History and Cytological Reassessment of *Rhododendron canadense*

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Summary
Rhododendron canadense represents a morphologically unique and taxonomically complicated species of azalea. Early attempts at classification commonly placed *R. canadense* and *R. vaseyi* together in clades based upon odd features such as extra stamens and tubeless flowers. However, more recent molecular studies suggest *R. canadense* shares a closer relationship with other deciduous azaleas including *R. luteum, molle,* and *calendulaceum*. Cytological and cytometric studies have yielded equally confusing results by reporting inordinately small genome sizes (DNA content) and both diploid and tetraploid individuals of *R. canadense*. This study utilized flow cytometry and traditional cytology to provided conclusive evidence that *R. canadense* exists primarily as a diploid species. A survey of relative genome size of *R. canadense* also revealed that this species has approximately 26% less DNA than most other deciduous azaleas. Further cytological investigation indicated that *R. canadense* may also possesses a lower base chromosome number with $2n = 2x = 24$ compared to most other *Rhododendron* with $2n = 2x = 26$. Additional investigation into *R. canadense* must be undertaken to reveal the full complexities of its cytogenetics, evolutionary history, and relationships within *Rhododendron*.

Introduction
Rhododendron canadense (L.) Torr., commonly known as Rhodora, is an unusual, primitive azalea that has been plucked from the rising grassy tussocks of sodden bogs for use in ornamental landscapes. Suited to the cold, wet conditions of northeast North America, *R. canadense* has charmed countless gardens with its bare, low-growing stems topped with trusses of rose-pink to purple flowers in the spring (Judd and Kron, 1995). In addition to providing a distinctive feature in the garden, *R. canadense* has also played an important cultural role in its native New England. After spending years exploring the natural landscape of Massachusetts, the renowned poet Ralph Waldo Emerson was so moved by this "rival of the rose" that he penned one of his most famous poems, "The Rhodora," in its honor (Wilson and Rehder, 1921). The rhodora was again honored in Massachusetts by becoming the namesake of the journal of the New England Botanical Club, a prestigious Harvard-affiliated scientific journal.

Rhododendron canadense has several morphological features that make it conspicuous among the deciduous azaleas. In April to early July, flowers occur before (or occasionally with) the leaves, and are borne on terminal, umbellate racemes of 3-9 rose-purple to pink (rarely white) flowers (Judd and Kron, 1995). The distinct feature of the *R. canadense* flower is the complete lack of a floral tube due to lack of fusion in its corolla. Flowers are two-lipped, with the fusion of the upper three corolla lobes contrasted by the flaring two lower lobes (Judd and Kron, 1995). Not only does *R. canadense* have the only tubeless flower of the deciduous azaleas, it also typically has ten or seven stamens instead of the usual five (though *R. vaseyi* Gray and *R. schlippenbachii* Maxium are nearly tubeless with more than five stamens) (Galle, 1987; Judd and Kron, 1995; Towe, 2004).

Rhododendron canadense typically grows as a rhizomatous shrub reaching a height of less than 1m. This unique species is the most northern of all the eastern North America azaleas, and can be found in glaciated areas along river banks, in moist woods, and in swamps from sea level to 1900 m. The geographic distribution of *R. canadense* stretches from Labrador and Newfoundland to southwestern Quebec, continuing down through New England into central New York, northern New Jersey, and northeastern Pennsylvania (Galle 1982; Judd and Kron, 1995; Towe, 2004) (Fig. 1).

Before its classification as *R. canadense* (Torrey, 1839), rhodora underwent several classifications including *Rhodora canadensis* (Linnaeus, 1762), *Hochenwartia canadensis* (Von Crantz, 1766), *Rhododendron rhodora* (Gmelin, 1791), and *Azalea canadensis* (Kuntze, 1891). The construction of phylogenies for *Rhododendron* and the particular placement of *R. canadense* have historically proven difficult. The first major classification of *Rhododendron* performed by George Don (1834) divided the genus into sections,

