Ploidy Levels and Relative Genome Sizes of Diverse Species, Hybrids, and Cultivars of Rhododendron

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Polyploidy has been an important pathway in the evolution of plants and can contribute to reproductive isolation, increased heterozygosity, novel gene combinations, modified gene expression, enzymatic multiplicity, and ultimately divergence and speciation (Soltis and Soltis, 1993; 2000; Wendel, 2000). The origins, adaptive significance, and genetic implications of polyploidy continue to be an active field of research (Bennett, 2004; Soltis et al., 2003; Chen and Ni, 2006).

For plant breeders, ploidy level is an important consideration because it can influence male and female fertility, cross fertility, plant vigor, and gene expression (Chahal and Gosal, 2002; Contreras et al., 2007; Ranney, 2006; Thomas, 1993). In some cases, polyploid plants, including rhododendrons, can have desirable characteristics including thicker leaves, enhanced vigor, and larger flowers with thicker petals that persist longer (Barlup, 2002; Hosoda et al., 1953; Kehr, 1996a; Leach, 1961). As a result, there continues to be interest in identifying naturally occurring polyploids and inducing (through mitotic doubling agents) artificial polyploids as a component of rhododendron breeding programs (Barlup, 2002; Kehr, 1996b; Paden et al., 1990; Pryor and Frazier, 1970; Leach, 1961).

Most of the more than 800 *Rhododendron* species have been reported to be diploid with $2n = 2x = 26$. However, polyploidy occurs naturally in some rhododendron species, particularly within the *Pentanthera* and *Rhododendron* subgenera, with ploidy levels ranging from three to twelve (Ammal, 1950; Ammal et al., 1950). Sax (1930) completed one of the first surveys of chromosome numbers of rhododendron, including sixteen species, and determined a base chromosome complement of x= 13 for the genus and identified both *R. calendulaceum* and *R. canadense* (deciduous azaleas in subgenus *Pentanthera*) as natural tetraploids. Nakamura (1931) surveyed fifteen Japanese species of rhododendron and found them all to be diploid. Ammal et al. (1950) completed an extensive survey of chromosome numbers and ploidy levels in 360 species of rhododendron and found the elepidote rhododendrons (subgenus *Hymenanthes*), evergreen azaleas (subgenus *Tsutsusi*), and the deciduous azaleas (with the exception of the tetraploid *R. calendulaceum* and *R. canadense*) to be predominantly diploid. Ammal et al. (1950) further reported a high frequency of polyploids in the scaly-leaved species of subgenus *Rhododendron*, with taxa ranging from triploids to dodecaploids. In a survey of fifteen deciduous azaleas from Eastern North America, Li (1957) reported that all of the species were diploid with the exception of the tetraploid *R. calendulaceum*. However, a single triploid *R. atlanticum* was also identified among the otherwise diploid species. Among lepidotes, chromosome counts for 27 species in the tropical subgenus *Rhododendron* section *Vireya* indicated that they were uniformly diploid (Atkinson et al., 2000).

Published information on chromosome counts of specific cultivars or clones of rhododendron is less extensive. Hosada et al. (1953) completed chromosome counts on twelve cultivars of Satsuki azaleas (*R. lateritium*) and identified diploid, triploid ('Bangaku'), and tetraploid ('Banka', 'Taihei', and 'Wako') plants. Pryor and Frazier (1970) determined that the evergreen azalea hybrids 'Redwing' and 'Ablaze' were triploids and also documented the existence of mixed ploidy cytochimeras resulting from colchicine treatment. Heursel and DeRoo (1981) completed chromosome counts on 47 cultivars of evergreen azaleas and found they were all diploid with the exception of the triploid, 'Euratom'.

The chromosomes in rhododendron are small and can be difficult to view and count (Eiselein, 1994; Tolstead and Glencoe, 1991). Light microscopy is therefore not a practical method for determining ploidy levels of large numbers of individual cultivars and clones. However, flow cytometry can provide a fast and accurate determination of nuclear DNA content (genome size) that is related directly to ploidy level among closely related taxa (de Laat et al., 1987; Doležel, 1991; Doležel et al., 1998; Galbraith et al., 1983). Flow cytometry is also effective for detecting mixaploidy or cytochimeras and individual histogenic layers can be analyzed by sampling appropriate tissue (DeSchepper et al., 2001). Flow cytometry has been used successfully to determine relative DNA content and ploidy levels of *Rhododendron* spp. (DeSchepper et al., 2001; Eeckhaut et al., 2004; Sakai et al., 2003, 2004a, 2004b, 2006; Ureshino and Miyajima, 1998; Väinölä, 2000). De Schepper et al. (2001), for example, determined the ploidy level for six species and 88 cultivars within the evergreen azalea subgenus *Tsutsusi* by using flow cytometry. The vast majority were found to be diploid with the exception of three triploids ('Red Wing', 'Euratom', and 'Euratom Orange'*) and one mixaploid ('Casablanca Tetra') that was found to be diploid in the LI and LII layers and tetraploid in the LIII. Eeckhaut et al. (2004) studied various Ghent and Rustica deciduous azalea hybrids by using flow cytometry and found them

