More Weighings: Exploring the Ploidy of Hybrid Elepidote Rhododendrons

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Rhododendron enthusiasts may not know it, but they love polyploid hybrid elepidotes. Check any flower show bench and look at the finalists for awards. Mention *Rhododendron* 'Cynthia', 'Gomer Waterer', 'Grace Seabrook', 'Horizon Monarch', 'Marinus Koster', 'Pink Pearl', 'Phyllis Korn', 'Point Defiance', 'Taurus', or 'Trude Webster', and gardeners quickly declare these as among their favorite rhododendrons or high on their wish list. Yes, these rhododendrons all display "something different" and are "highly desirable". They have larger than normal levels of genetic material. They are polyploids.

More than 80 rhododendrons with larger than normal ploidy levels are revealed below. We hope gardeners will see the connection of polyploidy to characteristics of thickness in the stem, leaf, and leaf petiole, along with firmness in flower substance. Indeed, remarkable vigor and substance overall, coupled with an outstanding floral performance at a young age, are often associated with a higher ploidy level. Even gardeners who never want to hear about ploidy love talking about their polyploid hybrid elepidote rhododendrons.

We (**Sally** and **John Perkins**) are not geneticists. We do have science backgrounds, combined with a passion for knowledge. Our ploidy journey began as simple curiosity combined with a willingness to coordinate with others, to scour the Rhododendron literature and the Web, and to do some field work, leading to more discoveries than we ever imagined. Engaging the expertise of João Loureiro and his research team from the University of Coimbra, Portugal, transformed this journey. We have continued this journey further, exploring the interesting comments and conclusions from multiple rhododendron breeders and enthusiasts. We hope you will attempt to grow the newer polyploid elepidotes, take up the challenge of hybridizing even more, and take an interest in the history of their development.

We keep a running list of possible higher ploidy hybrids that could be worthwhile testing should we find a source of samples for testing. These are parents, siblings, or progeny of known polyploid hybrids, visually suspicious plants, or plants suggested by knowledgeable rhododendron people. Thankfully, at the 2011 American Rhododendron Society Conference in Vancouver, Washington, Steve Hootman of the Rhododendron Species Botanical Garden in Federal Way, Washington, introduced us at the banquet table to Hartwig Schepker. Hartwig gave a talk the following evening and as Scientific Director of the Botanic Garden and Rhododendron-Park Bremen, Germany, home of one of the largest Rhododendron collections in the world, we immediately realized that here was a person who had access to additional rare material. Our ongoing polyploid journey would not have to end with so many unanswered questions. Hartwig also realized that this too could be the beginning of a mutually beneficial relationship, and all he needed to provide would be three floral buds per hybrid for flow cytometry testing by the research team at Portugal's University of Coimbra.

Background In Ploidy terms

When we refer to ploidy we mean the "size" of the plant's genetic material. In seed-bearing plants, the genetic material is found in the nucleus of the cell, packed into structures called chromosomes. There are two different techniques used to determine how much genetic material is in a cell and therefore, an estimate

of the number of chromosomes that are present in that cell. One can "count the ways" or "weigh the counts".

Count the ways: The classic way to determine the number of chromosomes in a plant is to "visualize" the chromosomes with stain where they are actively growing, as in a root tip, and then count the different pairs under the microscope. Reports are that this is very tedious (more so in rhododendrons), subject to error, and even eager graduate students are reluctant to cooperate. Chromosome counts are often not duplicated and rarely use multiple specimens.

Weigh the counts: With flow cytometry, it is possible to weigh the genetic material by taking healthy plant tissue (such as leaf, flower or seed) and measuring the "weight" of the genetic content. This technique is much less time consuming and, therefore, easier to verify by duplicating results.

Flow cytometry was developed to detect cancer cell mutations in humans. If the cells are normal and growing, a weighted value representing the normal number of chromosomes would be dominant. There also would be a small number of cells with double the weight of their chromosomes, the ones in the duplicated state, just prior to cell division. Any cells with less than or more than either of those two weights would be an indication of mutations of the amount of genetic material in the cell (i.e. cancer). Flow cytometry is used in this paper to detect the normal weight of genetic material in different *Rhododendron* species and elepidote hybrids.

Polyploidy: Beginning with 1, 2, 3, 4, and 5.

In most plant cells, i.e. leaves, stems, roots and some parts of the flower, the chromosomes are paired with a matching chromosome to form the **diploid** state. We say *most* cells because when it comes time to sexually reproduce, the gametes (cells that fuses with other cells during fertilization) that are the unfertilized seed and the pollen are formed by the splitting apart of the paired chromosomes during a process called meiosis. This forms a nucleus with a single set of chromosomes, the unpaired or **haploid** state. And just to make things complicated, "true seeds" undergo double fertilization, so that they have two haploid nuclei from the seed parent that are fertilized with a haploid pollen nucleus to form the endosperm of a seed. The fertilized endosperm therefore has three sets of chromosomes (two from the seed mother and one from the pollen father) and is triploid. This **triploid** material nourishes the germinating seedling's growth.

Most rhododendrons get one set of 13 chromosomes, denoted as 1x, from each parent (female and male) resulting in two sets of chromosomes. The **diploids** formed in this manner are denoted as 2x (1x + 1x = 2x = 26 chromosomes). However, some rhododendrons have four sets of chromosomes. These are commonly referred to as **tetraploids**, and denoted as 4x (2x + 2x = 4x = 52 chromosomes). **Triploids** have three sets of chromosomes and are denoted 3x. **Pentaploids** have five sets of chromosomes and are denoted 5x. Rhododendrons having more than two sets of chromosomes are referred to as **polyploid** rhododendrons (Ranney et al. 2008).

All species of elepidote rhododendrons that we have tested have been confirmed to be diploid with the noteworthy exception of *R. decorum* ssp. *diaprepes* 'Gargantua'. However, there is no evidence that the subspecies *diaprepes* is tetraploid; **only** the named form 'Gargantua' (selected from seed raised from Forrest 11958) has tested as tetraploid. To date, no wild elepidote population has tested as tetraploid, but this could change. We were hopeful and then disappointed that two relatively new species with stiff, thick leaves, *R. platypodum* and *R. yuefengense*, both tested as typical diploids.

In very rare instances, a parent will not go through the normal splitting process of meiosis and, as a result, the gametes are unreduced. Unreduced gametes donate the plant's full complement of chromosomes to the fertilized embryo. We are very interested in those rare occurrences. It is also possible to artificially induce a higher ploidy with chemicals that interfere with normal chromosome splitting. For example, Augie Kehr in North Carolina was able to form a tetraploid elepidote *R. maximum* 'Summer Joy' * and a lepidote *R. minus* 'Epoch' using colchicine. The terms **neotetraploid** or **neopolyploid** refer to a recent hybrid, whether man-made or natural, that is

a higher ploidy than the diploid state.

