by Chen and Tamura (2000a) and Nesom (2010). *Ophiopogon* included in this study formed two ploidy groups. The diploid group included *O. intermedius*, *O. jaburan*, *O. planiscapus*, and *O. umbraticola*. The tetraploid/hypotetraploid group consisted of *O. japonicus*. Monoploid genome sizes varied based on genus and species and ranged from 4.27 pg in *L. exiliflora* to 8.15 pg in *O. jaburan* (Table 3). Based on the taxa sampled, mean 1Cx genome sizes were smaller for *Liriope* (5.10 pg) than for *Ophiopogon* (6.27 pg) (Table 3). Although breeding efforts in the past have been limited by confusion over proper identification of germplasm and lack of information on ploidy levels and cytogenetics of available clones and cultivars, the reference collection established in this study will aid future revisions as well as assist in the development of breeding strategies for liriopogons.

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