to be either triploid ('Mina van Houtte', 'Daviesii', 'Quadricolor', 'Gloria Mundi', 'Van Houtte Flore Pleno', 'Norma', and 'Phébé') or tetraploid ('Nancy Waterer', 'Unique', 'Narcissiflorum', 'Jozef Baumann', 'Maja', 'Rosetta', 'Semiramis', 'Souvenir du President Carnot', 'Marie Verschaffelt', 'Batholo Lazarri'*, 'Guelder Rose', 'Coccineum Major', 'Raphael de Smet', 'General Trauff', 'Graf von Meran', 'Goldlack', 'Fénelon', and 'Racine'). In contrast to the survey by Ammal et al. (1950), Eeckhaut et al. (2004) found three clones of *R. luteum* to be tetraploid, not diploid. Sakai et al. (2006) identified twenty-three diploid, six triploid ('Daisetsuzan', 'Goko', 'Horiuchikanzaki'*, 'Issho-no-haru', 'Meicho', and 'Yuhime'*), nine tetraploid ('Ayaka'*, 'Eiko', 'Hoshuku'*, 'Hoshun', 'Sachi-no-haru'*, 'Shunka'*, 'Taihei', 'Taikonotsuki'*, and *R. kiusianum* × *R. eriocarpum* No. 5) and four mixaploid ('Koyo', 'Miharu'*, 'Shinsen', and 'Sulsen'*) evergreen azaleas and eight diploid and five tetraploid ('Golden Flare', 'Golden Sunset', 'Klondyke', 'Melford Yellow', and *R. japonicum* f. *flavum* No. 6) deciduous azaleas. Although flow cytometry can be used to directly compare relative genome sizes of tissue from related taxa, inclusion of an internal standard with a known genome size allows the calculation of the sample genome size (Doležel and Bartoš, 2005), which enables comparisons among studies of more divergent taxa.

The objectives of this project were to determine the ploidy level and relative genome size of a diverse collection of species, hybrids, and cultivars of rhododendron by using a combination of flow cytometry and traditional cytology in order to: 1) determine the ploidy level of suspected, but unconfirmed, polyploid taxa (both naturally occurring and chemically induced), 2) increase sampling among and within species, and 3) develop an extensive database for specific cultivars and clones for use by rhododendron breeders.

Materials and Methods

Flow cytometry. Holoploid, 2C genome sizes (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry (de Laat et al., 1987; Doležel, 1991; Galbraith et al., 1983; Greilhuber et al., 2005). Diverse species and cultivars were acquired from various sources that included taxa from the *Hymenanthes, Rhododendron, Tsutsusi*, and *Pentanthera* subgenera along with several inter-subgeneric hybrids (Table 1). Approximately 1 cm^2 of newly expanded leaf or petal tissue was finely chopped with a razor blade in a Petri dish with 500 mL of nuclei extraction buffer (CyStain UV Precise P Nuclei Extraction Buffer, Partec, Münster, Germany). The solution was incubated for 1 to 2 min at approximately 24 °C and then filtered through Partec CellTrics™ disposable filters with a pore size of 50 µm to remove tissue debris. Nuclei were stained with 1.5 mL 4', 6-Diamidino-2-phenylindole (DAPI) staining buffer (CyStain UV Precise P Staining Buffer, Partec). Stained nuclei were analyzed with a flow cytometer (Partec PA-I, Partec) to determine relative genome size. Counts exceeded a minimum of 3000 cells per sample. Genome sizes were determined by comparing mean relative fluorescence of each sample with an internal standard, *Pisum sativum* L. 'Ctirad', with a known genome size of 9.09 pg (Bennett and Smith, 1976; Doležel et al., 1998) and calculated as: 2C genome size of sample = 9.09 pg × (mean fluorescence value of sample/ mean fluorescence value of standard). The relationship between ploidy levels and genome sizes was initially determined for plants with documented chromosome numbers including diploid *R.* 'Fragrant Affinity', triploid *R.* 'Redwing' azalea, and the tetraploid Ilam azalea #HA L49-520 (Contreras et al., 2007; De Schepper et al., 2001; Krebs, 1997). Genome sizes were also determined for a range of species where ploidy levels and chromosome counts have been previously reported. Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 13) was calculated as 2C genome size / ploidy level. Data were subjected to analysis of variance and means separation by using the Waller procedure (PROC GLM; SAS version 8.02, SAS Institute., Cary, N.C.; SAS Institute, 1988).

Chromosome counts. In situations where cytometric results were not consistent with published research, chromosomes were counted by using standard cytological techniques (Contreras et al., 2007). Chromosomes were counted in mitotic cells from young root tips of rhododendron cuttings. Roots were collected before 11 a.m. and root tips were placed in a pre-fixative solution of 2mM 8-hydroxyquinoline for 4 hours at 12 °C in the dark. Root tissue was fixed in a 1 : 3 solution of propionic acid : 95% ethanol solution for 24 hours at room temperature and then hydrolyzed in 1N HCl for 15 minutes at room temperature and for 25 minutes at 60 °C, followed by a rinse in distilled water. Root tips were excised and placed on a glass microscope slide with a drop of 1% acetocarmine. Slides with tissue samples were heated to approximately 70°C for 10 to 15 s, squashed with a coverslip, and viewed under a light microscope (Nikon Eclipse 80i, Nikon, Melville, NY) at 1,500× using oil immersion.