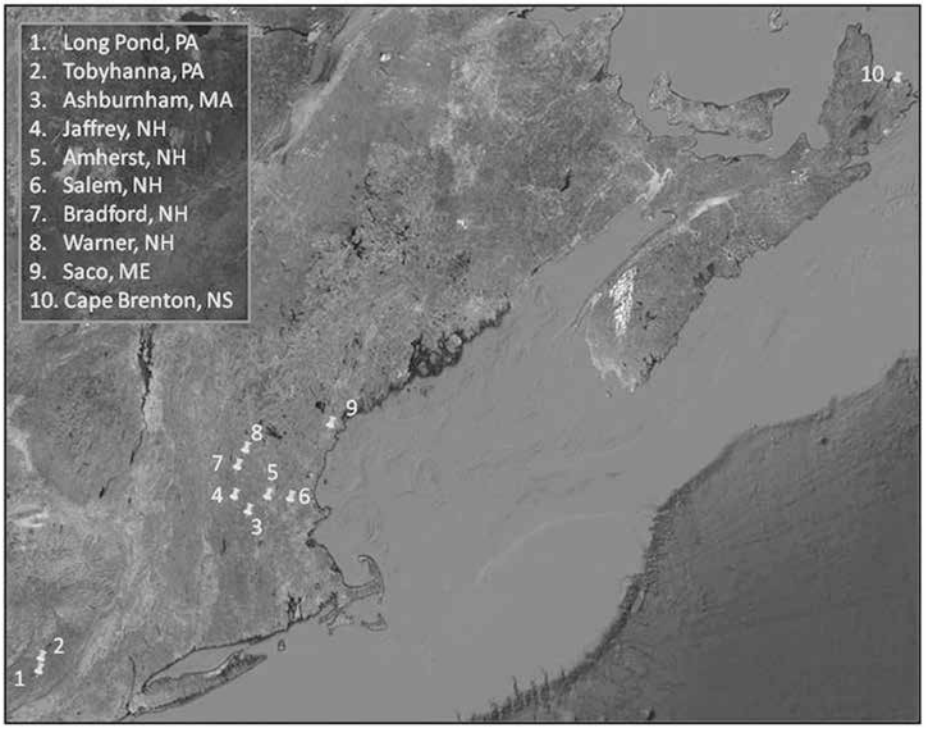

Figure 1. Locations of *R. canadense* sampling sites. Map compiled using Google Earth (Google Inc., Mountain View, CA).

with *R. canadense* belonging to section *Pentanthera* G. Don. Wilson and Rehder's monograph (1921) recognized the rank of subgenera, with subgenus *Anthodendron* (Endl.) Rehder section *Rhodora* (L.) G. Don containing *R. vaseyi*, *R. canadense*, *R. nipponicum*, *R. pentaphyllum* Maxim, and *R. albrechtii* Maxim. Philipson W. (1980), Philipson M. (1980), and Judd and Kron (1995) placed *R. canadense* in subgenus *Pentanthera* (G. Don) Poyarkova and further reduced section *Rhodora* to only *R. vaseyi* and *R. canadense*, taxa exhibiting a two-lipped corolla with a glabrous inner surface.

In addition to morphological studies of *Rhododendron*, studies using macromolecular data have helped determine relationships within the genus (Gao et al., 2002; Goetsch et al., 2005; Kurashige et al., 1998, 2001). Kurashige et al. (2001) and Gao et al. (2002) constructed phylogenies based on chloroplast matK and trnK intron sequences and nuclear ITS sequences, respectively; both placed *R. canadense* in subgenus *Pentanthera* section *Rhodora,* sister to section *Pentanthera.* More recently, Goetsch et al. (2005) evaluated 87 species based on analysis of the RPB2-I gene and found *Rhododendron canadense* was nested within section *Pentanthera* and more closely allied with *R. luteum* (L.) Sweet, *molle* (Bl.) G. Don, and *calendulaceum* (Michx.) Torr.

Rhododendron canadense does not hybridize readily with other species (Galle, 1987). However, limited hybrids have been reported including *R.* ×*seymourii* Herbert ex Lindley (*R. canadense* × *luteum*) and *R.* ×*fraseri* W. Watson (*R. canadense* × *japonicum* (A. Gray) Sur.)(Wilson and Rehder, 1921).

Studies on genome sizes and ploidy levels provide insights into the cytogentics of *Rhododendron*. Early efforts to verify ploidy level relied on counting chromosomes, though cytological studies of *Rhododendron* are notoriously difficult. The counting of *Rhododendron* chromosomes has historically proven a laborious, time consuming, difficult task because of the friability and small size of roots, the inordinately small chromosomes, interference of tannin granules, and the difficulty of obtaining meiotic pollen mother cells in the proper stage (Galle 1970, McAllister, 1993, Li 1957). For these reasons, relatively few studies have been conducted on cytogenetics and chromosome numbers of *Rhododendron*.