Although most rhododendron species are diploid, stable populations of tetraploid *Rhododendron* species do exist within deciduous azaleas and lepidotes (Zhou et al.2008). An individual triploid rhododendron can occur naturally where diploid and tetraploid species of Rhododendron are co-located, and they appear to be hybrids (Perkins et al.09/2010). For example, natural triploid deciduous azaleas exist in Audra State Park in West Virginia, Hurricane Creek in Georgia, and Wayah Bald in North Carolina.

Exploring: the Journey

In the fall of 1989, our Rhododendron polyploidy journey unknowingly started when we overheard at a local rhododendron meeting a statement that Frank Mossman wrote in 1972 concerning his hybridization efforts with *Rhododendron occidentale*:

> "We have found that *Rhododendron occidentale* will cross with many other rhododendrons or azaleas if *occidentale* is the seed parent, but *occidentale* as a pollen parent produces few seed." (Mossman 1972).

We wondered, "Why?"

In the fall of 2011 we read in the ARS online ejournal that in 1972 **Harold Greer** wrote the following concerning his hybridization of 'Countess of Derby' to produce 'Trude Webster':

> "If you are one of those who feels that there could be nothing outstanding produced in a pink rhododendron I would have been the first to agree with you. That was until I saw the first bud unfold on the original seedling of 'Countess of Derby' selfed" (**Greer** 1972).

Both Mossman and Greer had encountered the wonder accompanying the many puzzles presented by polyploid rhododendrons, so we were in good company.

Starting in the early 1990s, we unknowingly crossed deciduous azaleas involving different ploidy levels, leading in 2010 to collecting samples of diploid, triploid and tetraploid rhododendrons for ploidy testing at the University of Coimbra in Portugal. Each step on this pathway revealed more about the wonderful world of ploidy in our own rhododendron garden. Below is a summary of what we discovered, often based on the research, observations, and documentation of many others, about the ploidy of hybrid elepidote rhododendrons and the people encountered on our slow but wondrous journey.

Imagine if you will the following:

It is 1913 and a beautiful spring day in England, so what do you do? George V is the first Windsor King, Woodrow Wilson is serving his first term as President of the United States, and World War I is a future event. In bloom are the two most popular rhododendrons in the world at that time, 'Pink Pearl', an 1890s Waterer hybrid, and 'Cynthia', an 1850s Standish & Noble hybrid. On a beautiful spring day in 1913, if you were Henry 'Harry' White, a nursery manager in Sunningdale, England, you would have crossed 'Pink Pearl' with 'Cynthia', and later named a seedling from this cross 'Countess of Derby'.

It is spring 1961; John Kennedy is the handsome young President of the United States and Vietnam is a country unknown to most Americans. On a beautiful spring day in 1961, if you were **Harold Greer**, living in Eugene, Oregon, you selfed 'Countess of Derby' and later named a seedling from this cross 'Trude Webster'.

It is spring 1969; Richard Nixon is now the President of the United States, and Watergate is simply an office building in the DC area. On a beautiful spring day in 1969, if you were Robert Korn in Renton, Washington, you placed the pollen from 'Gomer Waterer', a 1900 Waterer 'Pink Pearl' hybrid, onto 'Diane' and later named a seedling from this cross 'Phyllis Korn'.

It is spring 1988; George H. Bush is the Vice President of the United States, and Iraq is simply a country somewhere in the Middle East. On a beautiful spring day in 1988 if you were Jim Barlup, living in Bellevue, Washington, you crossed 'Whitney's Late Peach' by 'Phyllis Korn'. You later named a seedling from this cross 'Summer Peach'.

It is spring 2001, George W. Bush is the President

of the United States and the Twin Towers in New York City are still standing. On a beautiful spring day in 2001 if you were Jim Barlup, living in Bellevue, Washington, you crossed 'Phyllis Korn' by 'Trude Webster' to create several viable offspring. You later named a seedling from this cross 'White Ginger'.

So what have you done by starting all this in 1913?

Well, you took two fertile triploids from the 19th century, namely 'Pink Pearl' and 'Cynthia' and pentaploid seedlings and from these selected 'White Ginger' (Fig. 1).

By doing so, you ended the myth that triploids are always sterile and showed that triploids can, in fact, be both seed and pollen parents. Moreover, triploids, when used in hybridization, produce **mostly reduced but some unreduced gametes**. You demonstrated that triploids provide a pathway for the bi-directional transfer of genes between diploids, triploids, tetraploids, and pentaploids. It took your imagination a few beautiful spring days