Results and Discussion

Flow cytometry was an effective method for determining genome sizes and ploidy levels of rhododendron. Mean 2C holoploid genome sizes varied as a function of subgenus and ploidy level (Tables 1 and 2). Analysis of variance demonstrated significant effects of both subgenus and ploidy level on 2C genome size ($P<0.05$). Genome sizes (2C) within ploidy levels for a given subgenus had a narrow range providing clear distinction among ploidy levels. Mean 1Cx monoploid genome size was conserved across ploidy levels within a subgenus, ranging from 0.72 to 0.75 pg for subgenus *Hymenanthes,* 0.67 to 0.83 pg for subgenus *Rhododendron,* 0.63 to 0.67 pg for subgenus *Tsutsusi,* and 0.80 to 0.83 for subgenus *Pentanthera* (Table 2). There did not appear to be a consistent reduction in base 1Cx genome size with increasing

ploidy level (i.e, genome downsizing) in rhododendron as has been commonly found in other genera with polyploid series (Leitch and Bennett, 2004). These results were based on cytometry methods using DAPI staining that provides consistent determination of relative genome size. However, it should be noted that other methods and stains may provide slightly different values and ranges (Doležel and Bartoš, 2005).

Hymenanthes. Genomic sizes (2C) in this subgenus ranged from 1.4 to 1.6 pg for diploids, from 2.1 to 2.2 pg for triploids, and from 2.9 to 3.4 pg for tetraploids (Table 2). As expected from earlier reports (Ammal et al., 1950; Nakamura, 1931), all of the sampled species fell within the diploid group (Table 1). However, some hybrids derived from species within this subgenus exhibited polyploidy. Barlup (2002) speculated on the possible polyploid nature of 'Taurus', ('The Honourable Jean Marie de Montague' × *R. strigillosum*) and we found it to be triploid, which most likely explains its low fertility. 'Hallelujah' ('The Honourable Jean Marie de Montague' × 'Kimberly') and an unnamed hybrid [('Nancy Evans' × ('Whopper' × 'Lem's Cameo')) × 'Point Defiance'] were also found to be triploids. These triploids may have arisen from either interploid crosses (particularly when the tetraploid 'Point Defiance' was a parent) or from an unreduced gamete from a diploid parent. Hybridity has been shown to increase formation of unreduced gametes even when the parental species might not exhibit the same characteristic (Ramsey and Schemske, 1998; Widrlechner et al. 1982). Other tetraploids arising from interspecific hybridization in this subgenus included 'Horizon Monarch' ('Nancy Evans × 'Point Defiance'), 'Lem's Monarch' ('Anna' × 'Marinus Koster'), 'Point Defiance' ('Anna' × 'Marinus Koster'), and 'Gentle Giant' ('Point Defiance' × 'Platinum Pearl'). 'Vulcan' tetraploid arose as somatic mutation (i.e., branch sport) on 'Vulcan' (Harold Greer, Eugene, Ore., per. comm.). Interestingly, we found 'Vulcan' tetraploid to be a 2x + 4x mixaploid that apparently arose from a mitiotic doubling event within a single histogenic layer.

Several chemically-induced tetraploids were also confirmed including 'Everlasting Tetra'*, 'Supernova', 'Briggs Red Star', and *R. fortunei* (NCSU 2005-175). 'Everlasting Tetra'* was developed from 'Everlasting' ('No Suchianum') (see Grant et al., 2004 for more history on this cultivar) at N.C. State University based on methods described by Contreras et al. (2007). 'Supernova' resulted from *in-vitro* colchicine treatment of 'Nova Zembla' at Briggs Nursery, Olympia, Wash. (Dan Meier, Olympia Wash., per. comm.). 'Briggs Red Star' was developed similarly at Briggs Nursery, but was found to be a 2x + 4x mixapoloid. *R. fortunei* NCSU 2005-175 was a colchicine treated plant developed by Dr. Max Byrkit, Williamsport, Md. (Kehr, 1996 b).

Rhododendron. Concordant with previous findings, polyploidy was prevalent among species and their hybrid derivatives from subgenus *Rhododendron* (Ammal et al., 1950). Genome sizes (2C) for diploids ranged from 1.3 to 1.9 pg, there was one triploid at 2.0 pg, tetraploids ranged from 2.8 to 3.3 pg, and hexaploids ranged from 4.4 to 4.6 pg (Table 2). The relationship between genome size and ploidy level above the hexaploid level was less clear. Two *R. maddenii* clones had genome sizes ranging from 5.4 to 5.8 pg that are most likely octoploids, but the plant with 5.4 pg could possibly be heptaploid. The only triploid found was 'White Ruffles', a cross made by Dr. August Kehr between the tetraploid *R. carolinianum* 'Epoch' (Kehr, 1996b) and *R. mucronulatum. Rhododendron augustinii* was found to be tetraploid as reported previously (Ammal et al., 1950) as were Dr. Kehr's *augustinii* hybrids: 37-1, 37-4, and 37-7 (Dr. Kehr, per. comm.). 'Shorty', a cross between a selfed 'Epoch' and 'Hi Tech' (Henry Schannen, Jackson, N.J., per. comm.), was a tetraploid indicating that 'Hi Tech' is either a tetraploid or produced unreduced pollen. 'Bubblegum' and 'Northern Starburst' were both tetraploids and were developed from *in-vitro* colchicine treatment of 'Weston's Aglo' and PJM Group respectively, at Briggs Nursery (Dan Meier, Olympia Wash., per. comm.).