The earliest cytological investigation into *Rhododendron* was performed by Moringa et al. (1929) on *R. quinquefolium* var. *speciosum* N. Yonez., finding the base chromosome compliment of *Rhododendron* to be $x = 13$. Sax (1930) confirmed this finding in a study that tested pollen mother cells of 16 species. Sax (1930) found all samples tested to be diploid ($2n = 2x = 26$) with the exception of 2 tetraploids ($2n = 4x = 52$), *R. calendulaceum* and *R. canadense*. In a large-scale study in 1950, Ammal et al. investigated 360 species of *Rhododendron* completing over 550 counts. They confirmed Sax's earlier findings of tetraploidy in *R. calendulaceum* and *R. canadense*, finding them to be the only polyploid *Rhododendron* in North America. However, for such a monumental study, no methods or materials were published, and subsequent studies have reached contradicting conclusions for certain species (Eeckhaut, 2004; Jones et al., 2007; Li, 1957; Zhou et al., 2008).

Numerous studies have utilized flow cytometry to quantify relative genome size and ploidy level in *Rhododendron* (De Schepper et al., 2001; Jones et al., 2007; Väinölä, 2000; Zhou et al., 2008). Flow cytometry measures fluorescence of individual nuclei, stained with a fluorescent dye, while suspended in a precise stream of fluid as a means to determine genome size (DNA content) relative to an internal standard (Doležel and Bartoš, 2005). Zhou et al. (2008) utilized flow cytometry to confirm tetraploidy in *R. luteum, R. atlanticum* Rehder, *R. calendulaceum, R. colemanii* R. Miller, and *R. austrinum* (Small) Rehder, but found *R. canadense* to be a diploid. However, only a single clone of *R. canadense* was tested, and the authors

Table 1. Genome sizes and ploidy levels of *Rhododendron canadense* samples from throughout its natural range.

Location/Source	2C Genome size (pg)[Z]	Ploidy Level (*x*)
Lincoln Bog, Ashburnham, MA, USA. Perkins[K].	1.13 ± 0.02	2*x*
Tobyhanna State Park. Tobyhanna, PA. Perkins[K].	1.07 ± 0.03	2*x*
Exit 1, Interstate 93. Salem, NH, USA. Perkins[K].	1.16 ± 0.02	2*x*
Exit 1, Interstate 93. Salem, NH, USA. Perkins[K].	1.14 ± 0.02	2*x*
Ponemah Bog. Amherst, NH, USA. Perkins[K].	1.17 ± 0.02	2*x*
Bradford Bog. Bradford, NH, USA. Perkins[K].	1.15 ± 0.04	2*x*
Mt. Kearsarge Bog. Warner, NH, USA. Perkins[K].	1.11 ± 0.03	2*x*
Saco Heath Bog. Saco, Maine, USA. Perkins[K].	1.13 ± 0.05	2*x*
Cape Brenton, Nova Scotia, Canada. Clyburn[W].	1.17 ± 0.03	2*x*
Mt. Monadnock, Jaffrey, NH. Newman[X] via Perkins[K]. NCSU 2009-164	1.23 ± 0.02	2*x**
Long Pond, Poconos Mts., PA. Plyler[Q] via Krebs[V]. NCSU 2009-173	1.22 ± 0.01	2*x**

[K]Perkins – Sally and John Perkins, Salem, NH.
[Q]Plyler – Jim and Bethany Plyler, Natural Landscapes Nursery, West Grove, PA.
[V]Krebs – Stephen Krebs, Holden Arboretum, Kirtland, OH.
[W]Clyburn – Bruce Clyburn, Cape Brenton, NS.
[X]Newman – George Newman, Bedford, NH.
[Z]8.75 picograms was use as the genome size for the internal standard, *Pisum sativum* 'Ctirad'.
*Ploidy confirmed with cytology.

proposed further investigation into the ploidy of *R. canadense* to confirm their findings (Zhou et al., 2008).

Due to the contradictions in the literature regarding the phylogeny and ploidy level assessment of *R. canadense*, the objective of this study was to perform a survey of genome size and ploidy level of *R. canadense* samples from throughout its natural range.

Methods and Materials

Flow Cytometry. To determine relative genome size of *R. canadense*, a survey composed of 11 *R. canadense* samples from across its natural range (Table 1) was conducted using flow cytometry. For each sample, approximately 1 cm^2 of newly expanded leaf tissue was placed in a petri dish containing 500 µL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extratcion Buffer®; Partec, Münster, Germany) and chopped finely with a razor blade until sample was completely incorporated into the buffer. After 1-2 minute incubation at room temperature (22°C), the resulting solution was filtered through a Partec CellTrics™ disposable filter with a pore size of 50 µm. Then, 1.2 mL of a nucleotide staining buffer (4', 6-diamidino-2-phenylindole; CyStain ultraviolet Precise P Staining Buffer; Partec) (DAPI) was added to the filtered solution. The resulting stained nuclei were analyzed with a flow cytometer (Partec PA II; Partec) with counts exceeding a minimum of 3,000 cells per analysis. The mean fluorescence for each sample was compared with an internal standard of known genome size (*Pisum sativum* 'Ctirad' 2C genome size = 8.75 pg) and holoploid, 2C genome size (i.e., DNA content of entire non-replicated chromosome compliment irrespective of ploidy) was calculated as 2C = DNA

