Ploidy Levels and Interploid Hybridization in Panicle Hydrangea (*Hydrangea paniculata*)

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Significance to Industry: *Hydrangea paniculata* is a medium to large shrub noted for its excellent floral displays throughout summer, followed by notable winter interest as the flowers dry and persist through the seasons. Although this species is native to Japan, Taiwan, and parts of China, it is grown in landscapes around the world (3, 9).Breeding efforts have been recently been directed towards smaller stature, quality architecture, panicle size, and coverage of sterile (showy) florets, among other attributes. In nature, *H. paniculata* occurs as diploid, tetraploid, and hexaploid cytotypes, where 1*x* = 18. There is evidence that most plants in cultivation are tetraploids (10). However, little is known about the ploidy levels of specific cultivars or potential for interploid hybridization and fertility of anisoploid progeny. To better understand the reproductive biology and to further breeding efforts within *H. paniculata* the objectives of this study were to: 1) determine the ploidy levels of diverse clones and cultivars, and 2) determine fertility and cytotypes of interploid and anisoploid hybrids. Although most cultivated plants were tetraploids, pentaploids and hexaploids were also found. Information on ploidy levels of specific cultivars will facilitate the development of more strategic and efficient breeding programs. The potential for interploid hybridization and maintenance of fertility of pentaploids may also allow for the introgression of traits between these cytotypes and the development of improved cultivars.

Nature of Work: Samples of cultivated *H. paniculata* were collected from public gardens and arboreta for ploidy determination. Interploid crosses were completed between unnamed tetraploid *H. paniculata* and hexaploid *H. paniculata* 'Dharuma', a 1989 introduction to the market (9) to create pentaploids. Pentaploids (H2008-081-010 and H2008-149-032) were open-pollinated to create the H2012-191, H2012-189, and H2012-185 populations. 'Jane' Little Lime® (4*x*) x H2008-076-030 (5*x*) generated the H2013-133 population. The reciprocal cross, H2008-076-030 x 'Jane' Little Lime® generated the H2013-132 population. H2008-081-010 (5*x*) x 'Phantom' (4*x*) generated the H2012-127 population. Due to self-incompatibility in *H. paniculata*, interploid crosses were completed by isolating parents in pollination cages.

Flow Cytometry. Relative genome sizes (DNA content) were determined using flow cytometry (4). Tissue samples (~1 cm^2) were collected from expanding leaves, placed in a plastic petri dish with ~0.1g of young floral bud tissue from *Magnolia virginiana* 'Jim

Wilson' serving as an internal standard with a known genome size of 2C = 3.92 pg (5). Samples were finely chopped with a razor blade in 0.4 mL of nuclei extraction buffer (Partec CyStain UV Precise P Nuclei Extraction Buffer; Partec GMBH, Munster, Germany) and filtered through a 50 µm nylon mesh filter. Nuclei were stained with1.6 mL 4',6-diamidino-2-phenylindole (DAPI) immediately prior to analysis with a flow cytometer (Partec PA II, Munster, Germany). Samples were run until 5000 nuclei were counted with a CV of less than 5%. Two subsamples were analyzed for each accession.

Cytology. 'Jane' Little Lime®, H2009-149-046, and 'Dharuma' were used to confirm and calibrate ploidy levels with genome sizes for 4X, 5X, and 6X plants, respectively. Actively growing root tips were excised from containerized plants in late July and placed into 3 mL of 2mM 8-hydroxyquinoline/0.248mM cycloheximide. Roots were incubated in hydroxyquinoline/cycloheximide solution for 2 HR at 23°C, and moved to 40°F for an additional 3 HR. Roots were then fixed in 3mL of 3 parts 95% Ethanol : 1 part propionic acid fixative solution at 70°F for 18 HR prior to examination.

