Pleasant Progress and Other Interploidy Pathways

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Native North American diploid (2x) and tetraploid (4x) deciduous azalea species interact to produce viable seedlings. This has given us the opportunity to explore interploidy interaction that could possibly occur in nature. Most people who are into hybridizing have mundane goals such as bigger flowers or more fragrance or brighter colors. For us these are secondary to our search for interploidy pathways. To be clear, we have done years of hybridizing using the diploid species *Rhododendron alabamense*, *R. arborescens*, *R. canescens*, *R. cumberlandense*, *R. eastmanii*, *R. flammeum*, *R. occidentale* (the Western azalea), *R. periclymenoides*, *R. prinophyllum*, *R. prunifolium*, *R. viscosum* and the tetraploid species *R. atlanticum*, *R. austrinum* (including the "pink austrinum"), *R. calendulaceum*, and *R. colemanii*. As an aside, none of this discussion involves the North American native species *R. vaseyi* and *R. canadense*, or the Asian *R. molle* or the European *R. luteum* species because there is little reason to think they interact in nature.

This direct interaction between diploid and tetraploid species is not bidirectional but has been shown to be one way. As shown in the Table of Ploidy Interactions (page 43), when the diploid species is the seed parent and tetraploid species is the pollen parent the result is viable seedlings. The blooms of these seedlings exhibit more of the tetraploid species characteristics. Flow cytometry has confirmed that the great majority of such

Photo 1. "Pleasant Progress" (4x), a tetraploid resulting from diploid × tetraploid interaction [(*R. cumberlandense* (2x) × *R. viscosum* (2x)) (2x) × *R. colemanii* (4x)].

Table of Ploidy Interactions

Seed Parent	Pollen Parent	Seedpods	Offspring	Offspring Flowered	Offspring Fertile
2x diploid	2x	Yes	2x	Yes	Yes
2x	4x	Yes	3x	Yes	Not Usually
2x	6x	Yes	4x	No	Not Tested
3x triploid	2x	Rarely Yes	2x to 4x	No	Not Tested
3x	4x	Rarely Yes	3x to 5x	Yes	Sometimes
4x tetraploid	2x	No			
4x	4x	Yes	4x	Yes	Yes
4x	5x	Yes	4x to 5x	No	Not Tested
4x	6x	Yes	5x	Yes	Yes
4x	8x	Yes	6x	Yes	Yes
5x pentaploid	4x	Yes	4x to 5x	Yes	Yes
5x	5x	Yes	Not Tested	No	Not Tested
5x	6x	Yes	Not Tested	No	Not Tested
6x hexaploid	4x	Yes	5x	Yes	Yes
6x	5x	Yes	Not Tested	No	Not Tested
6x	6x	Yes	6x	Yes	Yes
6x	8x	Yes	7x heptaploid	No	Not Tested
8x octoploid	Open-pollinated	Occasionally Yes	8x		

interactions with diploid X tetraploid deciduous azaleas produce the expected outcome of a triploid (3x). In all our hybridizing work with deciduous azaleas, seed has never been produced when the tetraploid species is the seed parent and the diploid species is the pollen parent.

"Pleasant Progress"

In this article we are reporting a very unusual result in a plant we call "Pleasant Progress" (See Photo 1). "Pleasant Progress" is the result of a hand cross between the diploid hybrid seed parent, (*R. cumberlandense* X *R. viscosum*) (See Photo 2.), with the tetraploid species pollen parent, *R. colemanii*. (See Photo 3.) What is surprising is that "Pleasant Progress" is a tetraploid and produces open pollinated seed.

