

Weigela species and cultivar genome size and ploidy estimations: shrub breeding[©]

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INTRODUCTION

Weigela are among the most popular flowering shrubs for temperate landscapes as they tolerate a wide range of cultural conditions, propagate easily from cuttings, and flower heavily in late spring. The genus is composed of 10 species native to China, Japan, Manchuria, and the Korean peninsula. Since the genus was brought to western horticulture near 1860, over two hundred cultivars have been introduced (Dirr, 2009; Sheffield Botanical Gardens, 2015). Introductions continue today with breeding work emphasizing the development of compact plants, novel foliage colors, and recurrent blooming characteristics. One cultivar, 'Courtalor', Carnaval[®] weigela is widely promoted as a reblooming polyploid (Pantin, 2015; Wood). Because polyploidy may be associated with ornamental characteristics that breeders may be selecting for, such as reblooming, we set out to investigate the presence of polyploidy in natural populations and extent of polyploidy in available cultivars. This manuscript reports genome size and ploidy estimations for 10 species and 46 cultivars, from a total of 74 accessions.

METHODS AND MATERIALS

Plant material was sampled from plants growing at The Morton Arboretum, the Chicago Botanic Garden, and the Arnold Arboretum of Harvard University. Genome sizes were determined by using a flow cytometer (CyFlow[®] PloidyAnalyser; Partec. Münster, Germany) with materials and protocols from Cystain PI absolute P test kits (Partec. Münster, Germany). Tissue samples were collected from expanding leaves and co-chopped with an internal standard, a leaf sample of *Pisum sativum* 'Ctirad', with a known genome size of 8.76 pg (Greilhuber et al., 2007). After chopping the sample was filtered through a 30-micron mesh filter (Celltrics[®]; Partec. Münster, Germany) and then stained with propidium iodide from the test kit. After staining the samples were immediately loaded and analyzed by the flow cytometer. Data was collected until at least 5000 nuclei of the unknown sample and at least 3000 nuclei of the internal standard were counted, CVs were maintained at less than 5% for the sample and the internal standard. Three replications were performed per genotype tested. Data was interpreted by one-way ANOVA ($P < 0.05$) and Fisher's LSD for means separation ($P < 0.05$). Our genome sizes were compared to reported chromosome counts to infer chromosome number and ploidy level.

RESULTS AND DISCUSSION

Genome sizes of our samples grouped from 1.91 to 2.32 pg of DNA; with one outlier, *W.* 'Courtalor', Carnaval[®] weigela at 3.03 pg of DNA (Table 1). Looking at literature, Duron and Decourtye report chromosome counts on the cultivar *W.* 'Newport Red' (syn. 'Vanicek') to be $2n=2x=36$, a diploid (1990); Sokolovskaya and Probatova (1985) report chromosome counts of *W. praecox* to be $2n=36$. Comparing these reports to our results we infer that the group with genome sizes of 1.91-2.32 pg of DNA are all diploid ($2n=2x=36$), and because *W.* 'Courtalor', Carnaval[®] weigela has approximately 1.5 times greater DNA content than the diploid group that it is a triploid (be $2n=3x=54$).

From our sampling across all ten species and from across some of the species ranges it appears that polyploidy does not occur or does not commonly occur in wild *Weigela* populations. Additionally our screening of 46 cultivars uncovered only one polyploid, suggesting that polyploidy among existing *Weigela* cultivars is also not common. On deeper

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investigation to the origins of *W.* 'Courtalor', Carnaval® weigela it was found that it had been derived from mutation breeding program in France. The breeders had artificially induced polyploidy (tetraploids, $2n=4x=74$) by in vitro colchicine applications and backcrossed tetraploids with diploids to recover triploids; leading to three selections *W.* 'Courtared', Lucifer® weigela, *W.* 'Courtamon', and *W.* 'Courtalor', Carnaval® weigela (Duron and Decourtye, 1990). In our work only *W.* 'Courtalor' CARNAVAL was tested and we did not confirm the ploidy level of these other two selections.

Table 1. Relative genome size and ploidy levels determined via flow cytometry for species and cultivars of *Weigela*.

