Weighing in: Discovering the ploidy of hybrid elepidote rhododendrons

SALLY PERKINS, JOHN PERKINS, JOSÉ CERCA DE OLIVEIRA, MARIANA CASTRO, SILVIA CASTRO & JOÃO LOUREIRO

TRIPLOID *RHODODENDRON* 'PHYLLIS KORN' is the offspring of a triploid (*R.* 'Gomer Waterer') and parent to a diploid (*R.* 'Summer Peach') SALLY & JOHN PERKINS

WHEN WE MENTION THE WORD 'PLOIDY' most gardeners' eyes glaze over. What does ploidy have to do with their beautiful garden specimens of rhododendrons?

Yet if we mention *Rhododendron* 'Cynthia', *R.* 'Gomer Waterer', *R.* 'Grace Seabrook', *R.* 'Horizon Monarch', *R.* 'Pink Pearl', *R.* 'Phyllis Korn', *R.* 'Point Defiance', *R.* 'Taurus' or *R.* 'Trude Webster', gardeners quickly add that these are among their favorite rhododendrons or high on their wish list. Yes, these rhododendrons display 'something different' and are 'highly desirable'.

WEIGHING IN

As dozens more rhododendrons with larger than normal ploidy levels are revealed below, we hope that gardeners begin to see the connection with characteristics of thickness in the leaf and firmness in flower substance. Indeed, remarkable vigor and substance overall, coupled with outstanding floral performance at a young age, starts to make sense. Even gardeners, who do not want to talk about ploidy, love talking about polyploids.

We are not geneticists. We do have science backgrounds though, 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 do some field work, leading to more discoveries than we ever imagined.

First, 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 cell's nucleus, 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), prone to error, and even eager graduate students are reluctant to co-operate. There are very few studies using this method, those that exist are mostly older, and there is even less duplication of results.

Weigh the counts: With flow cytometry it is possible to weigh the genetic material by taking healthy plant tissue 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 mutations in tumors and cancer cells. If the cells are normal and growing, there would be a small number of cells with double the weight of their chromosomes, as they would be in the phase prior to division. Any cells with less than or more than that weight would be an indication of mutations of the amount of genetic material in the cell (i.e. cancer). This valuable technique can also be used to detect the normal weight of genetic material in different species and hybrids of rhododendrons.

POLYPLOIDY: BEGINNING WITH 1, 2, 3, 4, 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 reproduce, the unfertilized seed and the pollen, called **gametes**, are formed by the splitting apart of the paired chromosomes during meiosis, forming a nucleus with a single set of chromosomes; the unpaired or **haploid** state. And just to make things complicated, true seeds have extra diploid tissue from the seed parent which merges with a haploid pollen nucleus to form the endosperm of a seed.

The fertilized endosperm therefore has 3 sets of chromosomes (two from the seed mother and one from the pollen father) and is **triploid**. This extra genetic material nourishes the germinating seedling. In rare instances a parent will not go through the normal splitting process of meiosis and as result the gametes are **unreduced**, donating the diploid number of chromosomes instead of the haploid.

Most rhododendrons get one set of chromosomes, denoted as **1x**, from each parent (female and male) resulting in two sets of chromosomes. They are commonly referred to as diploids, and denoted as **2x** (1x + 1x = 2x). However, some rhododendrons have four sets of chromosomes. These are commonly referred to as **tetraploids**, and denoted as **4x** (2x + 2x = 4x). **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 **polyploids**.[1]

Although most rhododendron species are diploids, tetraploid rhododendron species do exist.[2] Individual triploid rhododendrons, appearing to be hybrids, sometimes occur naturally where diploid and tetraploid species of *Rhododendron* are co-located.[3] The term **neotetraploid** or **neopolyploid** refer to a recent hybrid whether man-made or natural that is a higher ploidy than the diploid state.

TRIPLOID *R.* 'GOMER WATERER' is the offspring of a triploid (*R.* Pink Pearl) and a parent of a triploid (*R.* Phyllis Korn)

SALLY & JOHN PERKINS

DISCOVERING: THE JOURNEY

In the fall of 1989, our rhododendron polyploidy journey unknowingly started when we overheard at a local Rhododendron Meeting, a statement 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* Rhododendron occidentale *is the seed parent, but* Rhododendron occidentale *as a pollen parent produces few seed.'* [4]

We wondered why.