Results and Discussion: Ploidy levels and chromosome numbers for 'Jane' Little Lime®, H2009-149-046, and 'Dharuma' were confirmed to be tetraploid (2*n* = 4*x* = 72), pentaploid (2*n* = 5*x* = 90), and hexaploid (2*n* = 6*x* = 108), respectively. Ploidy levels were calibrated with genome sizes based on these representative cytotypes. Relative genome sizes were determined for 46 cultivars, 12 wild-collected accessions, and 61 interploid/anisoploid crosses of *H. paniculata* (Table 1). Mean genome sizes were found to be 7.4, 8.5, and 10.5 pg for 4*x*, 5*x*, and 6*x* plants respectively, clearly differentiating among ploidy levels. Overall mean1C_x genome size was1.80pg, slightly higher than values reported by Zonneveld (10) when using propidium iodide stain and *Agave americana* as an internal standard.

The vast majority of cultivated *H. paniculata* were found to be tetraploids (or near tetraploids) with 2C genome sizes ranging from 6.4 to 7.96 pg. 'Dharuma' and 'Praecox' were the only hexaploid cultivars found, though several wild-collected hexaploids from Japan were also identified. These two cultivars are noted for early blooming, although the panicles on 'Dharuma' have a flattened, less attractive appearance than other cultivars (2, 7, 8). The bloom-time differences and floral morphologies of 'Dharuma' and 'Praecox' have been noted before. However, these differences were thought to be a result of sub-specific variation, rather than the effect of ploidy (9). Three commercial cultivars were found to be pentaploid including 'Bulk' Quick Fire®, 'SMHPLQF' Little Quick Fire™, and 'Wim's Red' Fire and Ice. These most likely resulted from interploid hybridization, potentially with 'Dharuma' as a parent (9).

Interploid crosses between tetraploids and hexaploids produced pentaploid progeny with genome sizes ranging from 8.1 to 8.8 pg. Unlike many anisoploids, pentaploid *H. arborescens* maintained some fertility as both males and females. Crosses between pentaploids and tetraploids tended to produce progeny with genome sizes within or near the tetraploid range, suggestion a bias towards euploidy/isoploidy. Open pollenated pentaploids produced progeny with genome sizes ranging from 7.3 pg (~4X) to 9.0 pg

(~5X), as well as one plant with 12.8 pg (~7.5X), most likely resulting from an unreduced gamete from one parent. Reed (6) showed that *H. macrophylla* can produce unreduced gametes with some regularity. Triploids reported in by Funamoto (3) in *H. paniculata* could have been the result of unreduced gametes from diploid parents or potentially from interploid hybridization.

Another point of interest is the lack of diploid plants in commerce and from the wild-collected material. While Funamoto and Ogawa (3) suggested that there were no morphological differences between cytotypes of *H. paniculata*, it appears that tetraploids and to a lesser degree hexaploids have been preferentially selected for horticultural use.

These results document genome sizes and ploidy levels of diverse cultivars, hybrids, and wild-collected accessions of *H. paniculata*. Furthermore, it was found that interploid hybrids can be easily produced and that pentaploid hybrids maintain fertility and can produce offspring with a wide range of cytotypes including aneuploids, (near) euploids, and higher ploidy levels resulting from unreduced gametes.

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Table 1. Relative genome sizes and ploidy levels determined via flow cytometry for cultivars and hybrids of *H. paniculata.*

Source[z]	Taxa	Relative 2C genome size [mean ± SE (pg)]	Ploidy level (*x*)
SWG	2012SM12118 Taiwan	7.71 ± 0.02	4
MOR	417-99 'White Tiara'	7.51 ± 0.31	4
MOR	533-57 Kyoto U. Exp. Station, Japan	7.26 ± 0.07	4
MOR	101-99 Götenburg B.G., Sweden	7.67 ± 0.22	4
MOR	149-2011 'Paszam'	7.30 ± 0.17	4
CBG	'Angustipetala'	7.04 ± 0.15	4
CBG	'Barbara' The Swan™	7.96 ± 0.04	4
CBG	'Big Ben'	7.34 ± 0.11	4
PDN	BL 17-01	6.87 ± 0.08	4
CBG	'Bokrathirteen' Sweet Summer	7.30 ± 0.05	4
JCR	'Bokratorch' Magical™Flame	7.88 ± 0.53	4
CBG	'Bombshell'	7.29 ± 0.07	4
CBG	'Boskoop'	6.87 ± 0.05	4
CBG	'Brussel's Lace'	7.53 ± 0.13	4
CBG	'Burgundy Lace'	7.36 ± 0.17	4
CBG	'Chantilly Lace'	7.28 ± 0.02	4
CBG	'Dolly'	7.31 ± 0.03	4
CBG	'Dvppinky' Pinky Winky®	7.28 ± 0.13	4
CBG	'Floribunda'	7.35 ± 0.03	4
CBG	'Greenspire'	7.20 ± 0.07	4
CBG	'Hypmad I' White Diamonds®	7.06 ± 0.03	4
CBG	'Hypmad II' Tickled Pink®	7.31 ± 0.01	4
CBG	'ILVOBO' Bobo®	7.29 ±0.13	4
CBG	'Jane' Little Lime®	7.17 ±0.02	4
CBG	'Kyushu'	7.67 ± 0.17	4
CBG	'Le Vasterival' Great Star®	7.43 ± 0.08	4