Taxa	Source ¹	Accession #	Relative 2C genome size [mean ± SE (pg)]	2C ploidy level (x)
<i>W.</i> 'Courtalor', Carnaval® weigela	MOR	359-2015 ct	3.03 ± 0.02	3
<i>W. middendorffiana</i>	MOR	354-2015 ct	2.32 ± 0.00	2
<i>W. decora</i>	MOR	53-200*1	2.28 ± 0.02	2
<i>W. japonica</i>	ARN	1317-84-A	2.23 ± 0.00	2
<i>W. hortensis</i>	ARN	414-2007-B	2.20 ± 0.01	2
<i>W.</i> 'Sunset', My Monet® sunset weigela	MOR	221-2014*2	2.16 ± 0.00	2
<i>W. ×incarnata</i>	MOR	333-85*1	2.14 ± 0.02	2
<i>W. floribunda</i>	ARN	1019-90-rA	2.13 ± 0.05	2
<i>W.</i> 'Carlton', Ghost® weigela	MOR	348-2015 ct	2.13 ± 0.01	2
<i>W.</i> 'Verweig', My Monet® weigela	MOR	214-2007*2	2.12 ± 0.05	2
<i>W.</i> 'Bokratwo', Merlot Pink weigela PP#21763	MOR	357-2015 ct	2.09 ± 0.02	2
<i>W.</i> 'P. Duchartre'	MOR	1007-80*1	2.08 ± 0.03	2
<i>W.</i> 'Alexandra', Wine and Roses® weigela	MOR	426-2001*5	2.08 ± 0.02	2
<i>W.</i> 'Bristol Snowflake'	MOR	353-2015 ct	2.08 ± 0.04	2
<i>W.</i> 'Argento-marginata Variegata'	MOR	559-71*1	2.07 ± 0.01	2
<i>W.</i> 'White Knight'	MOR	1078-2004	2.07 ± 0.02	2
<i>W.</i> 'Bramwell', Fine Wine® weigela	MOR	164-2008	2.07 ± 0.02	2
<i>W. florida</i> 'Variegata'	MOR	905-62*1	2.06 ± 0.00	2
<i>W.</i> 'Bokraspiwi', Spilled Wine® weigela	MOR	358-2015	2.06 ± 0.01	2
<i>W.</i> 'Pink Delight'	CBG	236-1992	2.05 ± 0.02	2
<i>W.</i> 'Groenewegenii'	MOR	564-71*1	2.05 ± 0.02	2
<i>W.</i> 'Bokrashine', Shining Sensation™ weigela	CBG	639-2012	2.05 ± 0.01	2
<i>W.</i> 'Victoria'	CBG	709-2003*6	2.05 ± 0.02	2
<i>W.</i> 'Bokrafive' Merlot Rose	MOR	355-2015	2.05 ± 0.01	2
<i>W.</i> 'Pink Princess'	MOR	89-75*1	2.04 ± 0.02	2
<i>W.</i> 'Bokrafour', Flamingo Pink® weigela	MOR	356-2015	2.04 ± 0.01	2
<i>W.</i> 'Samba'	CBG	65-2012*3	2.04 ± 0.02	2
<i>W.</i> 'Centennial'	MOR	330-85*2	2.03 ± 0.00	2
<i>W. decora</i>	ARN	81-90-A	2.03 ± 0.01	2
<i>W.</i> 'Candida'	CBG	171-2003*1	2.03 ± 0.02	2
<i>W.</i> 'Elvera', Midnight Wine® weigela	CBG	501-2010	2.03 ± 0.02	2
<i>W. subsessilis</i>	ARN	906-77-E	2.02 ± 0.01	2
<i>W. coraeensis</i>	MOR	423-58*1	2.02 ± 0.02	2
<i>W.</i> 'Bristol Ruby'	MOR	1004-80*1	2.02 ± 0.01	2
<i>W.</i> 'Newport Red' (syn. 'Vanicek')	MOR	1009-80*3	2.02 ± 0.02	2
<i>W.</i> 'Tango'	CBG	66-2012*2	2.02 ± 0.04	2
<i>W.</i> 'Bokrasopea', Sonic Bloom® Pearl	CBG	1178-2014*4	2.01 ± 0.01	2
<i>W.</i> 'Olympiade', Briant Rubidor	CBG	898-1998	2.01 ± 0.02	2
<i>W.</i> 'Java Red' sport	CBG	61-2012	2.01 ± 0.02	2
<i>W.</i> 'Dark horse'	CBG	04R5293*03	2.01 ± 0.01	2
<i>W.</i> 'Red Prince'	MOR	1317-2004*1	2.00 ± 0.01	2
<i>W.</i> 'Walweigeve', Eyecatcher® weigela	CBG	Q4R5295*7	1.99 ± 0.01	2

Table 1. Continued.