In the fall of 2011 we noted from reading the ARS online ejournal, that in 1972 Harold Greer wrote the following concerning his hybridization with *R.* 'Countess of Derby' to produce *R.* '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 R. 'Countess of Derby' selfed.'[5]

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. Before *you* take this journey imagine 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. The two most popular rhododendrons in the world are *R.* 'Pink Pearl', an 1890s Waterer hybrid, and *R.* 'Cynthia', an 1850s Standish & Noble hybrid. On a beautiful spring day in 1913, if you are Henry 'Harry' White, a nursery manager in Sunningdale, England, you cross *R.* 'Pink Pearl' with *R.*

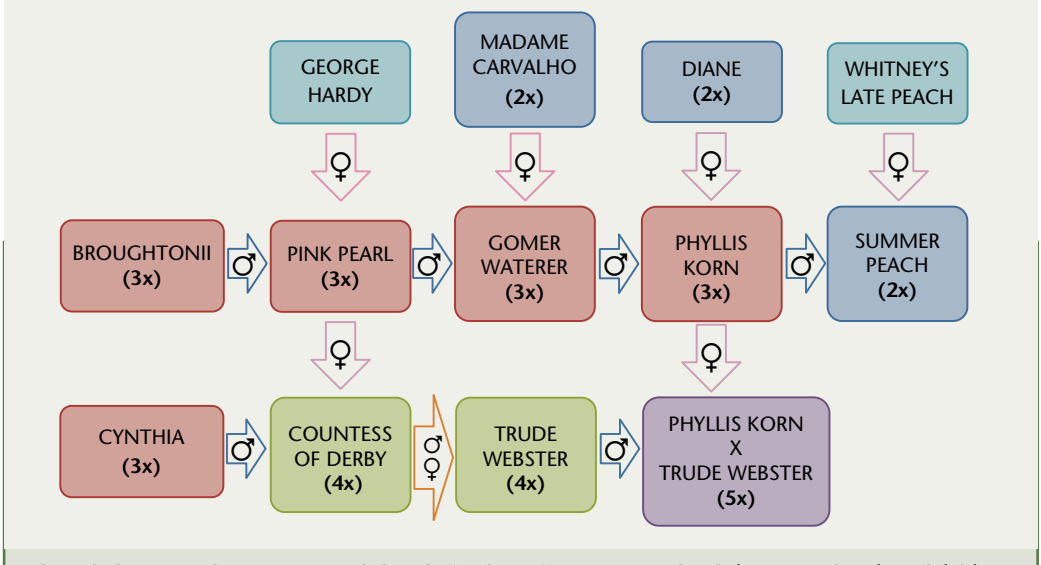

FIGURE 1: **GENERATIONAL BREEDING OF POLYPLOID HYBRID ELEPIDOTES** demonstrating that triploids can be fertile and can create a bi-directional pathway between ploidy levels

'Cynthia'. You later name a seedling from this cross *R.* 'Countess of Derby'.

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

In spring 1969, Richard Nixon is 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 are Robert Korn in Renton, Washington, you place the pollen from *R.* 'Gomer Waterer', a 1900 Waterer *R.* 'Pink Pearl' hybrid, onto R. 'Diane'. You later name a seedling from this cross *R.* 'Phyllis Korn'.

***RHODODENDRON* 'TAURUS'** ('The Honourable Jean Marie de Montague' x *strigillosum*) is a triploid produced by unreduced gametes probably from the diploid seed parent SALLY & JOHN PERKINS

In 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 are Jim Barlup, living in Bellevue, Washington, you cross *R.* 'Whitney's Late Peach' by *R.* 'Phyllis Korn'. You later name a seedling from this cross *R.* 'Summer Peach'.

In 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 are Jim Barlup, living in Bellevue, Washington, you cross *R.* 'Phyllis Korn' by *R.* 'Trude Webster' to create several viable offspring.

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

Well you took two fertile triploids from the 19th century, namely *R.* 'Pink Pearl' and *R.* 'Cynthia' and created a tetraploid, namely *R.* 'Countess of Derby'. You then selfed the tetraploid *R.* 'Countess of Derby' creating another tetraploid, namely *R.* 'Trude Webster'. You then placed pollen from the triploid *R.* 'Gomer Waterer' onto a diploid seed parent, namely *R.* 'Diane' and created a triploid, namely *R.* 'Phyllis Korn'. You then used the pollen of that triploid, *R.* 'Phyllis Korn' to create a diploid, namely *R.* 'Summer Peach'. You then placed the pollen of the tetraploid *R.* 'Trude Webster' onto the triploid seed parent *R.* 'Phyllis Korn' and produced a series of pentaploid seedlings. *(SEE 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 both reduced and unreduced gametes. You demonstrated that triploids provide a pathway for the bi-directional transfer of genes between diploids, triploids, tetraploids and pentaploids.