CBG	'Little Lamb'	7.28 ± 0.02	4
CBG	'Mega Pearl'	7.30 ± 0.19	4
CBG	'Interhydia' Pink Diamond	7.02 ± 0.16	4
PDN	PD 01-01	6.57 ± 0.03	4
PDN	PD 14-01	6.74 ± 0.06	4
PDN	PD 14-01	6.41 ± 0.02	4
CBG	'Pink Lady'	7.45 ± 0.08	4
CBG	'Renhy' Vanilla Strawberry™	7.29 ± 0.01	4
CBG	'Revival' Honeycomb™	7.48 ± 0.02	4
CBG	'Ruby' Angel's Blush™	7.21 ± 0.12	4
SWG	'Shadow'	6.92 ± 0.09	4
JCR	'Shikoku Flash'	7.54 ± 0.10	4
CBG	'Silver Dollar'	7.33 ± 0.04	4
CBG	'Skylight'	7.29 ± 0.07	4
CBG	'SMHPFL' Fire Light®	7.27 ± 0.13	4
PDN	TCM 12-666	7.40 ± 0.02	4
CBG	'Unique'	7.52 ± 0.13	4
CBG	'Webb's Grandiflora'	7.22 ± 0.05	4
CBG	'White Lace'	6.93 ± 0.00	4
CBG	'White Lady'	7.42 ± 0.02	4
CBG	'White Moth'	7.00 ±0.19	4
CBG	'White Tiara'	7.27 ± 0.04	4
CBG	'WRHPBB2' Polar Bear™	7.54 ± 0.11	4
CBG	'Zwijnenburg' = 'Limelight'	7.15 ± 0.06	4
CBG	'Bulk' Quick Fire® (1)	8.76 ± 0.21	5
CBG	'SMHPLQF' Little Quick Fire™	8.47 ± 0.14	5
CBG	'Wim's Red' Fire and Ice	8.79 ± 0.04	5
CBG	'Dharuma'	10.48 ± 0.01	6
MOR	30-98 – Mt. Iwo, Hokkaido, Japan.	10.56 ± 0.03	6
MOR	374-82 'Praecox'	10.52 ± 0.16	6
MOR	135-99 Kuta, Kyoto, Japan.	10.73 ± 0.05	6
MOR	140-2009 'Dharuma'	10.49 ± 0.17	6
MOR	180-2001 Chiba U. Japan.	10.24 ± 0.04	6
MOR	186-2000 Tohaka U. Sendai, Japan.	10.41 ± 0.04	6