The normal case for the diploid seed parent is to form the ovule (egg) by reducing chromosomes to a haploid (1x) gamete. We theorize that the most likely scenario in producing a tetraploid from this cross would be for the diploid seed parent, (*R. cumberlandense* X *R. viscosum*), to produce an unreduced gamete and therefore had a 2x gamete that was fertilized with a normal contribution from the tetraploid pollen gamete, also 2x, *R. colemanii*, This combination results in a tetraploid seedling (2x + 2x = 4x) rather than a triploid (1x +2x =3x). Flow cytometry shows that the siblings of "Pleasant Progress" tested, as expected, to be triploids (1x +2x =3x). The triploid siblings appear to be both seed and pollen sterile, again, as expected and more heavily favor the tetraploid parent *R. colemanii* also as expected. (See Photo 4.) "Pleasant Progress" is however both seed and pollen fertile. The backcross [(*R. cumberlandense* X *R. viscosum*) (2x) X "Pleasant Progress" (4x)] produces seed and viable seedlings. With "Pleasant Progress" we have a fertile tetraploid that can contribute diploid genes into the tetraploid species gene pool.

"Park's Deep Pink"

Despite our findings that crossing between diploid and tetraploid deciduous azalea species is unidirectional, in that diploid pollen is not accepted onto the tetraploid, that does not hold true when interploidy plants are produced. An unusual and

Photo 2. (***R. cumberlandense* × *R. viscosum***) (2x). The diploid seed parent of "Pleasant Progress" (4x).

Photo 3. ***R. colemanii*** (4x), the tetraploid pollen parent of "Pleasant Progress" (4x).

Photo 4. A typical triploid (3x) sibling of "Pleasant Progress" [(***R. cumberlandense* × *R. viscosum***) (2x) × ***R. colemanii*** (4x)] showing the higher influence of ***R. colemanii***.

fortuitous finding is a plant bred by the late Joe Parks of Dover, NH, called simply, "Park's Deep Pink" (See Photo 5). "Park's Deep Pink" is a seed fertile triploid deciduous azalea, producing seed when crossed with both diploids and tetraploids. When "Park's Deep Pink" is crossed with a diploid producing seedpods the flow cytometry testing of the seeds shows that there is a mix of diploid, triploid, and tetraploid seeds. To date, only a few long-term viable seedlings have been produced from the diploid crosses. Using flow cytometry, we have been able to document that seedlings from "Park's Deep Pink" X tetraploid tested in a range from triploid, tetraploid, aneuploid (an abnormal number of chromosomes between triploid and tetraploid), and occasionally pentaploids (5X). Our hybrid "Canobie Conundrum" (See Photo 6.) is the tetraploid pollen parent used in hybridizing with "Park's Deep Pink" to produce seedlings of different ploidy levels.

Photo 7 shows a pentaploid seedling that is the result of the cross ("Park's Deep Pink" X "Canobie Conundrum"). This pentaploid seedling from ("Park's Deep Pink" X "Canobie Conundrum") has proven to be seed fertile. So far, "Park's Deep Pink" seeds produced using tetraploid pollen have better germination than from diploid pollen. These results show that fertile triploids can be a mechanism for bidirectional gene flow between diploids and tetraploids and also a mechanism for the production of fertile pentaploids containing both diploid and tetraploid genes.

'Fragrant Star' and "Pleasant Pathway"

Another interesting finding involves using the plant 'Fragrant Star' in our breeding program. 'Fragrant Star' is an octaploid (8x) produced by Bruce Briggs of Olympia, Washington, who chemically treated the tetraploid *R. atlanticum* 'Snowbird'. 'Fragrant Star' is both seed and pollen fertile producing a 4x gamete but we have only been successful using it as a pollen parent (See Photo 8). Crosses of tetraploid with 'Fragrant Star' pollen produce, as expected, hexaploid (6x) seedlings (2x + 4x = 6x). An example of a hexaploid result is the plant we are calling "Pleasant Pathway" (See Photo 9). "Pleasant Pathway" is a hexaploid resulting from the tetraploid 'Chickasaw' crossed with pollen from the octoploid 'Fragrant Star'.