It took you a few beautiful spring days 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 PLOIDY OF NAMED HYBRID ELEPIDOTE RHODODENDRONS AS DETERMINED USING FLOW CYTOMETRY

SUMMARY

2x Diploids
1000 Butterflies
Alice
Anna (Lem, 1952) U
Betty Hume
Bibiani
Cheyenne *
Colonel Coen
Countess of Athlone
Diane
Duke Of York
Elegans
Everlasting *
Fantastica *
Furnivall's Daughter
Gillii
Gill's Triumph
Goldflimmer *
Goldsworth Orange #
Graf Zeppelin (van Nes, 1934) P
Horizon Lakeside
Hotei
Hurricane (Whitney, 1960) P
Isabel Pierce
Janet Blair *
J.G. Millais (Waterer, 1915) P
Jingle Bells #
Kathy Van Veen
Kupferberg #
Lady Bligh
Lady de Rothschild
Lady Eleanor Cathcart
Lem's Cameo
Loderi Venus
Madame Carvalho
Maxicat *
maximum Kalamity
Mindy's Love
Mother of Pearl (sport, 1925) P
Mrs A. T. de la Mare
Mrs Lindsay Smith
Mrs Furnival
Nancy Evans
Naselle
Norman Gill
Nova Zembla *
Olin O. Dobbs
Orange Leopard (Brack, 1988) P S
Peach Charm
Peach Recital (Barlup, 1996) P
Phipps Yellow
Pink Prelude
Polar Bear *
Puget Sound *
Red Olympia
Rendezvous (Hachmann, 1968) P S
Stony Brook (Brack, 1988) PS
Summer Peach (Barlup, 1988) P
Summer Wind (Barlup, 1996) P
The Honourable Jean Marie de Montague (van Nes, 1901) U
Viscy #
Voluptuous
Vulcan *
Vulcan's Flame *
White Pearl syn. Halopeanum
Wild Affair

3x Triploids
Anita Gehnrich (Waldman) UM
Anna Rose Whitney (Van Veen, 1954) F NM
August Lamken (Hobbie, 1942)
Beauty of Littleworth (Mangles, 1884)
Betty Wormald (Koster, 1907) F
Broughtonii (Broughton, 1840) F
Cotton Candy (Henny & Wennekamp, 1958) F UM
Cynthia (Standish &Noble, 1856) F
Dame Nellie Melba (Loder, 1926)
Django (Hachmann, 1985)
Ebony Pearl (sport, 1966)
El Camino (Whitney, 1976) NM
Gartendirektor Rieger (Hobbie, 1947)
Gomer Waterer (Waterer, 1900) F DM
Grace Seabrook (Seabrook, 1965) UM
Hallelujah (Greer, 1958)
Hank's Folly (Schannen) NM
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
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
Rothenburg (von Martin, 1944)
Rwain (Colombel, 1993) F NM
Solidarity (Schannen, 1969) F UM
Steredenn (Colombel) NM
Sugar Pink (Greer, 1960) NM
Super Dog (Bones) NM
Taurus (Mossman, 1962) F UM
Topsvoort Pearl (sport, 1935)
Val d'Aulnay (Croux and Fils, 1984) F
Van (Van Veen, 1930) NM

4x Tetraploids
Antoon van Welie (Endtz, 1930) 3X2
Brigg's Red Star (Briggs) T
Cherry Cheesecake (Briggs) T *
Countess of Derby (White, 1913) 3X3
diaprepes 'Gargantua' (Stevenson, 1923) †
Doreen Gale (Sanders) 4X4
Gentle Giant (Sanders, 1992) 4X3
Gorgeous George (Sanders) 4X4
Grand Slam (Greer, 1982) 4X3
Horizon Jubilee (Brockenbrough) *
Horizon Monarch (Brockenbrough, 1981) 2X4
Le Fouesnantais (Colombel, 1997) 4XQ
Legend (Barlup) 4X4
Lem's Monarch syn. Pink Walloper (Lem, 1965) 2X4
L Engin (de la Sablière) 4X2
Marinus Koster (Koster, 1937)
Point Defiance (Lem, 1970) 2X4
Summer Joy (Kehr) T
Super Nova (Briggs) T *
Trude Webster (Greer, 1960) 4x4
Very Berry (Greer, 1988) 4X2

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 triploid parents
4X4 indicates a tetraploid resulting from 2 tetraploid parents
4X3 indicates a tetraploid resulting from a tetraploid and a triploid parent
4XQ indicates a tetraploid resulting from a tetraploid parent
***** indicates flow cytometry ploidy testing was done by research team lead by Dr Ranney
indicates flow cytometry ploidy testing was done by Tom Eeckhaut
(,) indicates the name of the hybridizer and date of cross.

† Noteworthy is the named form *R. decorum* ssp. *diaprepes* 'Gargantua'. 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 elepidote species as a population has tested as tetraploid, but this could change.