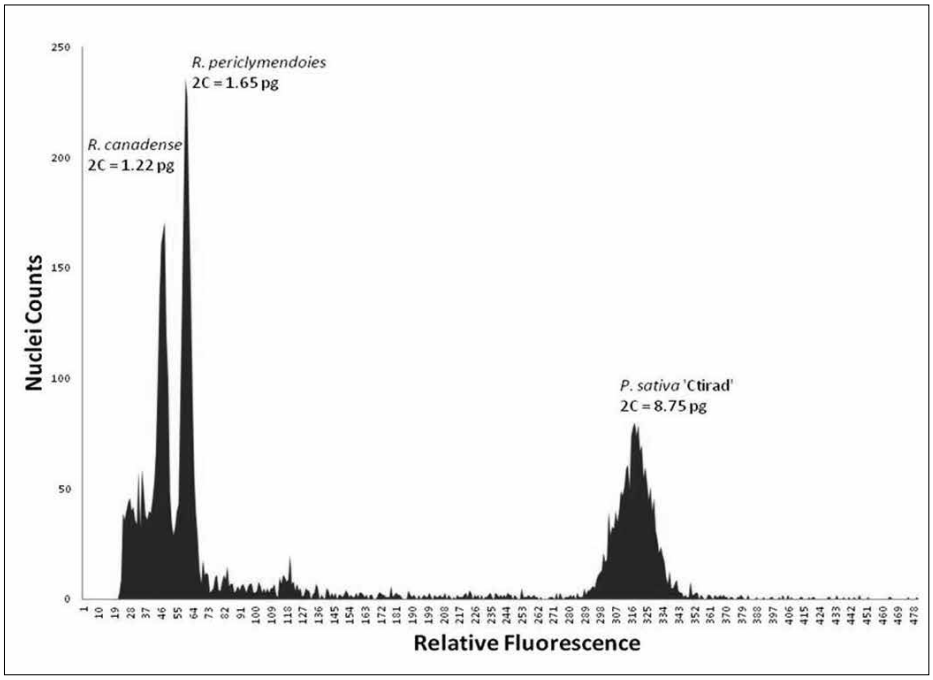

Figure 2. Comparison of genome size of *R. canadense* L. (NCSU 2009-164) and *R. periclymenoides* Michx. (NCSU 2011-027).

content of standard × (mean fluorescence of sample / mean fluorescence of standard).

Cytology. Over the course of a year, actively growing root tips were collected following a sunny day from rooted stem cuttings and mature plants of both *R. canadense* (NCSU 2009-164, Long Pond, PA) and *R. canadense* (NCSU 2009-173, Jaffrey, NH). Root tips containing mitotic cells were collected before 10AM and placed in freshly made vials of pre-fixative solution (2 mM 8-hydroxyquinoline + 70 mg L^{-1} cycloheximide). The compound 8-hydroxyquinoline has proven an effective method to condense chromosomes and destroy the mitotic spindle in order to arrest cells at metaphase in somatic plant cells (Grant and Owens, 1998; Grant and Owens, 2001; Watanabe and Orrillo 1993). Baszczynski et al. (1980) demonstrated that cycloheximide acts in the G2 phase and late metaphase, altering the movement of cells through the nuclear cycle as well as causing an increase in the mitotic index, an increase in metaphase frequency, a decrease in anaphase frequency, and an inhibition of the spindle fiber apparatus assemblage. After remaining in the dark for 3 hours, all vials were moved into a dark refrigerator at approximately 4°C for 3 hours yielding a total pre-fixative treatment of 6 hours. All root tips were rinsed with distilled water and transferred to a freshly made 1:3 fixative solution of propionic acid : 95% ethanol, and left overnight at room temperature (22°C). The next day, a 1:3 hydrolysis solution of 12M HCl : 95% ethanol was made for the root squash procedure.

For each root squash, a fresh root was removed from the fixative and hydrolyzed for approximately 30 seconds before being moved to a clean slide. The root tip was excised under a dissecting microscope and placed on a separate, clean slide with a drop of modified carbol-fuchsion stain. The stain used was a modification of the Carr and Walker (1961) carbol fuchsin stain for human chromosomes (Kao, 1975). The slide was tilted until the stain completely encompassed the excised root tip, and left for several minutes. Then, a clean cover slip was placed over the droplet of stain containing the excised root tip. A clean sheet of bibulous paper was placed over the cover slip while gently applying pressure with a pencil eraser (sometimes thumb) to squash the cells. The slide was then observed and photographed using a light microscope (Nikon Eclipse 80i, Nikon, Melville, NY). Layered images containing multiple depths of field were composed using Photoshop CS4 (Adobe Systems, San Jose, CA).