		4*x* × 6*x* Crosses	
MCI	H2008-076-030	8.53 ± 0.13	5
MCI	H2008-081-010	8.77 ± 0.01	5
MCI	H2008-081-018	8.12 ± 0.15	5
MCI	H2008-081-024	8.37 ± 0.10	5
MCI	H2008-082-011	8.53 ± 0.10	5
MCI	H2008-082-027	8.56 ± 0.03	5
MCI	H2008-095-002	8.45 ± 0.14	5
MCI	H2008-095-021	8.42 ± 0.08	5
MCI	H2008-095-027	8.67 ± 0.08	5
MCI	H2009-146-046	8.69 ± 0.04	5
MCI	H2009-149-032	8.28 ± 0.07	5
		5*x* × 4*x* Crosses	
MCI	H2013-127-001	7.52 ± 0.05	~4.2
MCI	H2013-127-002	7.52 ± 0.08	~4.2
MCI	H2013-127-003	7.48 ± 0.03	~4.2
MCI	H2013-127-004	7.57 ± 0.15	~4.2
MCI	H2013-127-005	7.51 ± 0.02	~4.2
MCI	H2013-127-009	7.62 ± 0.08	~4.2
MCI	H2013-127-010	7.66 ± 0.05	~4.3
MCI	H2013-127-015	7.58 ± 0.02	~4.2
MCI	H2013-127-016	7.62 ± 0.12	~4.2
MCI	H2013-127-025	7.63 ± 0.02	~4.2
MCI	H2013-127-028	7.51 ± 0.05	~4.2
MCI	H2013-132-001	7.61 ± 0.04	~4.2
MCI	H2013-132-002	8.19 ± 0.78	~4.6
MCI	H2013-132-003	7.61 ± 0.06	~4.2
MCI	H2013-132-004	8.17 ± 0.11	~4.5
MCI	H2013-132-005	7.47 ± 0.01	~4.2
MCI	H2013-132-006	7.68 ± 0.15	~4.3
MCI	H2013-132-007	9.71 ± 0.10	~5.4
MCI	H2013-132-008	8.10 ± 0.20	~4.5
MCI	H2013-132-009	7.64 ± 0.01	~4.3
MCI	H2013-132-010	8.85 ± 0.07	~4.9
		4*x* × 5*x* Crosses	
MCI	H2013-133-001	7.41 ± 0.02	~4.1
MCI	H2013-133-002	7.23 ± 0.01	~4.0
MCI	H2013-133-003	7.21 ± 0.07	~4.0
MCI	H2013-133-004	7.45 ± 0.01	~4.1

		5*x* Open Pollinated	
MCI	H2012-185-004	7.58 ± 0.21	~4.2
MCI	H2012-185-005	8.93 ± 0.33	~5.0
MCI	H2012-185-006	8.95 ± 0.01	~5.0
MCI	H2012-185-008	8.44 ± 0.19	~4.7
MCI	H2012-185-010	7.96 ± 0.23	~4.4
MCI	H2012-185-011	7.50 ± 0.31	~4.2
MCI	H2012-185-013	7.82 ± 0.09	~4.4
MCI	H2012-185-015	7.74 ± 0.04	~4.3
MCI	H2012-185-022	8.04 ± 0.04	~4.5
MCI	H2012-185-023	8.07 ± 0.03	~4.5
MCI	H2012-185-024	8.17 ± 0.30	~4.5
MCI	H2012-185-028	8.97 ± 0.13	~5.0
MCI	H2012-189-002	8.46 ± 0.02	~4.7
MCI	H2012-189-003	12.78 ± 0.04	~7.1
MCI	H2012-189-004	8.05 ± 0.03	~4.5
MCI	H2012-191-001	8.86 ± 0.21	~4.9
MCI	H2012-191-002	7.62 ± 0.02	~4.2
MCI	H2012-191-003	8.85 ± 0.00	~4.9
MCI	H2012-191-004	8.60 ± 0.06	~4.8
MCI	H2012-191-005	8.72 ± 0.15	~4.9
MCI	H2012-191-006	7.32 ± 0.02	~4.1
MCI	H2012-191-007	8.00 ± 0.15	~4.5
MCI	H2012-191-008	8.00 ± 0.15	~4.5
MCI	H2012-191-009	8.22 ± 0.06	~4.6
MCI	H2012-191-010	7.25 ± 0.05	~4.0

[z] Source Codes: CBG; Chicago Botanic Gardens. JCRA; JC Raulston Arboretum, Raleigh, NC. MCI; Mountain Crop Improvement Lab, Mills River, NC. MOR; Morton Arboretum. PDN; Plant Delights Nursery, Raleigh, NC. SWG; Smithgall Woodland Garden, Gainesville, GA.

[x] Also known by the name “Early Sensation” in Europe.