***R.* 'BEAUTY OF LITTLEWORTH'** (*griffithianum* x *campanulatum*) is a triploid from 1884. The species R. griffithianum is involved in a number of older polyploid breeding programs

SALLY & JOHN PERKINS

Historical evidence indicates that by 1910 the triploids *R.* 'Betty Wormald', *R.* 'Beauty of Littleworth', *R.* 'Broughtonii', *R.* 'Cynthia', *R.* 'Gomer Waterer' and *R.* 'Pink Pearl' would have been on most people's 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 *R.* 'Countess of Derby' and *R.* 'Marinus Koster'.

In 2008, the Siuslaw Chapter of American Rhododendron Society included on their list of best elepidote rhododendrons the triploids *R.* 'Cynthia', *R.* 'Dame Nellie Melba', *R.* 'Grace Seabrook' and *R.* 'Taurus', and the tetraploids *R.* 'Grand Slam', *R.* 'Lem's Monarch', *R.* 'Horizon Monarch', *R.* 'Point Defiance' and *R.* '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 *R.* 'Anita Gehnrich', *R.* 'Anna Rose Whitney', *R.* 'Cotton Candy', *R.* 'Ebony Pearl', *R.* 'Gartendirektor Rieger', *R.* 'Hallelujah', *R.* 'Markeeta's Prize', *R.* 'Platinum Pearl', *R.* 'Solidarity' and *R.* 'Super Dog', and the tetraploids *R.* 'Gentle Giant' and *R.* 'Trude Webster'.

***R.* 'ANNA ROSE WHITNEY'** is a triploid produced from a diploid species (*R. griersonianum*) and tetraploid pollen parent (*R.* 'Countess of Derby')

SALLY & JOHN PERKINS

TETRAPLOID *R.* 'LEM'S MONARCH' is a sister seedling of *R.* 'Point Defiance'

SALLY & JOHN PERKINS

R. 'Pink Pearl' won the first Award of Merit in 1897 and was selected Rhododendron of the Year in 2006 by the Southwestern Chapter of the American Rhododendron Society. In 1950, a large *R.* 'Cynthia', bred in 1858, was the first rhododendron planted in the Crystal Springs Rhododendron Garden. *R.* 'Trude Webster' 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. *R.* 'Broughtonii', bred in 1840, is still considered

to be among the best warm weather rhododendrons according to Don Burke who gardens in Australia.

In other words, over 25 of the 50 or so confirmed polyploid elepidote rhododendrons have appeared on lists of the best rhododendrons and once these polyploids appear on such lists they tend to make future such 'best' lists.

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

Barlup, Bones, Boulter, Bovees, Brack, Briggs, Brockenbrough, Broughton, Bruns, Colombel, Croux et Fils, de la Sablière, Drake, Elliott, Endtz, Evans, Farewell, Felix and Dijkhuis, Fennichia, Fujioka, Gill, Greer, Gehnrich, Hachmann, Hall, Hartman, Heinje, Henny and Wennekamp, Hobbie, Horlick, Horsley, Johnson, Kavka, Kehr, Korn, Koster, Larson, Laxdall, Lem, Loder, Lofthouse, Mangles, Markeeta, McCullough, Moynier, Mossman, Murcott, Naylor, Ostler, Patterson, Pearce, Perkins, Rabideau, Ragans, Reuthe, Sanders, Schannen, Seabrook, Shapiro, Smith, Standish & Noble, Stead, Stevenson, Stockman, Thacker, Thornton, van Nes, Van Veen, Vinson, von Martin, Waldman, Walton, Waterer, White, Whitney, Wilson, Weinberg & Smith, and Woodward.

TRIPLOID *R.* 'EBONY PEARL' is a sport of a triploid (*R.* 'Pink Pearl') that maintained the ploidy level SALLY & JOHN PERKINS

DIPLOID *R.* 'THE HONOURABLE JEAN MARIE DE MONTAGUE' dates from 1901; it probably produces unreduced gametes SALLY & JOHN PERKINS

Noteworthy is that more hybridizers have worked with confirmed elepidote polyploids than there are such confirmed polyploids. More importantly, some of the hybridizers on this list are best known for the polyploid elepidotes they have created. In fact, in a few instances polyploid elepidotes have been named in honor of a wife, a mother, or a grandparent.

Incidentally, Mossman, working with the diploid deciduous azalea species *Rhododendron 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 have addressed this topic in more detail elsewhere.[6]

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.'[7]

Breeding with polyploid elepidotes is not an easy task which explains why so few polyploids have been created to date, despite so many hybridizers having attempted to use them as parents.