Results and Discussion

Flow Cytometry. All 11 samples of *R. canadense* were found to be diploid (Table 1), in contrast to previous reports of tetraploid individuals (Ammal et al., 1950; Sax, 1930). Relative genome sizes (2C) in our study ranged between 1.07 pg and 1.23 pg (mean 1.15 pg). The fluorescent stain DAPI used in this study binds to adenine-thymine rich regions of DNA, though other stains are available that may provide slightly different estimates of absolute genome size (Doležel and Bartoš, 2005).

Though the sampling distribution covered multiple locations across nearly the entire geographic distribution of *R. canadense*, no tetraploids were found. These results augment the findings of Zhou et al. (2008) and further demonstrate that *R. canadense* is primarily a diploid species. In addition, relative genome sizes (2C) of all *R. canadense* tested proved unusually small (1.07 to 1.23 pg) when contrasted with diploid genome sizes of other deciduous azaleas in previous studies (Jones et al., 2007; Zhou et al. 2008). In the study by Zhou et al. (2008), diploid azalea genome sizes (not including *R. canadense*) ranged between 1.44 pg and 1.57 pg, and in Jones et al. (2007) the diploid azaleas ranged between 1.51 pg and 1.74 pg.

After the flow cytometry results revealed significant discrepancies in relative genome size (2C) between *R. canadense* and other diploid azaleas from previous studies, a separate cytometric analysis was performed by combining leaf tissue from both *R. canadense* (NCSU 2009-164, Long Pond, PA) and *R. periclymenoides* (NCSU 2011-027), as well as *Pisum sativum* 'Ctirad' (internal standard) into a single

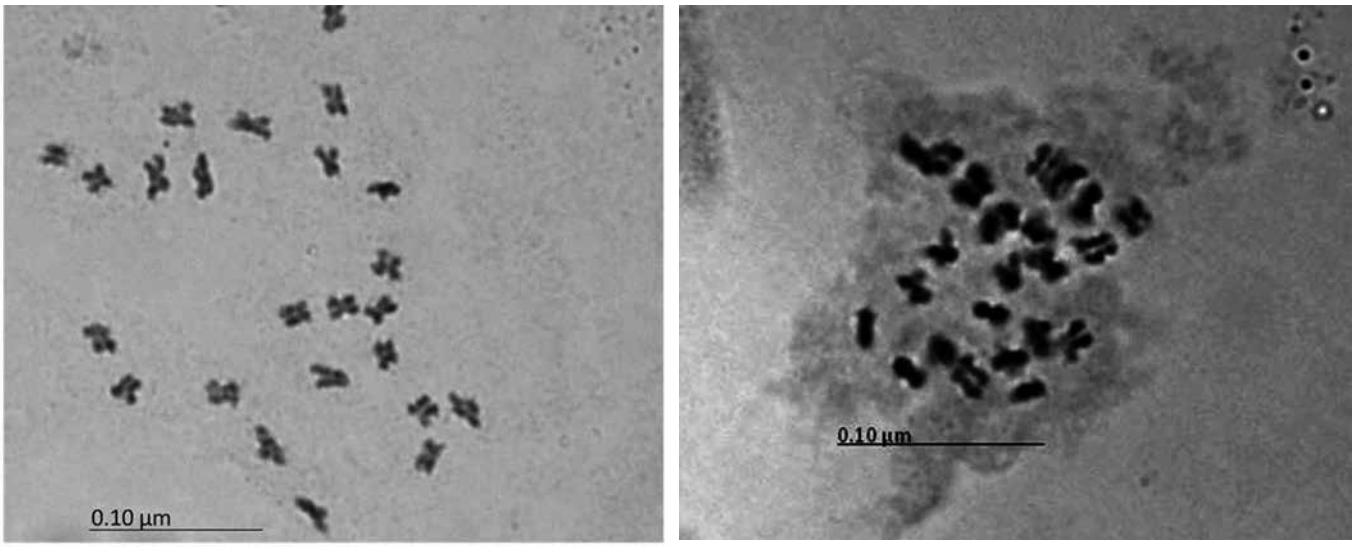

Figure 3. Photomicrographs of metaphase chromosomes from root tip cell of *R. canadense* L. (NCSU 2009-164) collected on Mt. Monadnock, Jaffrey, NH. Viewed at 1000x.