Pentanthera. Genome sizes for species and hybrids in subgenus *Pentanthera* ranged from 1.5 to 1.7 pg for diploids, 2.3-2.6 for triploids, 3.0-3.9 for tetraploids, and 6.3-6.5 for octoploids. The majority of deciduous azaleas, including *R. arborescens, alabamense, canescens, cumberlandense, periclymenoides, prinophyllum, prunifolium, serrulatum, vaseyi,* and *viscosum* were found to be diploids as has been reported previously (Ammal, 1950; Li, 1957; Sax, 1930). The more recently discovered *R. eastmanii* was also found to be a diploid (Kron and Creel, 1999). Also agreeing with past literature (Ammal et al., 1950; Li, 1957; Sax, 1930) was the confirmation of *R. calendulaceum* as a tetraploid, though one triploid *R. calendulaceum,* NCSU 2000-164, was found that most likely resulted from a natural hybrid with a diploid species. Three wild-collected accessions of Gregory Bald Hybrids were found to be diploids, confirming that their parentage does not include the tetraploid *R. calendulaceum.*

Our cytometric evidence suggests that natural polyploidy may be more prevalent among deciduous azalea species than previously thought. The data obtained for two selections of the Pontic azalea, *R. luteum* 'Bumb'* and 'Golden Comet' (Table 1) substantiate a finding by Eeckhaut et al. (2004) that this Central Asian species has tetraploid forms. All of the *R. atlanticum* and *R. austrinum* accessions tested in this study (Table 1) had polyploid genome sizes (mostly tetraploid and a few triploid), as did some of the *R. flammeum* and *R. occidentale* samples. This is notable because in all earlier reports, only one instance of polyploidy (triploid) in these four North American species has been reported (Ammal, 1950; Li, 1957; Sax, 1930). Cytometric results in the present study were confirmed by chromosome counts on somatic cells from fifteen accessions of both *R. atlanticum* and *R. austrinum*, which showed that they were tetraploids, $2n = 4x = 52$ (Figs. 1 and

2). Indirect evidence of tetraploidy in *R. atlanticum* is provided by the observation that *R. atlanticum* H2004-055 and H2004-056 readily hybridize with *R. calendulaceum* and produce fertile hybrids (Dr. Jim Ballington, N.C. State University, Raleigh, N.C., per. comm.). Fertile hybrids have also resulted from crosses between *R. calendulaceum* × *austrinum*, *R. calendulaceum* × *atlanticum*, *R. calendulaceum* × 'Marydel' (Mr. Ray Head, Rutherfordton, N.C., per. comm.).

No diploid *R. austrinum* or *R. atlanticum* was found despite extensive sampling of taxa from diverse sources and geographical origins (26 *R. austrinum* and 30 *R. atlanticum* accessions collected throughout the Southeast). The assessment of these species as diploids in previous studies was based on a much more limited sampling (Ammal, 1950; Li, 1957; Sax, 1930). Therefore it seems unlikely that the lack of diploid forms of *R. atlanticum* and *R. austrinum* in this survey represents a sampling limitation, but rather a predominance of polyploids in these species. This appears to be the case for *R. calendulaceum* as well, where there are no reports (present study included) of diploid populations. The two triploid *R. austrinum* accessions observed here (Table 1) may have resulted from a natural interploid cross between sympatric diploid and tetraploid populations—if so it would be informative to sample again from the areas where they were collected in order to document the presence of more diploid forms of this species.

The best example of a natural polyploid series in *Rhododendron* species appears to be *R. occidentale*, where both diploid and tetraploid accessions were observed (Table 1). These data suggest there is a range of ploidy levels found within this species as is naturally found in many other species, e.g., *Galax aphylla* (Nesom, 1983), representing an evolutionary progression (Arnold, 1997; Briggs and Walters, 1997). Multiple ploidy levels were also observed for *R. flammeum* and *R. flammeum* hybrids. However, since *R. calendulaceum* can appear very similar

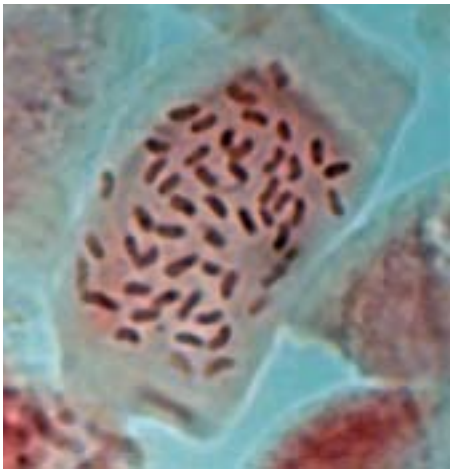

Figure 1. Photomicrograph of root tip cell of *R. austrinum* (2006-223) in prophase with 2*n* = 4x = 52 somatic chromosomes.

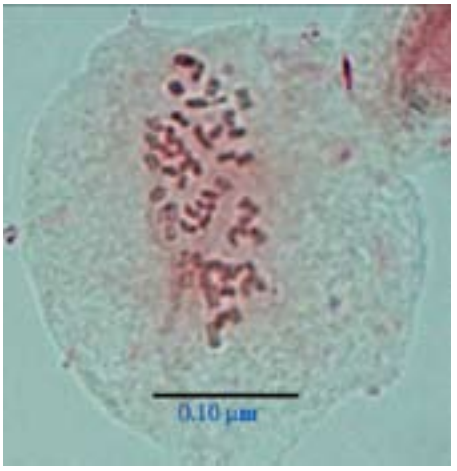

Figure 2. Photomicrograph of root tip cell of *R. atlanticum* (H2004-054-002) in prophase with 2*n* = 4x = 52 somatic chromosomes.

to *R. flammeum,* additional sampling from wild populations would be desirable to confirm this finding.

