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

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Index Words: Cytology, Flow Cytometry, Genome Size, Polyploidy

Significance to Industry: Polyploidy has been a central pathway in the evolution of plants and is an important consideration in plant breeding as it can influence fertility, crossability, plant vigor, and gene expression. In some cases, polyploid plants can also have desirable characteristics including thicker leaves and petals, enhanced vigor, and larger flowers that persist longer. This research provides an extensive survey of polyploidy in the genus *Rhododendron* L. and provides further insights into the genetics, evolution, and reproductive biology of rhododendron as well as serving as a valuable database for breeders.

Nature of Work: Many of the more than 800 *Rhododendron* species have been reported to be diploid with 2*n* = 2x = 26 (1). However, polyploidy occurs naturally in some rhododendron species, particularly within the *Pentanthera* and *Rhododendron* subgenera. Although some information has been published on polyploidy of *Rhododendron* species, there has been limited sampling and there is little data for specific clones or cultivars. The chromosomes in rhododendron are small and can be difficult to view and count. Determination of chromosome numbers by light microscopy is therefore not a practical method for establishing 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. Flow cytometry is also effective for detecting mixaploidy or cytochimeras. The objectives of this project were to determine the ploidy level and genome size of a diverse collection of species, hybrids, and cultivars of rhododendron using a combination of flow cytometry and traditional cytology. Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry. Two hundred diverse species and cultivars were acquired from various sources that included taxa from the *Hymenanthes* (Blume) K. Koch., *Rhododendron* L., *Tsutsusi* (Sweet) Pojarkova, and *Pentanthera* G. Don. subgenera. Stained nuclei from newly expanded leaf or petal tissue was analyzed using a flow cytometer (PA-I, Partec, Münster, Germany) to determine relative DNA content. 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 and calculated as: 2C DNA content of sample = 9.09 pg × (mean fluorescence value of sample/ mean fluorescence value of standard). The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers (3). 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) to assess variability in base genome size. Data were subjected to analysis of variance and means separation using the Waller procedure. In situations where cytometric results were not consistent with published research, chromosomes were counted using standard cytology techniques (2).

Results and Discussion: Flow cytometry was an effective method for determining genome size and ploidy levels of rhododendron. Analysis of variance demonstrated significant effects of both subgenus and ploidy level on 2C genome size ($P<0.05$). Genome sizes (2C) within ploidy level for a given subgenus had a narrow range providing clear distinction among ploidy levels (Table 1). Mean 1Cx monoploid genome size was conserved across ploidy levels within a subgenus (Table 1). As expected from past reports, all of the sampled species within the *Hymenanthes* were diploid. However, many interspecific hybrids were polyploids. Hybridity has been shown to increase formation of unreduced gametes even when the parental species might not exhibit the same characteristic (5). Tetraploids arising from interspecific hybrids included 'Horizon Monarch', 'Lem's Monarch', 'Point Defiance', and 'Gentle Giant'. 'Vulcan' tetraploid was found to be a 2x + 4x mixaploid that apparently arose from an asexual mitotic doubling event within a single histogenic layer. Several chemically induced tetraploids were found including 'Everlasting Tetra', R. *fortunei* Lindl. (NCSU 2005-175), 'Super Nova', and the mixaploid 'Briggs Red Star'. Concordant with previous findings, polyploidy was common among species and their hybrid derivatives from subgenus *Rhododendron*. *Rhododendron augustinii* Hemsl. and its hybrids were found to be tetraploids, while *R*. *maddenii* Hook. f. clones were found to be hexaploids and octoploids. 'Bubblegum' and 'Northern Starburst' were both tetraploids developed from *in-vitro* colchicine treatments. Polyploidy was not common among the evergreen azaleas (subgenus *Tsutsusi*) with the exception of two chemically induced tetraploids. The majority of deciduous azaleas (subgenus *Pentanthera*) were found to be diploids as has been reported previously and *R*. *calendulaceum* (Michx.) Torr. was confirmed as a tetraploid. However, our results indicated that natural polyploidy is more prevalent among deciduous azalea species than previously thought. All of the *R. atlanticum* (Ashe) Rehder and *R. austrinum* (Small) Rehder accessions tested in this study were polyploids (mostly tetraploid and a few triploid), as were some of the *R. flammeum* (Michx.) Sarg. This is notable because in all earlier reports, only one instance of polyploidy (triploid) in these three North American species has been reported (4). 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, 2*n* = 4x = 52 (Figs. 1 and 2). Both diploid and tetraploid accessions were observed for *R. flammeum* representing a natural polyploid series. Many deciduous azalea cultivars were found to be polyploids including the tetraploids 'Admiral Semmes', 'Gibralter', 'Gold Dust', 'Lemon Lights', 'MaryDel', 'My Mary', 'Klondyke', 'Snowbird', and the octoploid 'Fragrant Star'.