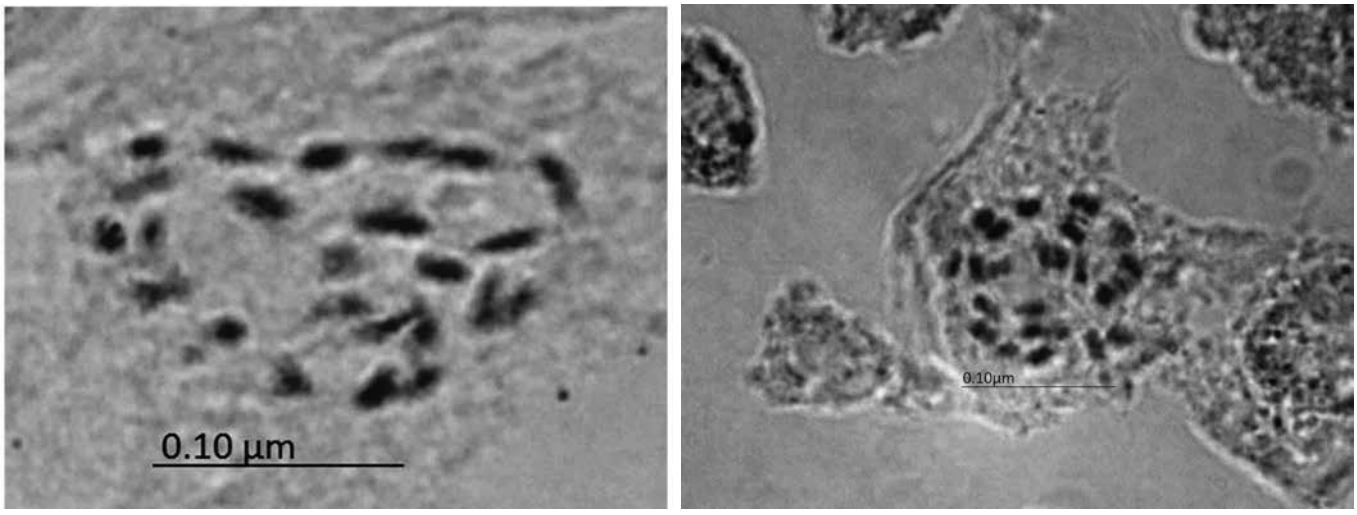

Figure 4. Photomicrographs of metaphase chromosomes from root tip cell of *R. canadense* L. (NCSU 2009-173) collected in the Pocono Mountains, Long Pond, PA. Viewed at 1000x.

sample. The resulting histograms showed there to be a significant difference in relative genome size between *R. canadense* and *R. periclymenoides* (Fig. 2), with *R. canadense* having approximately 26% less total DNA than *R. periclymenoides*.

Previous studies indicate that evolution of base genome size (i.e., DNA content of one complete set of chromosomes: 1Cx value) in angiosperms is a dynamic process of increases and decreases, with the general trend of small genome size representing the ancestral state and larger genome size representing derived state (Leitch et al., 1998, 2005; Soltis et al., 2003). Surprisingly, however, 1Cx genome size in *Rhododendron* (not including *R. canadense*) is highly conserved with 1Cx values between 0.61- 0.97 pg for the entire genus and between 0.72 – 0.97 pg for subgenus *Pentanthera* (Jones et al., 2007). We found *R. canadense* to have a particularly low relative (1Cx) genome size of 0.54-0.62 pg, considerably below the typical range for subgenus *Pentanthera*. Interestingly, Zhou et al. (2008) also found *R. luteum* to have a somewhat low 1Cx genome size of 0.65 pg, a species reported to be fairly closely allied to *R. canadense* in phylogenies based on macromolecular data (Gao et al., 2002; Goetsch, 2005; Kurashige et al., 2001). These results suggested further investigation into base chromosome number was warranted. Therefore, two samples of *R. canadense* (NCSU 2009-164, Long Pond, PA and NCSU 2009-173, Jaffrey, NH) were selected for further study using traditional cytological techniques to determine the

true diploid chromosome number of *R. canadense*.

Cytology. Despite extensive cytological examination performed over the course of a year, including observation of >20 cells with well-resolved chromosomes, no diploid cells were found where more than 24 chromosomes could be discerned (Fig. 3 and 4). These results confirm *R. canadense* to be a diploid and further suggest a base chromosome number of $x = 12$.