Many hybrid cultivars within this subgenus were found to be polyploids; most likely resulting from the hybridization of polyploid parents. Three Exbury azaleas of unknown parentage, 'Gibraltar', 'Gold Dust', and 'Klondyke', were tetraploids as were 'My Mary' ('Nacoochee' × 'Austrinum Gold'), 'Lemon Lights' (Northern Lights Series, unknown parentage), 'Admiral Semmes'* (Confederate Series, 'Hotspur Yellow' × *R. austrinum*), 'Marydel' (*R. atlanticum* or possible hybrid with *R. periclymenoides*) and an unnamed Ilam hybrid (HA L-46-520; unknown parentage) (Dirr, 1998; Galle, 1987). 'Snowbird' was determined to be a tetraploid and is believed to be a natural hybrid between *R. atlanticum* and *R. canescens* (Galle, 1987), suggesting an unreduced gamete from the *R. canescens* parent. 'Fragrant Star', developed through *in-vitro* colchicine treatment of 'Snowbird' at Briggs Nursery (Dan Meier, Olympia Wash., per. comm.) was found to be an octoploid as were open pollinated (selfed) seedlings from 'Fragrant Star'.

Tsutsusi. The ranges for 2C genome sizes in subgenus *Tsutsusi* were consistently lower than the other subgenera with the diploids ranging from 1.2 to 1.3 pg, the triploids from 1.9 to 2.0 pg, and the tetraploids from 2.6 to 2.8 pg. We found 'Red Wing' to be a triploid which was consistent with the findings of Pryor and Frazier (1970), but contrary to the findings of Heursel and Roo (1981), who found it to be a diploid, suggesting that multiple clones may exist under the same name. The purple-leaved 'Crimson Majesty'*, a sport of 'Red Formosum' was also found to be a triploid as was an unnamed hybrid between 'Pink Gloria Tetra' × 314-1 (NCSU 2000-171). The clone 314-1, a colchicine-treated seedling (open-pollinated seedling of 'Perle de Swynaerde' × 'Pryor Dwarf'*) developed by Dr. August Kehr, was also found to be a tetraploid, as was the unnamed hybrid 'Anytime Tetra' × 314-1 (NCSU 2000-167). We did not have access to 'Pink Gloria Tetra' and could not determine its ploidy. However, upon further investigation, we found the original 314-1 specimen, provided by Dr. Kehr, to be a mixture of diploid and tetraploid shoots with diploid shoots arising from below the treated crown. If flowers from these diploid shoots were used in breeding with the presumed tetraploid 'Pink Gloria Tetra', a triploid could have resulted.

Inter-subgeneric Hybrids. Several hybrids were examined that were the result of crosses between subgenera. In agreement with Contreras et al. (2007), we confirmed that 'Fragrant Affinity'* (*R. viscosum* × *R. ponticum*) was a diploid and its allopolyploid complement, 'Fragrant

Affinity Tetra',* was a tetraploid. The hybrids *R. calendulaceum* × '314-1' and 'Briggs Red Star' × 'Fragrant Affinity Tetra' were tetraploids as expected given that all parents were also tetraploids.

Conclusion

This study provides extensive information on genome sizes and ploidy levels for a broad range of species, cultivars, and hybrids of rhododendron including naturally occurring and induced polyploids. Flow cytometry was an efficient and effective method for determining genome size of rhododendron. Genome sizes (2C) within ploidy levels for a given subgenus had a narrow range providing clear distinction among ploidy levels. Polyploidy was found to be common in the genus *Rhododendron* and considerably more prevalent in the subgenus *Pentanthera* than previously known. Particularly noteworthy were the findings that *R. occidentale* includes both diploid and tetraploid individuals and that *R. atlanticum* and *R. austrinum* are predominantly tetraploid species. This information provides further insights into the genetics, evolution, and reproductive biology of rhododendron as well as serving as a valuable database for breeders.

Table 1. Relative genome size and estimated ploidy level, determined by flow cytometry, for a diverse collection of rhododendron species and cultivars.