<i>Taxa</i>	Source ^z	Accession #	Relative 2C genome size [mean ± SE (pg)]	2C ploidy level (x)
<i>W. subsessilis</i>	ARN	317-2001-C	1.99 ± 0.01	2
<i>W.</i> 'Dart's pink lady'	CBG	79-1999*5	1.99 ± 0.01	2
<i>W.</i> 'Brigela' French Lace™ weigela	MOR	785-2005*1	1.99 ± 0.02	2
<i>W. florida</i>	ARN	82-2010-A	1.98 ± 0.03	2
<i>W.</i> 'Kolmagira', Rainbow Sensation™ weigela	MOR	360-2015	1.98 ± 0.01	2
<i>W.</i> 'Rumba'	CBG	64-2012*10	1.97 ± 0.03	2
<i>W.</i> 'Kosteriana Variegata'	CBG	382-2001*8	1.97 ± 0.03	2
<i>W. subsessilis</i>	ARN	587-53-A	1.97 ± 0.01	2
<i>W. maximowiczii</i>	ARN	167-97-B	1.97 ± 0.01	2
<i>W. praecox</i>	MOR	554-79*11	1.97 ± 0.03	2
<i>W. subsessilis</i>	CBG	249-2008-A	1.96 ± 0.01	2
<i>W. hortensis</i>	MOR	178-85*2	1.96 ± 0.01	2
<i>W.</i> 'Verweil-4', Sonic Bloom® Red	CBG	1202-2013*1	1.96 ± 0.02	2
<i>W.</i> 'Java Red'	CBG	612-2012*5	1.96 ± 0.02	2
<i>W.</i> 'Bokrasopin', Sonic Bloom™ Pink	CBG	961-2013*3	1.95 ± 0.03	2
<i>W.</i> 'Suzanne'	CBG	481-2003	1.95 ± 0.03	2
<i>W. florida</i>	ARN	132-96-B	1.94 ± 0.01	2
<i>W. florida</i>	ARN	422-93-A	1.94 ± 0.02	2
<i>W. florida</i> var. <i>venusta</i>	ARN	817-84-B	1.94 ± 0.05	2
<i>W.</i> 'Folius Purpurius'	CBG	957-1991*1	1.94 ± 0.00	2
<i>W. florida</i>	MOR	319-94*1	1.94 ± 0.00	2
<i>W. hortensis</i>	ARN	279-84-B	1.94 ± 0.01	2
<i>W. praecox</i>	ARN	966-85-D	1.93 ± 0.01	2
<i>W.</i> 'Sunny Princess'	CBG	191-2013*1	1.93 ± 0.03	2
<i>W.</i> 'Styriaca'	CBG	638-2003*3	1.92 ± 0.01	2
<i>W. looymansii</i> 'Aurea'	CBG	1423-2002*2	1.90 ± 0.00	2
<i>W. praecox</i>	ARN	843-84-B	1.90 ± 0.01	2
<i>W. florida</i>	ARN	404-86-B	1.90 ± 0.02	2
<i>W.</i> 'Abel Carriere'	CBG	76-1999	1.90 ± 0.01	2
<i>W. florida</i>	ARN	125-2003-B	1.89 ± 0.00	2
<i>W. hortensis</i>	ARN	30-2001-C	1.88 ± 0.01	2
<i>W. praecox</i> 'Korean Sunrise'	CBG	482-2003*6	1.87 ± 0.03	2

¹Source Codes: MOR, The Morton Arboretum, Lisle Illinois; ARN: Arnold Arboretum of Harvard University, Boston, Massachusetts; CBG: Chicago Botanic Garden, Glencoe, Illinois.

At the beginning of our investigation we had thought that recurrent blooming may be linked to polyploidy in weigela, but this does not necessarily appear to be the case. Although 'Courtalor' CARNAVAL is a recurrent blooming polyploid, other repeat or re-blooming cultivars such as the SONIC BLOOM series ('Verweil-4' SONIC BLOOM Red, 'Bokrasopin' SONIC BLOOM Pink, and 'Bokrasopea' SONIC BLOOM Pearl), 'Red Prince', and 'White Knight' all are diploid. Mutation breeding and ploidy manipulation may be viable methods for further improvement in *Weigela*, including further improvement in flower size, heavier recurrent bloom, and improvement in plant stature. The new plant development program at The Morton Arboretum has a weigela improvement program underway.

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