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

NAMED ELEPIDOTES SUSPECTED OF BEING POLYPLOID (AS YET UNTESTED)

Adriaan Koster
Aggie
Aibette
Alibaby
Annie E. Endtz
Arden Primrose
Ariel Sherman
Aristide Briand
Arnold Piper *
Arthur Ostler
Babar
Bellevue
Bernard Crisp
Bernard Shaw
Boskoop Concorde
Bruns Sirius
Canadian Beauty *
Cara Meg
Caruso
Castanets
Charis
Courtenay Duke
Dagmar
Denali
Diane Marie
Diane Titcomb
Direktor Siebert
Doctor A. Blok
Doctor Arnold W. Endtz
Doctor H.C. Dresselhuys
Don Juan
Donald Waterer
Doris Nolan
Double Drake
Dr. V.H. Rutgers
Edward Cornelius
Elizabeth Titcomb
Ester Grace
Eureka Maid
Fiona Wilson
Forever Violet
Fragrant Sensation *
Francis Augustus Storey *
Frentano
Friesland
Garnet
George Hardy
Germania *
Gill's Gloriosa
Goliath
Grab Ya *
Gunborg
Gwen Bell
Hachmann's Anastasia
Hachmann's Kristina
Haithabu
Halfdan Lem
Heat Wave
Heinje's Venezia
Helen Druecker
Hollandia
Horizon Serenity *
Ilam Apricot
Inheritance
Irmelies
Isadora
Isobel Baillie
Jan Dekens
Janet Ward
Jean Lennon
Jean Marie Variegated
Jeanne Yvonne
Jenice Coffey
Johnny Bender
Julie Titcomb
Justa Pink
Kareness
Kathe Heinje
Kathy Ann Pieries
Kay Too
KSW
Lady Longman
Leonardslee Giles
Lilian
Loder's White
Lou-John Gem
Madah Jean
Margaret Mack
Marion
Mary-Ed
Maureen Ostler
Melville
Miss Kitty
Mistake
Mrs E. C. Stirling (sister of Pink Pearl)
Newcomb's Sweetheart
Nicandra Newman
Orrie Dillie
Patricia Jacobs *
Peggy Bannier
Pink Goliath
President Kennedy
Pride of Roseburg *
Princess Debiann
Professor Hugo de Vries
Professor J. H. Zaayer
Qualicum's Pride
Queen Mary
Record
Reverend Paul *
Red Walloper *
Rhododendron niveum
Robert Korn
Romilda
Rotha
Scandinavia
Seraphine
Shalom
Shari Laurel
Sheer Enjoyment *
Sierra Sunrise
Sigrid
Souvenier de Doctor S. Endtz
Standishii
Titness Belle
TT116
Twins Candy
Virgo *
Vonnie Stockman
Walküre
Walloper *
Whidbey Island
White Swan
William Avery *

* Almost certainly polyploid

''Francis Augustus Storey' – Best of grex of four plants from weak germination. One died in 2000 and another in 2001.'[8]

Dick Murcott wrote the following about the plant he calls 'TT116':

'TT116 – [('Jean Marie' x 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.'*[9]

Barlup, Murcott and Naylor each encountered both the wonder and puzzles 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 normally low.

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'[10] or ''Margaret Abbott' is a Tetraploid'.[11]

SUSPECTED PLOIDY

Despite having created the suspected polyploid list *(opposite)*, we believe that over 25% would not test as polyploid. However, *R.* 'Fragrant Sensation', *R.* 'Grab Ya', *R.* 'Pride of Roseburg' and *R.* 'Sheer Enjoyment', having both parents as tested tetraploids, are almost certainly polyploids. We have marked using an * the dozen or so we think are the most likely (almost certainly) polyploids.

***R.* 'MARGARET ABBOTT'** is a tetraploid deciduous azalea produced from a diploid species (*R. prinophyllum*) and a tetraploid species (*R. calendulaceum*) SALLY & JOHN PERKINS

***R.* 'POINT DEFIANCE'** is a tetraploid produced by the diploid seed parent's (*R.* 'Anna') unreduced gametes and the tetraploid pollen parent (*R.* 'Marinus Koster') SALLY & JOHN PERKINS

Most in this 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 are known to be capable of producing diploid offspring when the other parent is a diploid. Many hybrids on our suspected polyploid list 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 insure either parent is a polyploid.

If one excludes the known or suspected polyploid hybrids listed, creating a list of 100 suspected polyploid named elepidote hybrids, where more than 20% would test as polyploid, would be difficult. In fact, it is highly likely that most attempts at such a list of 100 named elepidote hybrids would include very few if any additional polyploids.