Taxa	Source[1]	Relative 2C genome size (pg)[2]	Estimated ploidy (x)
Subgenus Hymenanthes			
Species			
catawbiense 'Catalgla'	HA	1.44±0.03	2
fortunei	NCSU 2003-144	1.55±0.02	2
maximum	NCSU 2006-281	1.44±0.01	2
maximum	NCSU 2005-243	1.53±0.12	2
ponticum (variegated)	NCSU 2006-047	1.46±0.01	2
sinogrande	NCSU 2006-038	1.54±0.04	2
Hybrids			
'Cheyenne'	NCSU 2002-086	1.41±0.03	2
'Everlasting'*	NCSU 2000-162	1.52±0.03	2
'Fantastica'	NCSU 2004-285	1.45±0.00	2
'Goldflimmer'	JCRA 040681	1.64±0.01	2
'Janet Blair'	NCSU 2004-291	1.44±---	2
'Maxecat'	NCSU 2005-238	1.52±0.00	2
'Nova Zembla'	NCSU 2006-093	1.53±0.01	2
'Polar Bear'	NCSU 2002-089	1.55±0.02	2
'Puget Sound'	NCSU 2005-015	1.47±0.00	2
'Queen Anne's' × 'Gold Dust'	NCSU 2000-270	1.43±0.02	2
'Vulcan'	NCSU 2006-095	1.49±0.03	2
'Vulcan's Flame'	NCSU 2004-134	1.55±0.01	2
'Taurus'	NCSU 2006-026	2.06±0.06	3
'Hallelujah'	NCSU 2005-009	2.22±0.05	3
[Nancy Evans × (Whopper × Lem's Cameo)] × Point Defiance	Brockenbrough	2.22±0.06	3
'Gentle Giant'	NCSU 2006-020	3.37±0.11	4
'Grand Slam'	NCSU 2006-021	3.03±0.02	4
'Horizon Monarch'	NCSU 2006-022	2.89±.0.07	4
'Horizon Monarch' × 'Point Defiance' (clone R)	Brockenbrough	2.93±0.00	4
'Lem's Monarch'	Brockenbrough	2.90±0.01	4
'Point Defiance'	Brockenbrough	2.93±0.02	4
'Vulcan Tetraploid'*	NCSU 2004-103	1.51±0.02 3.03±0.07	2+4
Induced polyploids			
'Briggs Red Star'	NCSU 2002-260	1.53±0.02 3.04±0.05	2+4
'Everlasting Tetra'*	NCSU 2005-149	2.86±0.02	4
'Supernova'	NCSU 2002-263	2.98±0.04	4
fortunei	NCSU 2005-175	3.14±0.03	4
Subgenus Rhododendron			
Species			
edgeworthii 'Bodnant'*	NCSU 2005-361	1.75±0.01	2
edgeworthii 'Ice'*	NCSU 2006-053	1.76±0.04	2
augustinii	NCSU 2000-170	3.10±0.01	4
maddenii	NCSU 2006-162	4.41±0.04	6
maddenii	NCSU 2006-160	4.45±0.02	6
maddenii subsp. *crassum*	NCSU 2006-256	4.39±0.01	6
maddenii subsp. *maddenii*	NCSU 2006-037	4.47±0.01	6
maddenii	NCSU 2006-161	5.97±0.01	8
maddenii subsp. *crassum*	NCSU 2006-258	5.42±0.01	8
Hybrids			
'Aglo'	NCSU 2006-045	1.49±0.05	2
'April Rose'	NCSU 2006-018	1.36±0.02	2
'California Gold'	NCSU 2006-259	1.71±0.02	2
'Coastal Spice'	NCSU 2005-355	1.86±0.01	2
'Dora Amateis'	NCSU 2005-222	1.62±0.06	2
'Improved Fragrantissimum'*	NCSU 2002-088	1.72±0.05	2
'McNabii'	NCSU 2006-039	1.61±0.02	2
'Mysterious Maddenii'*	NCSU 2006-262	1.82±0.00	2
PJM Group	NCSU 2006-012	1.32±---	2
'Reine Long'	NCSU 2006-264	1.76±0.02	2
'Southern Cloud'	NCSU 2006-265	1.65±0.04	2
'White Ruffles'*	NCSU 2006-113	2.01±0.03	3
'Blue Target'	NCSU 2000-168	3.10±0.00	4
'Epoch' × *augustinii*	NCSU 2006-044	3.25±0.02	4
'Gletschernacht'	NCSU 2003-143	2.78±0.07	4

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Table 1 cont.

37-1	NCSU 2000-267	3.11±0.02	4
37-4	NCSU 2000-169	3.12±0.10	4
37-7	NCSU 2000-269	3.19±0.00	4
'Shorty'	NCSU 2006-042	3.22±0.04	4
'Bernice'	NCSU 2006-255	4.57±0.01	6
'Pink Trumpets'*	NCSU 2006-263	4.61±0.02	6
Induced polyploids			
'Bubblegum'	NCSU 2006-046	2.90±0.07	4
'Northern Starburst'	NCSU 2006-011	2.81±---	4
Subgenus Pentanthera			
Species			
alabamense	2004-114	1.66±0.05	2
arborescens	NCSU 1998-454	1.65±0.05	2
austrinum (OP)	BE	1.59±0.00	2
austrinum (pale yellow)	BE	1.64±0.02	2
canescens 'Crains Creek'*	TNCA 1995-26*B	1.72±0.02	2
canescens 'Sp. Found'*	TNCA 1989-60*A	1.61±0.01	2
canescens 'White Canescens'*	TNCA 1989-59*A	1.65±0.01	2
cumberlandense	TNCA 1994-10*B	1.63±0.02	2
eastmanii (Newberry, SC)	Cantrell	1.58±0.04	2
eastmanii (York Co, SC)	Cantrell	1.60±0.04	2
flammeum (ES Selection)	TNCA 1994-454*B	1.68±0.01	2
flammeum	TNCA 1995-31*B	1.72±0.02	2
occidentale 'Homboldt Picotee'	Cavender	1.51±0.04	2
occidentale 'Tatum's Deep Pink'*	Cavender	1.51±0.06	2
periclymenoides (*nudiflorum*)	NCSU 2000-419	1.68±0.00	2
prinophyllum	TNCA 1994-20*A	1.64±0.00	2
prunifolium	TNCA 1994-22*B	1.56±0.01	2
prunifolium	NCSU 1998-455	1.58±0.00	2
serrulatum	TNCA 1989-78*A	1.74±0.03	2
vaseyi	NCSU 1998-447	1.56±0.01	2
viscosum	TNCA 1995-460*A	1.67±0.04	2
austrinum	TNCA 1989-40*A	2.52±0.05	3
austrinum 'Firecracker'*	TNCA 1995-451*A	2.48±0.01	3
calendulaceum	NCSU 2000-164	2.30±0.07	3
atlanticum	JCRA 050431	3.01±0.01	4
atlanticum	TNCA 1998-0103a	3.05±---	4
atlanticum	NCBG 1994-0093b	3.10±---	4
atlanticum	NCBG 1986-2041a	3.12±---	4
atlanticum	NCSU H2004-054-002	3.15±0.01	4
atlanticum	TNCA 1994-9*B	3.16±0.00	4
atlanticum	NCSU H2004-056-002	3.20±0.00	4
atlanticum	NCSU H2004-055-003	3.20±0.02	4
atlanticum	NCSU H2004-055-001	3.21±0.01	4
atlanticum	NCSU H2004-056-001	3.24±0.00	4
atlanticum	NCSU H2004-055-002	3.24±0.02	4
atlanticum	NCSU H2004-054-004	3.24±0.07	4
atlanticum	NCSU H2004-054-001	3.26±0.00	4
atlanticum	NCSU H2004-054-003	3.26±0.01	4
atlanticum	TNCA 1989-33*A	3.27±0.00	4
atlanticum #1	HA	3.16±0.02	4
atlanticum #1(Del Mar Pen.)	HA	3.13±0.06	4
atlanticum #2	HA	3.10±---	4
atlanticum #2	HA	3.33±0.02	4
atlanticum #3	HA	3.10±0.01	4
atlanticum #3	HA	3.29±0.01	4
atlanticum #4	HA	3.12±0.03	4
atlanticum #4	HA	3.14±0.01	4
atlanticum #5	HA	3.08±.00	4
atlanticum #6	HA	3.06±---	4
atlanticum #7	HA	3.19±0.05	4
atlanticum #8	HA	3.32±---	4
atlanticum 'Choptank Pink &White'*	TNCA 1995-467*B	3.22±0.01	4
atlanticum	TNCA 1998-34*A	3.20±0.02	4
atlanticum 'Winterthur'*	JCRA 000609	3.18±0.02	4
austrinum	JCRA 020494	3.11±0.03	4
austrinum	NCBG 1991-0301a	3.12±---	4
austrinum	TNCA 1996-0374a	3.21±---	4