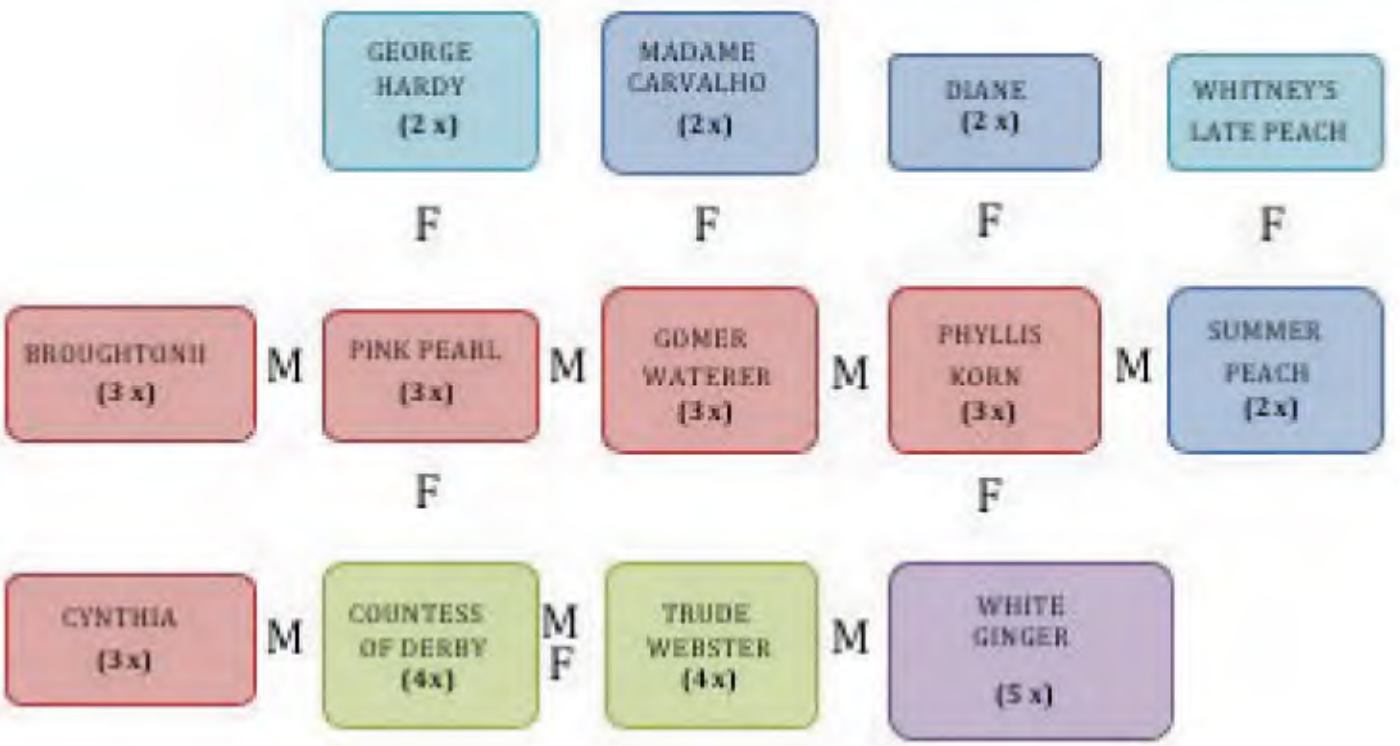

Figure 1: GENERATIONAL BREEDING OF POLYPLOID HYBRID ELEPIDOTES demonstrating that triploids can be fertile and can create a bi-directional pathway between ploidy levels.

created a tetraploid, namely 'Countess of Derby'. You then selfed the tetraploid 'Countess of Derby' creating another tetraploid, namely 'Trude Webster'. You then placed pollen from the triploid 'Gomer Waterer' onto a diploid seed parent, namely 'Diane' and created a triploid, namely 'Phyllis Korn'. You then used the pollen of that triploid, 'Phyllis Korn' and crossed it with 'Whitney's Late Peach' (untested but probably a diploid) to create a diploid, namely 'Summer Peach'. You then placed the pollen of the tetraploid 'Trude Webster' onto the triploid seed parent 'Phyllis Korn' and produced a series of doing crosses, a few changes of names and addresses, nearly 90 years, and a team of young researchers at the University of Coimbra in Portugal to confirm your results, but all in all not a bad piece of work!

The following ploidy list of named hybrid elepidote Rhododendrons as determined using flow cytometry, all by João Loureiro, Silvia Castro, José Cerca Oliveira, and Mariana Castro, Plant Ecology and Evolution Group, Centre for Functional Ecology, Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, Portugal, unless otherwise indicated.

Summary of Ploidy

Key:

F indicates a fertile triploid.
T indicates a chemically induced tetraploid.
P indicates a diploid with a polyploid ancestor.
S indicates a diploid with a tetraploid parent.
U indicates a diploid with a tendency to produce unreduced gametes.
DM indicates a triploid resulting from a triploid parent.
NM indicates a triploid resulting from a tetraploid parent.
UM indicates a triploid resulting from 2 diploid parents.
2X4 or 4X2 indicates a tetraploid resulting from a diploid and a tetraploid parent.
3X2 indicates a tetraploid resulting from a triploid and a diploid parent.
3X3 indicates a tetraploid resulting from 2 triploids parents.
3XQ indicates a tetraploid resulting from at least one triploid parent.
4X4 indicates a tetraploid resulting from 2 tetraploid parents.
4X3 indicates a tetraploid or pentaploid resulting from a tetraploid and a triploid parent.
4XQ indicates a tetraploid or pentaploid resulting from a tetraploid parent.
% indicates flow cytometry ploidy testing was done by research team led by **Tom Ranney.**
# indicates flow cytometry ploidy testing was done by Tom EeckHaut.
(,) indicates the name of the hybridizer and date of cross related to use or production of a polyploid.
* indicates not registered.

2x Diploids (89 Total, 22 from Polyploid Parents)

'1000 Butterflies'
'Adriaan Koster'
'Alice '
'Anna' (Lem, 1952) U
'Bethany Jade'
'Betty Hume'
'Bibiani'
'Bruns Sirius' (Bruns, 1955) P
'Cheyenne'
'Colonel Coen'
'Countess of Athlone'
'Dagmar' (Kavka, 1965) P
'Diane'
'Doctor H.C. Dresselhuys'
'Doctor V.H. Rutgers'
'Donald Waterer' (Waterer, 1916) P
'Don Juan'
'Double Besse'
'Duke Of York'
'Elegans'
'Everlasting' %
'Fantastica' %
'Furnivall's Daughter'
'George Hardy' (Mangles, 1884) U
'Gillii'
'Gill's Triumph'
'Goldflimmer' %
'Goldsworth Orange' #
'Gotham Rheingold'
'Graf Zeppelin' (van Nes, 1934) P
'Gunborg' (Sagemuller, 1964) P
'Heinje's Venezia' (Sagemuller, 1964) P
'Horizon Lakeside'
'Hotei'
'Hurricane' (Whitney, 1960) P
'Irmelies' (Hachmann, 1962) P S
'Isabel Pierce'
'Isadora' (Sagemuller, 1962) P
'Janet Blair' %
'J.G. Millais' (Waterer, 1915) P
'Jingle Bells' #
'Käthe Heinje' (Heinje, 1964) P
'Kathy Van Veen'
'Kupferberg' #
'Lady Bligh'
'Lady de Rothschild'
'Lady Eleanor Cathcart'
'Lady Longman' (White, 1929) P
'Lem's Cameo'
'Loderi Venus'
'Loder's White'
'Madame Carvalho'
'Maxecat' %

R. maximum 'Kalamity'
R. maximum 'Delp's Red Max'
'Mindy's Love'
'Mother of Pearl' ('Pink Pearl' sport, 1925) P
'Mrs A.T. de la Mare'
'Mrs E.C. Stirling' (Waterer, 1906) P
'Mrs Furnivall'
'Mrs Lindsay Smith'
'Nancy Evans'
'Naselle'
'Norman Gill'
'Nova Zembla' %
'Olin O. Dobbs'
'Orange Leopard' (Brack, 1988) P S
'Peach Charm'
'Peach Recital' (Barlup, 1996) P
'Phipp's Yellow'
'Pink Prelude'
'Polar Bear' %
'Professor J.H. Zaaijer' (Endtz, 1958) P
'Puget Sound' %
'Red Olympia'
'Rendezvous' (Hachmann, 1968) P S
'Scandinavia' (Koster, 1950) P
'Shoreham'
'Sir Robert Peel'
'Stony Brook' (Brack, 1988) P S
'Summer Peach' (Barlup, 1988) P
'Summer Wind' (Barlup, 1996) P
'The Honourable Jean Marie de Montague' (van Nes, 1901) U
'Voluptuous'
'Vulcan' %
'Vulcan's Flame' %
'Wanna Bee'
'White Pearl' (syn 'Halopeanum')
'Wild Affair'