In other words, we speculate that nearly all (over 90%) named polyploid elepidote hybrids named prior to this article appear in this single article. This is almost certainly the case for polyploid elepidote hybrids named prior to 2000. The chances there were more than 200 polyploid elepidote hybrids named prior to that

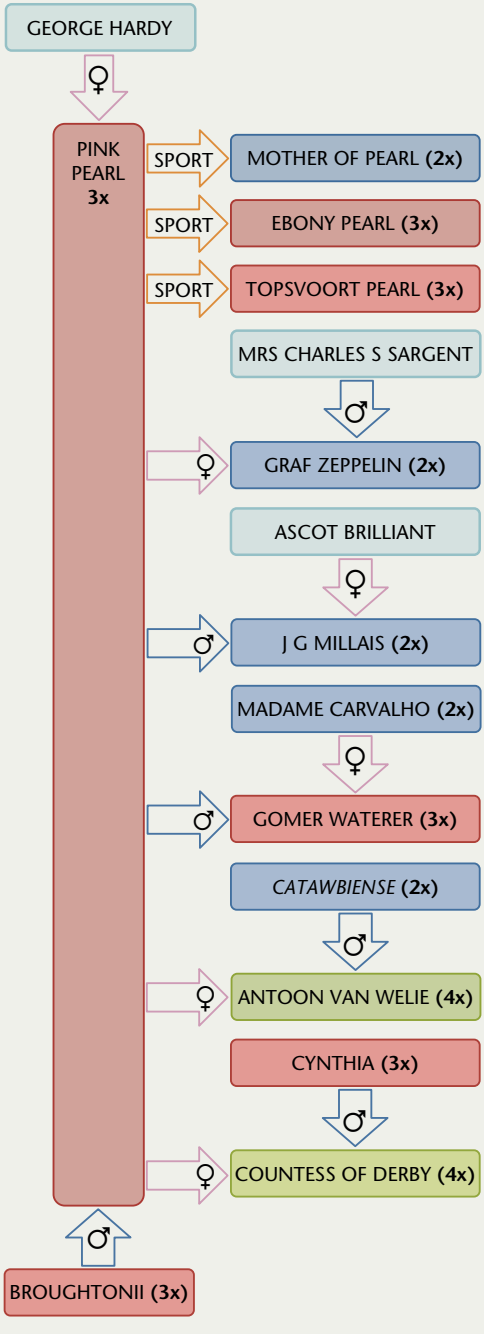

FIGURE 2: **OFFSPRING FROM TRIPLOIDS:** *Rhododendron* 'Pink Pearl'

are low. The chances there were more than 50 tetraploid elepidote hybrids named prior to 2000 are even lower.

In short, there are no rules of thumb for guessing 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, are known to often 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 3 sets of chromosomes. This is commonly referred to as the **unreduced mechanism for creating triploids**.

Ploidy results suggest that triploids such as *R.* 'Anita Gehnrich', *R.* 'Grace Seabrook', *R.* 'Markeeta's Flame', *R.* 'Markeeta's Prize', *R.* 'Solaridity' and *R.* '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 3 sets of chromosomes. This is referred to as the **normal meiosis interploidy mechanism for creating triploids**.

Ploidy results suggest that triploids such as *R.* 'Anna Rose Whitney', *R.* 'Cotton Candy', *R.* 'El Camino', *R.* 'Hank's Folly', *R.* 'Julia Caroline, *R.* 'Lady of Spain', *R.* 'Lucky Strike', *R.* 'Lydia', *R.* 'Opal Thornton', 'Platinum Pearl', *R.* 'Rwain', *R.* 'Steredenn', *R.* 'Sugar Pink', *R.* 'Super Dog', and *R.* 'Van' were most likely created by this normal meiosis interploidy mechanism.

Triploids are commonly believed to always be sterile as both seed parents and pollen parents. Yet offspring are documented for triploids such as *R.* 'Anna Rose Whitney', *R.* 'Betty Wormald',

R. 'Broughtonii', 'Cotton Candy', *R.* 'Cynthia', *R.* 'Gomer Waterer', *R.* 'Lydia', *R.* 'Pearce's American Beauty', *R.* 'Phyllis Korn', *R.* 'Pink Pearl', *R.* 'Platinum Pearl', *R.* 'Rwain', *R.* 'Solidarity', *R.* 'Taurus' and *R.* 'Val d'Aulnay'. *(SEE FIG. 1)*

Triploids such as *R.* 'Pink Pearl', *R.* 'Phyllis Korn', *R.* 'Rwain' and *R.* 'Taurus' appear to be partially fertile as both seed and pollen parents. *(SEE FIG. 2)* In fact, triploids can be the progeny of triploids. Based on parental documentation, *R.* 'Broughtonii', *R.* 'Pink Pearl, *R.* 'Gomer Waterer' and *R.* 'Phyllis Korn' represent four consecutive generations of triploids. *(SEE FIG. 1)*

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

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

***R.* 'HORIZON MONARCH'** is a tetraploid produced from a diploid seed parent (*R.* 'Nancy Evans') and a tetraploid pollen parent (*R.* 'Point Defiance') SALLY & JOHN PERKINS