(Table 1 continued on next page.)

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Table 1 cont.

austrinum	JCRA L20	3.21±0.05	4
austrinum	TNCA 1994-339*B	3.24±0.01	4
austrinum	TNCA 1989-221*E	3.43±0.03	4
austrinum	NCBG 1998-0104a	3.47±---	4
austrinum	TNCA 1989-221*A	3.41±0.01	4
austrinum	NCBG 1998-0188a	3.31±---	4
austrinum	NCSU 2005-062	3.33±0.03	4
austrinum	NCSU 2006-223	3.34±0.04	4
austrinum	NCSU 2004-117	3.36±0.01	4
austrinum	NCSU 2005-063	3.37±0.02	4
austrinum #1 (Nat. For. Ala.)	HA	3.33±0.00	4
austrinum #10	HA	3.37±0.09	4
austrinum #12	HA	3.30±0.00	4
austrinum #2	HA	3.23±0.01	4
austrinum #3	HA	3.27±0.05	4
austrinum #4	HA	3.88±0.59	4
austrinum #5	HA	3.32±0.00	4
austrinum #6	HA	3.29±0.02	4
austrinum 'Austrinum Gold'*	TNCA 1989-38*A	3.28±0.01	4
austrinum 'Flame'*	TNCA 1990-22*A	3.28±0.01	4
austrinum 'Millie Mac'	TNCA 1993-327*A	3.33±0.07	4
calendualaceum 'Deliverance'*	TNCA 1989-55*A	3.28±0.01	4
calendulaceum	NCSU H2000-048	3.14±0.09	4
flammeum	NCSU 2007-001	3.14±0.03	4
flammeum 'Pink Surprise'*	TNCA 1994-332*B	3.24±0.03	4
luteum 'Bumb'*	NCSU 2005-101	3.00±0.01	4
luteum 'Golden Comet'	NCSU 2006-006	3.00±0.01	4
occidentale 'Double Dig Twelve'	Cavender	2.94±.08	4
Hybrids			
'August Beauty'*	NCSU 2006-118	1.58±0.00	2
'Lemon Drop'	NCSU 2006-119	1.51±0.04	2
'Millennium'	NCSU 2005-122	1.61±0.03	2
'Popcorn'	NCSU 2005-123	1.51±0.01	2
'Summer Lyric'	NCSU 1998-453	1.64±0.04	2
'Weston's Parade'*	NCSU 2005-121	1.57±0.06	2
Gregory Bald Hybrid	TNCA 1992-515*M	1.62±0.03	2
Gregory Bald Hybrid	TNCA 1992-212*E	1.65±0.01	2
Gregory Bald Hybrid	TNCA 1992-213*B	1.67±0.03	2
flammeum × *canescens*	TNCA 1994-2*F	2.60±0.05	3
'Admiral Semmes'*	NCSU 2005-081	3.15±0.04	4
flammeum × *calendualaceum*	TNCA 1996-325*A	3.39±0.01	4
'Gilbralter'	NCSU 2005-356	3.38±0.01	4
'Gold Dust'	NCSU 2005-111	3.27±0.04	4
'Klondyke'	NCSU 2005-357	3.26±0.16	4
'Lemon Lights'	NCSU 2005-113	3.03±0.00	4
'Marydel'	NCSU 1998-456	3.43±0.03	4
'My Mary'	NCSU 2006-117	3.15±0.08	4
'Snowbird'	NCSU 2006-048	3.24±0.03	4
Ilam hybrid	HA L49-520	3.17±0.01	4
Induced polyploids			
'Fragrant Star'	NCSU 2004-293	6.32±0.03	8
'Fragrant Star' selfed	NCSU H2006-007-003	6.39±0.11	8
'Fragrant Star' selfed	NCSU H2006-007-001	6.41±0.08	8
'Fragrant Star' selfed	NCSU H2006-007-004	6.46±0.03	8
Subgenus Tsutsusi			
Species			
stenopetalum 'Linearifolium'	JCRA 050534	1.27±0.01	2
Hybrids			
'Conles' Autumn Express ™	NCSU 2002-237	1.27±0.02	2
'Glacier'	NCSU 2005-064	1.24±0.03	2
'Hardy Gardenia'*	NCSU 2005-023	1.22±0.00	2
'Polar Bear'	NCSU 2005-196	1.26±0.00	2
'Secret Wish'	NCSU 2005-097	1.30±0.01	2
'Crimson Majesty'*	NCSU 2004-245	1.94±0.03	3
'Pink Gloria Tetra' × '314-1'	NCSU 2000-171	1.98±0.01	3
'Redwings'	NCSU 2006-094	1.88±0.02	3