3x Triploids (51 Total)

'Anita Gehnrich' (Gehnrich) UM
'Anna Rose Whitney' (Van Veen, 1954) F NM
'Annie E. Endtz' (Endtz, 1939) DM
'August Lamken' (Hobbie, 1942)
'Beauty of Littleworth' (Mangles, 1884)
'Betty Wormald' (Koster, 1907) F
'Broughtonii' (Broughton, 1840) F
'Caruso' (Hachmann, 1990) NM
'Charis' (Hachmann, 1990) NM
'Cotton Candy' (Henny & Wennekamp, 1958) F UM
'Cynthia' (Standish & Noble, 1856) F
'Dame Nellie Melba' (Loder, 1926)
'Denali' (Elliott, 1987) NM
'Django' (Hachmann, 1985)
'Ebony Pearl' ('Pink Pearl' sport, 1966)
'El Camino' (Whitney, 1976) NM
'Frentano' (Hachmann, 1989) NM
'Gartendirektor Rieger' (Hobbie, 1947)
'Gomer Waterer' (Waterer, 1900) F DM
'Grace Seabrook' (Seabrook, 1965) UM
'Halfdan Lem' (Lem, 1967) UM
'Hallelujah' (Greer, 1958)
'Hank's Folly' (Schannen) NM *
'Hollandia' (Endtz, 1938) DM
'Johnny Bender' (Seabrook, 1960) F UM
'Julia Caroline' (Brockenbrough, 1990) NM
'Lady of Spain' (Lofthouse, 1966) NM
'Lucky Strike' (Van Veen, 1958) NM
'Lydia' (Greer, 1963) F NM
'Markeeta's Flame' (Markeeta, 1960) UM
'Markeeta's Prize' (Markeeta, 1970) UM
'Newcomb's Sweetheart' (Newcomb, 1968) NM
'Opal Thornton' (Thornton) NM
'Pearce's American Beauty' (Pearce, 1930) F
'Phyllis Korn' (Korn, 1969) F DM
'Pink Pearl' (Waterer, 1892) F DM
'Platinum Pearl' (Greer, 1983) F NM
'Queen Mary' (Felix & Dijkhuis, 1950) DM
'Romilda' (Hachmann, 1990) NM
'Rothenburg' (von Martin, 1944)
'Rwain' (Colombel, 1993) F NM
'Solidarity' (Schannen, 1969) F UM
'Souvenir de Doctor S. Endtz' (Endtz, 1924) DM
'Steredenn' (Colombel) NM
'Sugar Pink' (Greer, 1960) NM
'Super Dog' (Bones) NM *
'Taurus' (Mossman, 1962) F UM
'Topsvoort Pearl' ('Pink Pearl' sport, 1935)
'Val d'Aulnay' (Croux & Fils, 1984) F

'Van' (Van Veen, 1930) NM
'Viscy' (Hobbie, 1950) UM

4x Tetraploids (31 Total)

'Antoon van Welie' (Endtz, 1930) 3X2
'Aristide Briand' (Endtz, 1950) 3XQ
'Brigg's Red Star' (Briggs) T
'Cherry Cheesecake' (Briggs) T % *
'Countess of Derby' (White, 1913) 3X3
R. diaprepes 'Gargantua' (Stevenson, 1923)
'Doctor A. Blok' (Endtz, 1937) 3XQ
'Doctor Arnold W. Endtz' (Endtz, 1927) 3XQ
'Doreen Gale' (Sanders) 4X4
'Friesland' (Endtz, 1958) 3XQ
'Gentle Giant' (Sanders, 1992) 4X3
'Germania' (Hobbie, 1956) 4X2
'Gorgeous George' (Sanders) 4X4
'Grand Slam' (Greer, 1982) 4X3
'Horizon Jubilee' (Brockenbrough) % *
'Horizon Monarch' (Brockenbrough, 1981) 2X4
'Jan Dekens' (Endtz, 1940) 3XQ
'Le Fouesnantais' (Colombel, 1997) 4XQ
'Legend' (Barlup) 4X4
'Lem's Monarch' (Lem, 1965) 2X4
'L'Engin' (de la Sablière) 4X2 *
'Marinus Koster' (Koster, 1937)
'Pink Goliath' (van Nes, 1958) 4XQ
'Point Defiance' (Lem, 1970) 2X4
'Professor Hugo de Vries' (Endtz, 1958) 3X2
'Reverend Paul' (Sumner, 1975) 4X4
'Summer Joy' (Kehr) T *
'Supernova' (Briggs) T %
'Trude Webster' (Greer, 1960) 4x4
'Very Berry' (Greer, 1988) 4X2
'XXL' (ID'Flor) *

5x Pentaploids (2 Total)

'Haithabu' (Hachmann, 1991) 4XQ
'White Ginger' (Barlup, 2001) 4X3

'Pink Pearl' a triploid, won the first Award of Merit in 1897 and continues as a garden-worthy plant as Rhododendron of the Year in 2006 by the Southwestern Chapter of the American Rhododendron Society. In 1950, a large 'Cynthia', a triploid originally bred in 1858, was the first rhododendron planted in Portland Chapter's Crystal Springs Rhododendron Garden. 'Trude Webster', a tetraploid, won the American Rhododendron Society's first Superior Plant Award in 1971 and is still found on lists of Proven Performers for the West Coast. 'Broughtonii', a triploid bred in 1840, is still considered to be among the best warm weather rhododendrons according to Don Burke, who gardens in Australia.

Historical evidence indicates that by 1910, the triploids 'Betty Wormald', 'Beauty of Littleworth', 'Broughtonii', 'Cynthia', 'Gomer Waterer' and 'Pink Pearl' would have been on most lists of best elepidote rhododendrons. In 1958, George Grace's list of best elepidote rhododendrons included all but one of these triploids plus the tetraploids 'Countess of Derby' and 'Marinus Koster'. In 2008, the Siuslaw Chapter of the American Rhododendron Society included on their list of best elepidote rhododendrons the triploids 'Cynthia', 'Dame Nellie Melba', 'Grace Seabrook', and 'Taurus', and the tetraploids 'Grand Slam', 'Lem's Monarch', Horizon Monarch', 'Point Defiance' and 'Very Berry'.