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Table 1. Summary of means and ranges for 2C, holoploid genome size (ρg) and 1Cx monoploid genome size (ρg) by sub-genus and ploidy level.

	Ploidy level				
Sub-genus	Diploid (2x)	Triploid (3x)	Tetraploid (4x)	Hexaploid (6x)	Octoploid (8x)
Hymenanthes	2C = 1.50 ± 0.01[1] 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)	2C = 5.70 ± 0.28 E (5.42-5.97) 1 Cx = 0.72 ± 0.03 AB (0.68-0.75)
Pentanthera	2C = 1.63 ± 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 = 3.24 ± 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 the entire data set. Means followed by different letter, within a row, are significantly different, P<0.05.

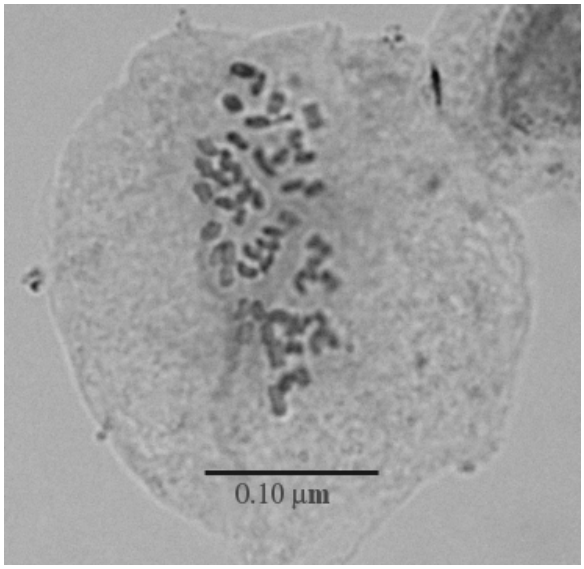

Figure 1. Photomicrograph of root tip cell of *R. atlanticum* (H2004-054-002) in prophase with 2*n* = 4x = 52 somatic chromosomes.

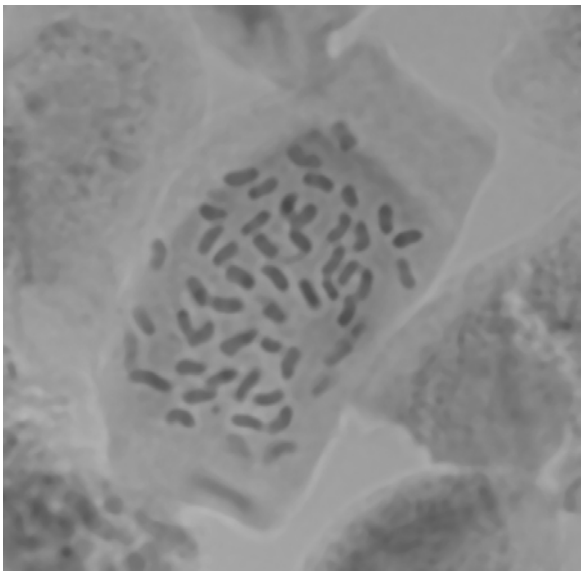

Figure 2. Photomicrograph of root tip cell of *R. austrinum* (2006-223) in prophase with 2*n* = 4x = 52 somatic chromosomes.