***R.* 'PEARCE'S AMERICAN BEAUTY'** is an example of a very cold hardy triploid; the source of the higher ploidy is unknown

SALLY & JOHN PERKINS

Tetraploids can be the progeny of triploids: *R.* 'Countess of Derby', a tetraploid, is documented to have 2 triploid parents, namely *R.* 'Pink Pearl' and *R.* 'Cynthia'. The tetraploids *R.* 'Antoon van Welie', *R.* 'Gentle Giant' and *R.* 'Grand Slam' are documented to have a triploid parent. In the case of *R.* 'Antoon van Welie', the triploid *R.* 'Pink Pearl' is documented as the seed parent. *(SEE FIG. 2)*

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. *R.* 'Rwain' the seed parent, is a triploid. *R.* 'L'Engin', the pollen parent, is a tetraploid. Moreover, three seedlings of 'Horizon Monarch' x 'Rwain' tested as tetraploids but one seedling tested as triploid. *R.* 'Horizon Monarch' is a tetraploid.

Fig. 2 suggests that a triploid parent, for instance *R.* 'Pink Pearl', can produce offspring that are diploids, triploids and tetraploids. Fig. 1 suggests that pentaploids are also possible from a triploid parent.

In the 1930s, CJ Darlington showed that triploids could be fertile. Moreover Darlington confirmed a third mechanism for creating triploids. Darlington showed that, during

meiosis, triploids chromosomes may split forming a bell-shaped curve distribution. This means that although there are a few cells formed with 1x and 2x chromosomes, most are closer to the midpoint of 1.5x. So in a few cases, a triploid parent can act as a diploid contributing 1 set of chromosomes or as a tetraploid contributing 2 sets of chromosomes.

Our ploidy results, 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 as viable as any hybrid diploid pollen.

TETRAPLOIDS AND DIPLOIDS AS THE PROGENY OF TETRAPLOIDS

Tetraploids such as *R.* 'Doreen Gale', *R.* 'Gorgeous George', and *R.* 'Legend' have been created by the normal meiosis mechanism where both parents are tetraploids.

Tetraploids such as *R.* 'Horizon Monarch', *R.* 'Lem's Monarch', *R.* 'L'Engin', *R.* 'Point Defiance' and *R.* 'Very Berry' may have been created by the unreduced mechanism of a diploid parent with the other parent being a tetraploid.

***R.* 'ANITA GEHNRICH'** is a triploid probably produced by the diploid seed parent's (*R.* 'The Honourable Jean Marie de Montague') unreduced gametes SALLY & JOHN PERKINS

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

Noteworthy is that one seedling of *R.* 'Horizon Monarch' which had been open pollinated, tested as diploid. The actual plant of *R.* 'Horizon Monarch' that was the parent of this particular diploid seedling tested as tetraploid. Other seedlings from the same seedpod tested as tetraploid.

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, 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. 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 is that other researchers found that the offspring of triploids are often **aneuploids** (having an abnormal number of chromosomes). For rhododendrons, an aneuploid would have a number of chromosomes 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 diploids, triploids, or tetraploids do not have exactly 26, 39, or 52 chromosomes but instead may have a chromosome count close to these numbers. Flow cytometry being a method of weighting *sets* of chromosomes rather than counting the *number* of chromosomes is not well suited to separating **euploids** (a normal number of chromosomes) from aneuploids when the samples tested involve interactions between a wide range of species within the same genus.

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 desired by gardeners since their introduction by Broughton, Standish & Noble and Waterer.

The ploidy of more than 100 named hybrid elepidote rhododendrons is listed above.

Although to date all species of elepidote rhododendrons have tested as diploid, more than 50 named hybrid elepidote rhododendrons have tested as polyploids.

Approximately two-thirds of the named hybrid elepidote rhododendrons which tested as polyploids tested as triploids with the remaining third testing as tetraploids.

Triploids can be fertile as both seed and pollen parents. Triploids are able to produce diploid, triploids, tetraploid and pentaploid offspring.

Tetraploids are able to produce diploid, triploid, tetraploid and pentaploid offspring.

The mechanisms of normal, distributive, unreduced and super-reduced meiosis are discussed.