By 2011, Rhododendrons of the Year, Proven Performers, Awards of Garden Merit and Best in Show trusses were added to the 'bests' mentioned above, taking in the triploids 'Anita Gehnrich', 'Anna Rose Whitney', 'Cotton Candy', 'Ebony Pearl', 'Gartendirektor Rieger', 'Hallelujah', 'Markeeta's Prize', 'Platinum Pearl', 'Solidarity' and 'Super Dog', and the tetraploids 'Gentle Giant' and 'Trude Webster'. In fact, over 30 of the 84 confirmed polyploid elepidote rhododendrons have appeared on lists of the best rhododendrons. Once these polyploids appear on such lists, they are likely to stay.

The following hybridizers have worked with or produced polyploid elepidote hybrids:

Arends, Barlup, Blaauw, Bohlken, Bones, Boulter, Bovees, Brack, Briggs, Brockenbrough, Broughton, Bruns, Colombel, Croux et Fils, de la Sablière, Drake,

Dürre, Elliott, Endtz, Evans, Farewell, Felix & Dijkhuis, Fennichia, Fujioka, Gehnrich, Gill, Grall, Greer, Guyens, Hachmann, Hall, Hartman, Heinje, Henny & Wennekamp, Hobbie, Horlick, Horsley, Hübbers, Johnson, Kavka, Kehr, Korn, Koster, Larson, Laxdall, Lem, Loder, Lofthouse, Mangles, Markeeta, McCullough, Moynier, Mossman, Murcott, Naylor, O'Rourke, Ostler, Patterson, Pearce, Perkins, Peste, Poulsen, Rabideau, Ragans, Reuthe, Sagemuller, Sanders, Schannen, Schnupper, Seabrook, Shapiro, Smith, Standish & Noble, Stead, Stevenson, Stockman, Sumner, Thacker, Thornton, van Nes, Van Veen, Vinson, von Martin, Waldman, Walton, Waterer, Wieting, White, Whitney, Wilson, Winberg & Smith, and Woodward.

It is noted that nearly as many hybridizers have worked with confirmed elepidote polyploids as there are confirmed polyploid hybrids. More importantly, some of the hybridizers on this list are best known for the polyploid elepidotes they have created. In fact, polyploid elepidotes have been named in honor of wives, mothers, grandparents, and queens.

Incidentally, Mossman, working with the diploid deciduous azalea species *R. occidentale,* discovered what Barlup later discovered working with hybrid elepidotes: diploids are much more likely to accept pollen from tetraploids than tetraploids are to accept pollen from diploids. We found this to be true for our crosses and have addressed this topic in more detail elsewhere(**Perkins** et al. 12/2009). Breeding with polyploid elepidotes is not an easy task, and is fraught with low fertility in seed set and low viability in seed germination. This explains why so few polyploids have been created to date, despite so many hybridizers having attempted to use them as parents.

Jim Barlup wrote the following about using polyploid elepidotes as parents:

> *"I continue to test the pollen and plants which I doubt for 3 or 4 years to determine their fertility or sterility. If you cross a diploid with tetraploid pollen you can achieve beautiful seedpods but their germination is very difficult. 3% seed germination for 'Point Defiance'. Obtained are both diploid or tetraploid offspring.'*(Barlup 1999)

Ron Naylor wrote the following about his best plant, 'Francis Augustus Storey', from a cross involving the tetraploid 'Point Defiance':

> *'Francis Augustus Storey' - Best of grex of four plants from weak germination. One died in 2000 and another in 2001.'* (Naylor 2010)

Dick Murcott wrote the following about the plant he called 'TT116'*:

> *'TT116 – [('Jean Marie de Montegue' X degronianum var. yakushimanum) X 'Grand Slam']. Only one seed from this cross germinated. Looks like a tetraploid. Pink. Looks like 'Trude Webster' but is definitely a seedling.'* (Murcott 2010)

Barlup, Murcott, and Naylor each encountered both the wonder and puzzlement presented by polyploid rhododendrons. We have discovered for deciduous azaleas that seed produced from tetraploid X tetraploid normally has high rates of germination, but germination from diploid X tetraploid crosses varies greatly **but** is often poor. To read about Frank Abbott's encounter with the wonders of working with deciduous azaleas of different ploidy levels see 'Frank Abbott's Village of Azaleas' (Perkins et al. 09/2009) or 'Margaret Abbott' is a Tetraploid' (Perkins et al. 2011).

The following people and organizations donated samples for this research without whose generous donation of time and material this work could not have progressed:

John Abbott, Jane Adams of White Cloud Nursery, **Charles Andrews**, Vivian Abney of East Fork Nursery, Living Collection of **Arnold Arboretum**, Natural Collection of Audra State Park, Living Collection of Bartlett Arboretum, Jim Barlup, Norman Beaudry of American Rhododendron Society Seed Exchange, Jane Brooks, Joe Bruso, Werner Brack, Ned Brockenbrough, Natural Collection of Canobie Lake NH, Dick Cavender, Clarice Clark of Western North American Rhododendron Species Project, Living Collection of Connecticut College Arboretum, Marc Colombel, Alfred Cook, **Mike Creel, Bruce**

Untested Named Elepidote Hybrids We Suspect of Being Polyploid:

'Adelheid' *
'Aggie'
'Aibette'
'Alibaby'
'Andantino'
'Aperitif'
'Arden Primrose'
'Ariel Sherman'
'Arnold Piper' ##
'Arthur Ostler'
'Babar'
'Bel Air'
'Bellevue'
'Bernard Crisp'
'Bernard Shaw'
'Bohlken's Kronjuwel'
'Boskoop Concorde'
'Canadian Beauty' ##
'Cara Meg'
'Castanets'
'Comte du Parc'
'Courtenay Duke'
'Diane Marie'
'Diane Titcomb'
'Diderk'
'Direktor Siebert'
'Doris Nolan'
'Dorothy Peste Anderson'
'Double Drake'
'Edward Cornelius'
'Elizabeth Titcomb'
'Ester Grace'
'Eureka Maid'
'Fiona Wilson'
'Flensburg'
'Forever Violet'
'Fragrant Sensation' ##
'Francis Augustus Storey' ##
'Garnet'
'Gill's Gloriosa'
'Ginette'
'Goliath'
'Grab Ya' ##
'Gwen Bell'
'Hachmann's Anastasia'
'Hachmann's Kristina'
'Hachmann's Pinguin'
'Hachmann's Veronika'
'Heat Wave'
'Heinje's Schneewittchen'
'Heinje's Venezia'
'Helen Druecker'
'Horizon Serenity' ##
'Humoreska'
'Ilam Apricot'
'Ilam Orange'
'Inheritance'
'Isobel Baillie'
'Janet Ward'
'Jean Lennon'
'Jean Marie Variegated'
'Jeanne Yvonne'
'Jenice Coffey'
'Julie Titcomb'
'Justa Pink'
'Kareness'
'Kathy Ann Pieries'
'Kay Too'
'Kranenburg'
'Kranenkorn'
'Kranenrosette'
'KSW'
'Leonardslee Giles'
'Lilian'
'Lou-John Gem'
'Madah Jean'
'Maimorgen'
'Malaga'
'Margaret Mack'
'Marie Oliva Schlicková'
'Marion'
'Mary-Ed'
'Maureen Ostler'
'Melville'
'Miss Kitty'
'Mistake'
'Nicandra Newman'
'Onkel Dines'
'Orrie Dillie'
'Patricia Jacobs' ##
'Peggy Bannier'
'Pink Perfection'
'Pink Petticoats'
'Pink Titan'
'President Kennedy'
'Pride of Roseburg' ##
'Princess Debiann'
'Prinzessin Inka'
'Professor Horst Robenek' ##
'Qualicum's Pride'
'Record'
'Red Walloper' ##
'Rheinzauber'
'Robert Korn'
'Rosandra'
'Rosa Millennium'
'Rose Marie'
'Rotha'
'Rubinpracht'
'Seraphine'
'Serengeti'
'Shalom'
'Shari Laurel'
'Sheer Enjoyment' ##
'Siegfried Sommer'
'Sierra Sunrise'
'Sigrid'
'Standishii'
'Tausendschön'
'The Duchess'##
'Titness Belle'
'TT 116' *
'Twins Candy'
'Vincent Van Gogh'
'Virgo' #
'Vonnie Stockman'
'Walküre'
'Walloper' ##
'Whidbey Island'
'White Swan'
'Wilhelm Schacht'
'William Avery' ##

- Almost certainly polyploid based on parentage and/or fertility

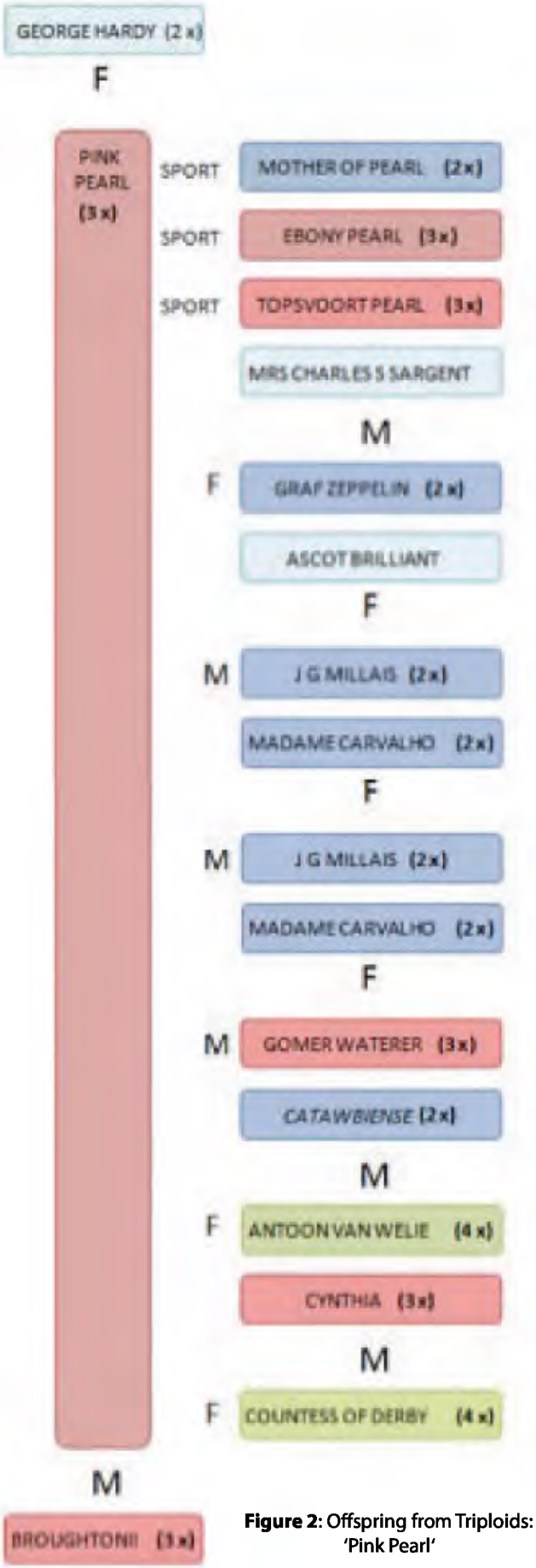

Figure 2: Offspring from Triploids: 'Pink Pearl'

Clyburn, Hans Eiberg, Al Fitzburg, Robert Fox, **Harold Greer of Greer Gardens**, George Hibben, Living Collection of Highstead Arboretum, Steve Hootman of Rhododendron Species Botanical Garden, **Don Hyatt, J. Jackson of Appalachian Native Plants, Lindy Johnson of Azalea Society of America Seed Exchange,** Richard Jaynes of Broken Arrow Nursery, Doug Jolley, Fred Knippel, Johnny Larsen, Living Collection of **Longwood Gardens**, Robert MacIntyre, Ron Miller, Dick Murcott, Michael Medeiros of Planeview Nursery, Wayne Mezitt of Weston Nurseries, George Newman, Peter Norris, **John and Sally Perkins, Ron Rabideau of RareFind Nursery**, Ellie Sather of Whitney Gardens, Hartwig Schepker of Rhododendron-Park Bremen, Natural Collection of Stoddard Bog NH, Kristian Theqvist, Patrick Thompson of Donald E Davis Arboretum, **John Thornton**, Hendrik Van Oostand of Azaleatuin, **Kathy Van Veen of Van Veen Nursery.**

Despite having created this new suspected polyploid list, our four-year journey of testing suspected polyploids has shown that only about half will turn out to be polyploids. Some such as 'Fragrant Sensation', 'Grab Ya', 'Pride of Roseburg', and 'Sheer Enjoyment', having both parents as tested tetraploids, are almost certainly polyploids. In the list above,. we have marked, using the double pound sign (##), a dozen or so we think are the most likely polyploids based on parentage and fertility. The only way you can know for certain whether a plant is an actual polyploid is to obtain a fresh sample from a reliable source and test it under laboratory conditions.