The plants in the genus *Rhododendron* have been thought to have a uniform base chromosome number of $x = 13$, with the exception of plants in the basal subgenus *Therorhodion* (Maxim.) Small with $x = 12$ (Kron and Judd, 1990; Kurashige et al., 2001; Gao et al., 2002, Stevens, 1971). Curiously, *R. canadense* (subgenus *Pentanthera*) and *R. camtschaticum* Pall. (subgenus *Therorhodion*) share certain traits, such as rhizomatous growth, July blooms of speckled pink/purple flowers, connate petals with a tubeless corolla, and 10 stamens (Hutchinson, 1921). Perhaps *R. canadense* retained the ancestral chromosome number of $2n = 2x = 24$, similar to plants in subgenus *Therorhodion*, but that is unlikely, based on recent phylogenetic analyses (Kurashige et al., 2001; Gao et al., 2002; Goetsch et al., 2005). Alternatively, various processes such as translocations, inversions, insertions or deletions can lead to chromosome fusion (Da Silva, 2005; Luo et al., 2009; Lysak et al., 2006; Schubert, 2007) resulting in reduced chromosome numbers. This may also be the case in *R. canadense*.

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Works Cited

Ammal, E.K.J, I.C. Enoch and M. Bridgwater. 1950. Chromosome numbers in species of rhododendron. *Rhododendron Yrbk.* 5: 78-91.

Baszczynski, C.L., D.B. Walden and B.G. Atkinson. 1980. Cycloheximide-induced nuclear alteration in maize root tips. *Can. J. Genet. and Cytol.* 22 (3): 319-331.

Carr, D.H. and J.E. Walker. 1961. Carbol fuchsin as a stain for human chromosomes. *Stain Technology* 36: 233-236.

De Schepper, S., L. Leus, M. Mertens, E. Van Bockstaele and M. De Loose. 2001. Flow cytometric analysis of ploidy in Rhododendron (subgenus Tsutsusi). *HortScience* 36 (1): 125-127.

Doležel, J. and J. BartoŠ. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Ann. Bot.* 95: 99-110.

Don, G. 1834. *A General History of Dichlamydeous Plants. Vol. 3, Calyciflorae.* J.F. and F. Rivington, London.

Eeckhaut, T.G.R., L.W.H. Leus, A.C. De Raedt and E.J. Van Bockstaele. 2004. Occurrence of polyploidy in *Rhododendron luteum* Sweet, Hardy Ghent, and Rustica hybrids. *The Azalean* 26: 32-37.

Galle, F.C. 1970. 'Bear Facts' on Gregory Bald. *J. Amer. Rhododendron Soc.* 24 (4): 199-200.

Galle, F.C. 1987 (Reprinted 1991, 1995). *Azaleas: Revised and Enlarged Edition.* Timber Press; Portland, Oregon.

Gao , L.M., D.Z. Li, C.Q. Zhang and J.B. Yang. 2002. Infra-generic and sectional relationships in the genus Rhododendron (Ericaceae) inferred from ITS sequence data. *Acta Botanica Sinica* 44: 1351-1356.

Grant, W. F. and E. T. Owens. 1998. Chromosome aberration assays in Crepsis for the study of environmental mutagens. *Mutation Res.* 410: 291-307.

Grant, W. F. and E. T. Owens. 2001. Chromosome aberration assays in Pisum for the study of environmental mutagens. *Mutation Res.* 488: 93-118.

Gmelin, J.F. 1791. *Systema Naturae. ed.* 1791 (2)1: 694.

Goetsch, L.A., A.J. Eckert and B.D. Hall. 2005. The molecular systematic of Rhododendron (Ericaceae): a phylogeny based upon RPB2 gene sequences. *Sys. Bot.* 30: 616-626.

Hutchinson, J. 1921. The genus Therorhodion. *Bulletin of Miscellaneous Information* (Royal Gardens, Kew) 5: 201-205.

Jones, J.R., T.G. Ranney, N.P. Lynch and S.L. Krebs. 2007. Ploidy levels and relative genome sizes of diverse species, hybrids and cultivars of Rhododendron. *J. Amer. Rododendron Soc.* 61(4): 220-227.

Judd, W.S. and K.A. Kron. 1995. A revision of Rhododendron VI. Subgenus Pentanthera (Sections Sciadorhodion, Rhodora and Viscidula). Edinburgh *J. Bot.* 52: 1-54.

Kao, K.N. 1975. A nuclear staining method for protoplasts. In: O.L.Gamborg and L.Z. Wetter (ed.) *Plant Tissue Culture Methods.* L.R. National Research Council of Canada. Praire Regional Labratory. Saskatoon, Saskatchewan, Canada 1975, p. 60-64.

Kron, K. A. and W.S. Judd. 1990. Phylogenetic Relationships within the Rhodoreae (Ericaceae) with Specific Comments on the Placement of Ledum. *Systematic Bot.* 15(1): 57-68.