(Table 1 continued on next page.)

Table 1 cont.

'Anytime Tetra'* × '314-1'	NCSU 2000-167	2.75±0.07	4
Induced polyploids			
'314-1'	NCSU 2000-165	2.60±0.01	4
Inter-Subgeneric Hybrids			
'Fragrant Affinity'*	NCSU H2003-003	1.59±0.12	2
'Briggs Red Star' × 'Fragrant Affinity Tetra'*	NCSU H2005-085	2.99±0.03	4
R. calendulaceum × '314-1'	NCSU H2006-008-001	2.81±.00	4
Induced polyploids			
'Fragrant Affinity Tetra'*	NCSU H2003-002	3.11±0.04	4

*Name is not registered.

[1]BE – Biltmore Estate, Asheville, N.C.
Brockenbrough – Mr. Ned Brockenbrough, Hunts Point, Wash.
Cantrell – Mr. Allen Cantrell, Chesnee, SC.
Cavender – Mr. Dick 'Red' Cavender, Sherwood, Oregon.
HA = Holden Arboretum, Kirtland and Madison, Ohio.
TNCA = The North Carolina Arboretum, Asheville, N.C.
NCBG = North Carolina Botanical Garden, Chapel Hill, NC.
NCSU = North Carolina State University, Mountain Horticultural Crops Research and Extension Center, Fletcher, N.C.
2Values represent mean 2C holoploid genome size ± SEM for two samples. Values with no SEM indicate only one sample was analyzed.

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*Name is unregistered.

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Table 2. Summary of means and ranges for 2C, holoploid genome size (pg) and 1Cx monoploid genome size (pg) by subgenus and ploidy level.

Subgenus	Ploidy level				
	Diploid (2x)	**Triploid (3x)**	**Tetraploid (4x)**	**Hexaploid (6x)**	**Octoploid (8x)**
Hymenanthes	2C = 1.50 ± 0.01 A (1.41-1.64) 1Cx = 0.75 ± 0.01 A (0.71-0.82)	2C = 2.17 ± 0.05 B (2.06-2.22) 1Cx = 0.72 ± 0.02 A (0.69-0.74)	2C = 3.01 ± 0.04 C (2.89-3.37) 1Cx = 0.75 ± 0.01 A (0.72-0.84)	NA	NA
Rhododendron	2C = 1.65 ± 0.05 A (1.32-1.86) 1Cx = 0.83 ± 0.02 A (0.66-0.93)	2C = 2.01 ± -- B (NA) 1Cx = 0.67 ± -- B (NA)	2C = 3.06 ± 0.05 C (2.78-3.25) 1Cx =0.77 ± 0.01 AB (0.70-0.81)	2C = 4.48 ± 0.04 D (4.39-4.61) 1Cx = 0.75 ± 0.01 AB (0.73-0.77)	5.70 ± 0.28 E (5.42-5.97) 1 Cx = 0.72 ± 0.03 AB (0.68-0.75)
Pentanthera	2C = 1.62 ± 0.01 A (1.51-1.74) 1Cx = 0.81 ± 0.01 A (0.76-0.87)	2C = 2.48 ± 0.06 B (2.30-2.60) 1Cx = 0.83 ± 0.02 A (0.77-0.87)	2C = 3.23 ± 0.02 C (3.00-3.88) 1Cx = 0.81 ± 0.00 A (0.75-0.97)	NA	2C = 6.40 ± .03 D (6.32-6.46) 1Cx =0.80 ± 0.00 A (0.79-0.81)
Tsutsusi	2C = 1.26 ± 0.01 A (1.22-1.30) 1Cx = 0.63 ± 0.01 A (0.61-0.65)	2C = 1.93 ± 0.03 B (1.88-1.98) 1Cx = 0.65 ± 0.01 AB (0.63-0.66)	2C = 2.68 ± 0.08 C (2.60-2.75) 1Cx = 0.67 ± 0.02 B (0.65-0.68)	NA	NA

[1]Values represent means ± SEM followed by (ranges) derived from Table 1. Means followed by different letter, within a row, are significantly different, P<0.05.