Hexaploids will reduce in gamete formation to a 3x pollen or 3x ovule. These hexaploid plants are both seed and pollen fertile and, incidentally, very fragrant. Remarkably, we crossed tetraploids with these hexaploid plants in both directions and produced pentaploid (5x) offspring (3x + 2x = 5x) or (2x + 3x = 5x). Pentaploid seedlings from such crosses have proven to be both seed and pollen fertile also. (See Table 1). Hand crossing of diploids with hexaploid pollen produced seedpods and viable tetraploid seedlings (1x + 3x = 4x). None of these hexaploid pathway tetraploid seedlings to date has matured to flowering size. Since 2020, we have demonstrated that crossing pentaploids with other pentaploids produces seedpods. The resulting seeds have not been tested for ploidy. Since pentaploid seed parents produce viable offspring, we are hopeful that subsequent years' seed crops will also prove viable.

Flow cytometry from the Portugal group has been used to verify the ploidy levels of seed and seedlings resulting from all crosses mentioned above and was used to create the Table of Ploidy Interactions.

Conclusions

The interploidy pathways demonstrated in our hand crosses may play a role in the bidirectional flow of genes between diploid and tetraploid deciduous azalea species of North America. Possible mechanisms are 1) unreduced gametes from diploids, 2) variable sized gametes from fertile triploids, and 3) variable sized gametes from fertile pentaploids. Lepidote rhododendrons do have naturally occurring hexaploid and octaploid species based on flow cytometry. However, the higher ploidy of hexaploid and octaploid are of academic interest in deciduous azaleas, as there is no evidence to date that suggests hexaploid or octaploid deciduous azaleas occur naturally in North America or elsewhere.

We have demonstrated that fertile pentaploids can be produced via a triploid pathway or a hexaploid pathway. We will continue this work by looking at flow cytometry of fertile pentaploids offspring. Planned future hand crosses will explore the diploids interaction with pentaploids.

Photo 5. "Park's Deep Pink" (3x), a seed fertile triploid deciduous azalea.

Photo 6. "Canobie Conundrum" ('My Mary' (4x) × 'Marydel' (4x)) is the tetraploid pollen parent used in hybridizing with "Park's Deep Pink" (3x) to produce seedlings of different ploidy levels.

Photo 7. An example of a pentaploid (5x) seedling of "Park's Deep Pink" (3x) × "Canobie Conundrum" (4x)

Photo 8. 'Fragrant Star' (8x), the octoploid, chemically created from *R. atlanticum* 'Snowbird' (4x).

Photo 9. "Pleasant Pathway" (6x), a hexaploid result from 'Chickasaw' (4x) × 'Fragrant Star' (8x)).

About the Authors:

Sally and John Perkins garden in Salem, NH, 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 Massachusetts Chapter American Rhododendron Society for 30 years in various roles, now mostly with the Plants for Members program and the seed exchange. They also maintain a Vaseyi Chapter membership in the ASA and contribute to its seed exchange. Sally and John are both happily retired from their former lives and enjoy spending winters not in New Hampshire.

Mariana Castro, Silvia Castro, and João Loureiro are all members of the Centre for Functional Ecology - Science for People & the Planet, University of Coimbra, Coimbra, Portugal. João Loureiro is Assistant Professor at the Department of Life Sciences (Plant Physiology and Bioinformatics). Ten years ago, Mr. Loureiro offered to do the flow cytometry if the Perkins could provide the samples and if the University of Coimbra would have the right to use the data. They have run almost 3,000 samples to date.

All photos in this article are by John and Sally Perkins.

Additional Resources on Ploidy Interactions and Definitions of Terms

• Perkins, John and Sally. 2010. "Rules of Engagement: Have Pollen—Will Travel." *The Azalean*, 32(2): 28-33.

• Perkins, John and Sally. 2015. "More Weighing: Exploring the Ploidy of Hybrid Elepidote Rhododendrons." *The Azalean*, 37(1&2): 28-42.