Kuntze, O. 1891. *Revisio Generum Plantarum. Vol. 2.* Leipzig. Pg. 386.

Kurashige, Y., M. Mine, N. Kobayashi, T. Handa, K. Takayangi and T.

Yukawa. 1998. Investigation of sectional relationships in the genus Rhododendron (Ericaceae) based on matK sequences. *J. Jpn. Bot.* 73: 143-154.

Kurashige, Y., J.I. Etoh, T. Handa, K. Takayanagi and T. Yukawa. 2001. Sectional relationships in the genus Rhododendron (Ericaceae): evidence from matK and trnK intron sequences. *Plant Systematics and Evolution* 228: 1-14.

Li, H. 1957. Chromosome studies in the azaleas of eastern North America. *Am. J. Bot.* 44(1): 8-14.

Linnaeus, C. 1762. *Species plantarum, ed.* 2. Stockholm. Pg 561.

McAllister, H.A. 1993. Chromosome numbers in rhododendrons. In: *Rhododendrons with Camellias and Magnolias.* Royal Hort. Soc. London, 24-30.

Moringa, T., E. Fukushima, T. Kanui and Y. Tamasaki. 1929. Chromosome numbers of cultivated plants. *Botanical Mag.* (Tokyo) 43: 589-594.

Philipson, M.N. 1980. Cotyledons and Rhododendron classification. In: Luteyn, J. and M. O'Brien (eds) *Contributions toward a classification of Rhododendron.* Proc. Intl. Rhododendron Conf., pp. 75-88. New York Botanical Garden, Bronx, NY.

Philipson. W.R. 1980. Problems in classification of the azalea complex. In: Luteyn, J. and M. O'Brien (eds) *Contributions toward a classification of Rhododendron.* Proc. Intl. Rhododendron Conf., pp. 53-62. New York Botanical Garden, Bronx, NY.

Sax, K. 1930. Chromosome stability in the Genus Rhododendron. *Amer. J. Bot.* 17(4): 247-251.

Stevens, P.F. 1971. A classification of the Ericaceae: subfamilies and tribes. *Bot. J. Linnean Soc.* 64: 1-53.

Torrey, J. 1839. Catalogue of Plants. In: *Geological Survey, New York Assembly* 50: 151.

Towe, L.C. 2004. *American Azaleas.* Timber Press, Portland and Cambridge, 146pp.

Väinölä, A. 2000. Polyploidization and early screening of *Rhododendron* hybrids. *Euphytica* 112: 239-244.

Von Crantz, H.J.N. 1766. *Institutiones rei herbariae: juxta nutum natur naturae digestae ex habitu. Vol. II.* Vienna. Pg 469.

Wilson, E.H. and A. Rehder. 1921. A monograph of the azaleas. Publ. of the Arnold Arboretum 9: 125-206.

Watanabe, K. N. and M. Orrillo. 1993. An alternative pretreatment method for mitotic chromosome observation in potatoes. *Amer. J. Potato Res.* 70 (7): 543-548.

Zhou, W., T. Gibbons, L. Goetsch, B. Hall, T. Ranney, R. Miller. 2008. Rhododenron colemanii: A new species of deciduous azalea (Rhododendron section Pentanthera; Ericaceae) from the coastal plain of Alabama and Georgia. *J. Amer. Rhododendron Soc.* 62(4): 72-78.

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[3]Research Specialist

R. coriaceum: Subgenus *Hymenanthes*, Sect. *Ponticum*, Subsect. *Falconera*

Rhododendron coriaceum (epithet: leathery) was first found by the Abbé Soulié in 1895 in northwest Yunnan, and was described by Adrien René Franchet (National Museum of Natural History in Paris) in 1898. It was introduced into cultivation in 1918 by Forrest, with later introductions by Forrest, Rock and McLaren. It also occurs in mid-west Yunnan and southeast Tibet. It grows in thickets in rhododendron, pine and spruce forests, and among rocks at elevations of 3000 to 4200 m (10,000 to 13,600 ft) and grows as a broadly upright or rounded spreading shrub or tree to 1.2-7.6 m (4-25 ft) in the wild.

The foliage is variable but typically oblanceolate to elliptic, matted olive green on the upper surface and with a thick, soft, smooth, typically creamy, two-layered indumentum on the lower surface. Trusses have 10-20 flowers with five to seven-lobed campanulate white to rose flowers that have a crimson blotch at the base, and are with or without crimson spots.

Two distinct colour forms are grown, white and rose, and the new growth is charmingly white. Most specimens in cultivation are easily grown and are hardy, with flowering in April/May, making it a choice rhododendron. It has not been used much in hybridizing, but the clone 'Morocco' won an Award of Merit in 1953.