Most in this suspected list are known to have at least one polyploid parent, be a sibling of a polyploid, or be a parent of one or more polyploids. However, both triploid and tetraploid hybrid elepidotes have been shown to be capable of producing diploid offspring when the other parent is a diploid. Many hybrids on our suspected polyploid list do have one parent suspected of being a diploid. In other words, a diploid can have a polyploid parent or sibling. Moreover, two diploid parents can produce a polyploid offspring, so having a polyploid offspring does not ensure either parent is a polyploid. Having a polyploid parent or

Figure 3: The pedigree of 'The Duchess', a suspected polyploid, showing the 23 non-distinct polyploids in its ancestry.

sibling greatly increases the chances that a plant is a polyploid but is no guarantee. Whereas, being highly fertile reduces the chances that a plant is a polyploidy, especially if one of that plant's parents is a known diploid. A popular hybrid with polyploid ancestry and few named offspring is an excellent candidate for our list of suspected polyploids.

Based on looking at parentage and the number of offspring of named hybrids plus confirming 24 polyploid hybrids with research done on 48 additional suspected polyploids in 2012, we conjecture that our lists of known (84) and suspected (132) polyploids contain the vast majority of what is almost certainly less than 200 polyploid elepidote hybrids registered prior to 2000.

In short, there are no rules of thumb for "knowing" the ploidy of the offspring for hybrid elepidotes if the parents are of mixed ploidy levels or either parent is a triploid or pentaploid. Diploid X diploid will almost always (but not always) create diploid offspring. Tetraploid X tetraploid will almost always (but not always) create tetraploid offspring. However, diploid X tetraploid and tetraploid X diploid, which are normally associated with producing triploid offspring, have been shown to produce a combination of diploids, triploids, and tetraploids when working with hybrid elepidotes.

Triploids, Fertile Triploids and Triploids as the Progeny of Triploids

Triploids are normally believed to be produced by one of two mechanisms. Two diploids can cross where one diploid parent, instead of providing one set of chromosomes, provides two, resulting in an offspring that has three sets of chromosomes. This is commonly referred to as the **unreduced mechanism for creating triploids.**

Ploidy results suggest that triploids such as 'Anita Gehnrich', 'Grace Seabrook', 'Markeeta's Flame', 'Markeeta's Prize', 'Solaridity', and 'Taurus' were most likely created by this unreduced mechanism.

On the other hand, a diploid parent and a tetraploid parent can cross where the diploid parent provides one set of chromosomes and the tetraploid parent provides two sets of chromosomes, resulting in an offspring with three sets of chromosomes. This is referred to as the **normal meiosis interploidy mechanism for creating triploids**. Ploidy results suggest that triploids such as 'Anna Rose Whitney', 'Cotton Candy', 'El Camino', 'Hank's Folly', 'Julia Caroline', 'Lady of Spain', 'Lucky Strike', 'Lydia', 'Opal Thornton', 'Platinum Pearl', 'Rwain', 'Steredenn', 'Sugar Pink', 'Super Dog', and 'Van' were most likely created by this normal meiosis interploidy mechanism.

Offspring are documented for the following triploids: 'Anna Rose Whitney', 'Betty Wormald', 'Broughtonii', 'Cotton Candy', 'Cynthia', 'Gomer Waterer', 'Lydia', 'Pearce's American Beauty', 'Phyllis Korn', 'Pink Pearl', 'Platinum Pearl', 'Rwain', 'Solidarity', 'Taurus', and 'Val d'Aulnay' (Fig. 1). The common belief that triploids are always sterile as both seed parents and pollen parents is challenged by these findings. Triploids such as 'Pink Pearl', 'Phyllis Korn', 'Rwain' and 'Taurus' appear to be partially fertile as both seed and pollen parents (Fig. 2). In fact, triploids can be the progeny of triploids. Based on parental documentation, 'Broughtonii', 'Pink Pearl, 'Gomer Waterer' and 'Phyllis Korn' represent

four consecutive generations of triploids (Fig. 1).

Three sports of the triploid 'Pink Pearl' were ploidy tested: 'Ebony Pearl' and 'Topsvoort Pearl' tested as triploid whereas intriguingly, 'Mother of Pearl' tested as diploid (Fig. 2).

Diploids can be the progeny of triploids. Diploids such as 'Graf Zeppelin', 'Hurricane', 'J.G. Millais', and 'Summer Peach' are documented to have a triploid parent. In the case of 'Graf Zeppelin', the triploid 'Pink Pearl' is documented as the seed parent (Fig. 2). Although a diploid, 'Graf Zeppelin' exhibits characteristics often associated with named polyploids.

Tetraploids can be the progeny of triploids: 'Countess of Derby', a tetraploid, is documented to have two triploid parents, namely 'Pink Pearl' and 'Cynthia' (Fig.1). Tetraploids such as 'Antoon van Welie', 'Gentle Giant', and 'Grand Slam' are documented to have a triploid parent. In the case of 'Antoon van Welie', the triploid 'Pink Pearl' is documented as the seed parent (Figs. 2 and 3).

Marc Colombel donated some of his suspected polyploid hybrid seedlings for testing. Noteworthy is that four seedlings of 'Rwain' X 'L'Engin' tested as tetraploid. 'Rwain', the seed parent, is a triploid. 'L'Engin', the pollen parent, is a tetraploid. Moreover, three seedlings of the cross of the tetraploid 'Horizon Monarch' with the triploid 'Rwain' tested as tetraploids but one seedling tested as triploid.

Figure 2 suggests that a triploid parent, for example 'Pink Pearl', can produce offspring that are diploids, triploids, and tetraploids. Figure 1 suggests that pentaploids such as 'White Ginger' are also possible from a triploid parent.

In the 1930s, C. D. Darlington showed that triploids could be fertile. Moreover, Darlington confirmed a third mechanism for creating triploids. Darlington showed that the chromosome set in triploids may split unevenly during meiosis to form a bell-shaped curve distribution. This means that most gamete cells form near the midpoint of 1.5x, with a few having 1x and 2x chromosomes. So in a few cases, a triploid parent can act as a diploid contributing one set of chromosomes or as a tetraploid contributing two sets of chromosomes. Our ploidy results, (Perkins et al. 10/2010) when combined with the documentation of parentage, strongly suggest this third distributive meiosis mechanism does occur for fertile triploid elepidote rhododendrons. Hans Eiberg has determined in controlled lab experiments that for rhododendrons, hybrid triploid pollen is sometimes just as viable as any hybrid diploid pollen.

Tetraploids and Diploids as the Progeny of Tetraploids

Tetraploids such as 'Doreen Gale', 'Gorgeous George', and 'Legend' have been created by the normal meiosis mechanism where both parents are tetraploids. Tetraploids such as 'Horizon Monarch', 'Lem's Monarch', 'L'Engin', 'Point Defiance', and 'Very Berry' may have been created by the unreduced mechanism of a diploid parent with the other parent being a tetraploid.