This research used as a foundation, work done by the following:

Hybridization of Rhododendron Elepidote Polyploids by Jim Barlup www.rhododendron.fr/articles/article35c.pdf

Rules of Engagement: Have Pollen – Will Travel by John and Sally Perkins 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 R. Jones, Thomas G. Ranney, Nathan P. Lynch and Stephen L. Krebs http://www.holdenarb.org/education/documents/Jonesetal2007.pdf

Ploidy Breeding and Interspecific Hybridization in Spathiphyllum and Woody Ornanamentals by Tom Eeckhaut 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 http://www.springerlink.com/content/d017424p78822l13/

Neopolyploidy in Flowering Plants by Justin Ramsey and Douglas W. Schemske 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.

http://rosebayblog.blogspot.com/search/label/U of Coimbra

ACKNOWLEDGEMENTS

We wish to thank the following people and organizations who donated samples for this research: John Abbott, Vivian Abney of East Fork Nursery, Charles Andrews, Living Collection of Arnold Arboretum, Natural Collection of Audra State Park, Living Collection of Bartlett Arboretum, Jim Barlup, Bruce Clyburn, Jane Brooks, Joe Bruso, Werner Brack, Ned Brockenbrough, Natural Collection of Canobie Lake, NH, Dick Cavender, Living Collection of Connecticut College Arboretum, Marc Colombel, Mike Creel, Al Fitzburg, Robert Fox, Harold Greer of Greer Gardens, George Hibben, Living Collection of Highstead Arboretum, Don Hyatt, Lindy Jackson of Appalachian Native Plants, Richard Jaynes of Broken Arrow Nursery, Doug Jolley, Fred Knippel, Living Collection of Longwood Gardens, Ron Miller, Dick Murcott, Michael Medeiros of Planeview Nursery, Portsmouth, RI John and Sally Perkins, Ron Rabideau of RareFind Nursery, Ellie Sather of Whitney Gardens, Natural Collection of Stoddard Blog, NH, John Thornton, Hendrik Van Oostand of Azaleatuin, Kathy Van Veen of Van Veen Nursery.

Sally & John Perkins

garden in Salem, NH, USA on a small wooded lot full of wildflowers and rhododendrons made possible by the excellent drainage, afforded by the slope down to the shoreline of Canobie Lake. Both have been members of the American Rhododendron Society Massachusetts Chapter for over 20 years and Sally is also the District 6 director. John is a software architect and Sally a part-time program manager for the US Department of Veterans Affairs research

José Cerca De Oliveira

is a Graduate Student in the Life Sciences Department at University of Coimbra, Portugal concentrating on the taxonomy and morphology of Portuguese flora

Mariana Castro

is a Masters Student in the Life Sciences Department at University of Coimbra, Portugal

Dr Sílvia Castro

is a Post-Doctoral Researcher in the Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra concentrating on plant-animal interactions (pollination and seed dispersal) and evolutionary dynamics of diploid-polyploid plant groups

Dr João Loureiro

is an Assistant Professor in the Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra concentrating on plant evolution

REFERENCES ■ 1. Understanding Polyploidy: Insights Into the Evolution and Breeding of Azaleas, Thomas G. Ranney & Jeff R. Jones – Mills River, North Carolina **http://www.ces.ncsu.edu/fletcher/staff/tranney/understanding_polyploidy2008.pdf**
2. Rhododendron colemanii: A New Species of Deciduous Azalea (Rhododendron section Pentanthera; Ericaceae) from the Coastal Plain of Alabama and Georgia **http://www.ces.ncsu.edu/fletcher/mcilab/publications/zhou-etal-2008.pdf**
3. Audra State Park: A Ploidy Haven **http://rosebayblog.blogspot.com/2010/09/audra-state-park-ploidy-haven.html**
4. With Camera, White Umbrella, and Tin Pants. In *R. occidentale* Heartland. Frank Mossman, Vancouver, Wash. **http://scholar.lib.vt.edu/ejournals/JARS/v26n4/v26n4-mossman.htm**
5. R. 'Trude Webster' Harold Greer, Eugene, Oregon, **http://scholar.lib.vt.edu/ejournals/JARS/v26n2/v26n2-greer.htm**
6. Rules of Engagement: Have Pollen - Will Travel. John and Sally Perkins, Salem, NH, **http://rosebayblog.blogspot.com/2009/12/rules-of-engagement.html**
7. L'hybridation de rhododendrons élépidote polyploides. Jim Barlup, **http://www.rhododendron.fr/articles/article35c.pdf**
8. Ron Naylor Rhododendrons **http://219.88.101.105/e16.html**
9. Selected Rhodo Seedlings. Rich Murcott. **http://www.murcottgarden.com/select-seedlings**
10. Frank Abbott's Village of Azaleas. John and Sally Perkins, Salem, NH, **http://rosebayblog.blogspot.com/2009/09/abbott-azaleas.html**
11. 'Margaret Abbott' is a Tetraploid. John and Sally Perkins, Salem, NH, **http://rosebayblog.blogspot.com/2011/06/margaret-abbott-is-tetraploid.html**
12. 'Pink Pearl' is a Fertile Triploid, **http://rosebayblog.blogspot.com/2010/10/pink-pearl-is-fertile-triploid.html**