Justin Ramsey's work with newly created neotetraploids suggests that such neotetraploids may experience irregular meiosis. Ramsey suggests that in some instances a **neotetraploid** may contribute only one set of chromosomes to the offspring. For the purposes of this article, we refer to this as the **super-reduced mechanism.**

Diploids such as 'Rendezvous', 'Irmelies', 'Orange Leopard', and 'Stony Brook' may have been created by this super-reduced mechanism. In the case of 'Rendezvous', the tetraploid 'Marinus Koster' is documented as the seed parent.

Noteworthy is that one seedling of 'Horizon Monarch' that had been open-pollinated tested as diploid. The actual plant of 'Horizon Monarch' that was the parent of this particular diploid seedling tested as tetraploid. Other seedlings from the same seedpod tested as tetraploid. 'Pink Goliath', a tetraploid (4x), is the result of (['Antoon van Welie' (4x) X 'Professor J. H. Zaaijer' (2x)] X 'Annie E. Endtz' (3x)), where each parent contains 'Pink Pearl' (3x) in its heritage.

The pentaploids, 'Haithabu' and 'White Ginger', have a tetraploid parent. Our ploidy results suggest that tetraploids may produce diploid, triploid, tetraploid, and pentaploid offspring.

Normal, Unreduced, Super-reduced, and Distributive Meiosis: By the Numbers

A diploid rhododendron has 26 chromosomes. Normally a diploid rhododendron as a parent splits in half during meiosis, contributing 13 chromosomes to the offspring. A tetraploid rhododendron has 52 chromosomes. Normally a tetraploid rhododendron as a parent splits in half, contributing 26 chromosomes to the offspring. A triploid rhododendron has 39 chromosomes. Half of 39 is between 19 and 20. Darlington showed that if a triploid having 39 chromosomes were to split, it would split mainly 19/20 but also, to ever decreasing occurrences, 18/21, 17/22, 16/23, 15/24, 14/25, and 13/26, where the splitting as 13/26 occurs the least frequently. This splitting would form a bell shaped curve between 13 and 26.

Thus, in principle, for rhododendrons:

diploid x diploid usually results in a diploid since 13 + 13 = 26.
tetraploid x tetraploid usually results in a tetraploid since 26 + 26 = 52.
diploid x tetraploid usually results in a triploid since 13 + 26 = 39.
diploid x unreduced diploid can in a few instances result in a triploid since 13 + 26 = 39.
unreduced diploid x tetraploid can in a few instances result in a tetraploid since 26 + 26 = 52.
diploid x super-reduced tetraploid can in a few instances result in a diploid since 13 + 13 = 26
diploid x triploid can in a few instances result in a diploid since 13 + 13 = 26 or in a triploid since 13 + 26 = 39.
triploid x tetraploid can in a few instances result in a triploid since 13 + 26 = 39 or in a tetraploid since 26 + 26 = 52.

Noteworthy, other researchers have found that the offspring of triploids are often **aneuploids**. For rhododendrons, an aneuploid would have a chromosome count slightly more or less than 26 (2x), 39 (3x), 52 (4x), 65 (5x) or other multiples of 13 (x = 13). The unstable meiosis associated with triploids and neotetraploids most likely means that some of the rhododendrons listed above as diploid, triploid, or tetraploid do not have exactly 26, 39, or 52 chromosomes but instead, have close to these counts. Flow cytometry being a method of weighing sets of chromosomes rather than counting the number of chromosomes is not well suited to separating **euploids,** which have a normal chromosome count, from aneuploids.

Summary

Named hybrid elepidote polyploid rhododendrons have played an important role in the garden for more than 150 years. The physical characteristics associated with polyploid rhododendrons have proven to be highly desirable by gardeners since their introduction by Broughton, Standish & Noble, and Waterer. The ploidy of more than 170 named elepidote rhododendrons is listed above. Although all species of elepidote rhododendrons have tested as diploid to date, more than 80 named hybrid elepidote rhododendrons have tested as polyploid. Eighty-nine samples tested as diploid, 51 as triploid, 31 tested as tetraploid, and two as pentaploid. Twenty-two tested diploids are documented to have a polyploid parent. Four tested diploids are documented to have a tetraploid parent.

Triploids can be fertile as both seed and pollen parents, and triploids are able to produce diploid, triploid, tetraploid, and pentaploid offspring. Tetraploids also are able to produce diploid, triploid, tetraploid, and pentaploid offspring. The mechanisms of normal, distributive, **unreduced and super-reduced** meiosis are discussed. All ploidy results presented here are based on flow cytometry.

This research was guided by the work contained in the following:

Hybridization of Rhododendron Elepidote Polyploids by Jim Barlup pg 1-4 1999
http://www.rhododendron.fr/articles/article35c.pdf

Rules of Engagement: Have Pollen - Will Travel by John and Sally Perkins 2009
http://rosebayblog.blogspot.com/2009/12/rules-of-engagement.html

Ploidy Levels and Relative Genome Sizes of Diverse Species, Hybrids, and Cultivars of Rhododendron by Jeff Jones, Thomas G. Ranney, Nathan P. Lynch, and Stephen L. Krebs pg 1-8 2007 http://www.holdenarb.org/education/documents/Jonesetal2007.pdf

Ploidy Breeding and Interspecific Hybridization in Spathiphyllum and Woody Ornanamentals by Tom Eeckhaut pg 1-184 2003 http://lib.ugent.be/fulltxt/RUG01/000/788/476/RUG01-000788476_2010_0001_AC.pdf

Meiosis in Polyploids Part I. Triploid and Pentaploid Tulips by W. C. F. Newton and C. D. Darlington pg 1-15 1929 http://www.springerlink.com/content/d017424p78822ll3/

Neopolyploidy in Flowering Plants by Justin Ramsey and Douglas W. Schemske pg 1-52 2002 http://www.botany.wisc.edu/courses/botany_940/07Polyploidy/papers/RamseySchemske02.pdf

Posts for each sample ploidy tested are available on the Rosebay Blog. Posts have been grouped using tags to promote easy viewing of related posts. Please weigh in by exploring these posts to discover the wonderful world of ploidy in the Rhododendron Garden. 2012
http://rosebayblog.blogspot.com/search/label/U of Coimbra/

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Zhou, W., Gibbons, T, Goetsch, L, Hall, B. Ranney, T, and Miller, R. 2008 Rhododendron colemanii: A NewSpecies of Deciduous Azalea (Rhododendron section Pentanthera; Ericaceae) from the Coastal Plain of Alabama and Georgia.
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