Rules of Engagement: Have Pollen—Will Travel

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The conventional wisdom, passed along in books, articles, and word-of-mouth, states "North American native deciduous azaleas, excluding *Rhododendron vaseyi* and *canadense*, interbreed freely"; however, the rules of engagement for these azaleas are far more complex when crossing diploid and tetraploid azalea species.

The Two Clades

The latest work by Benjamin Hall and Tom Ranney indicates that native deciduous azaleas break into two major clades that correspond to their ploidy levels. For those unfamiliar with the discussion of clades and ploidy we will digress and explain that when genetic material is used to try to determine how closely related species are, the term clade refers to species that have a common ancestor. The analysis done so far strongly suggests that there are two different clades when examining the North American deciduous azaleas. These two clades seem to separate according to their ploidy level. The ploidy level refers to the number of chromosomes. Most rhododendrons and azaleas have a base number of 13 chromosomes (x=13) which is the number found in pollen and unfertilized ovule. Diploid deciduous azaleas are classified as having 2x=26 chromosomes, triploid deciduous azaleas are classified as having 3x=39 chromosomes, and tetraploid deciduous azaleas are classified as having 4x=52 chromosomes. The tetraploids have twice as much genetic material as the diploids, but they do not, based on genetic studies from Hall, have simply a duplication of the genetic material alon;, and the tetraploids and diploids have not been freely exchanging genes with each other for a long time. Triploids often result as a result of a cross between a diploid and a tetraploid.

The tetraploid clade includes *Rhododendron atlanticum*, *austrinum*, *calendulaceum*, *colemanii*, *luteum* (from Europe), and possibly, a pink-flowered azalea similar to *austrinum* (see Figure 1). The diploid clade includes *R. alabamense*, *arborescens*, *canescens*, *cumberlandense*, *eastmanii*, *flammeum*, *occidentale*, *periclymenoides*, *prinophyllum*, *prunifolium*, and *viscosum* (see Figure 2).

▲ Figure 1 Species of Tetraploid Clade. Center: *Rhododendron calendulaceum*. Clockwise from top left: *R. colemanii, luteum, atlanticum*, an austrinum-like pink azalea, *austrinum*

Although each is diploid, the two native deciduous azaleas *R. canadense* and *vaseyi*, as well as the Chinese and Japanese species of *R. molle*, do not belong to the diploid clade as described above. Benjamin Hall, Hans Eiberg, and K. A. Kron each found *R. vaseyi* to be only a distant relative of the other North American deciduous azaleas. Of the three, *R. molle* is the only one that has been used extensively to produce commercial hybrids involving the two clades described above. George Fraser and Harold Pellett each successfully crossed *R. canadense* x *R. molle*.

Rules of Engagement for Crossing Species in the Two Clades

Rule No. 0

The rules of engagement for deciduous azaleas within these two clades are highly generalized guidelines. The rules focus on the ability to successfully create seedpods. Exceptions to these rules will occur. The only way to know for certain is to do the cross and let nature take its course.

However, such exceptions are rare enough that hand crosses violating these rules are worthy of documentation, and attempts should be made to see if the same result is repeatable between the same parents and other members of the two clades.

▲ Figure 2 Species of Diploid Clade. Center: *Rhododendron cumberlandense.* Clockwise from top left: *periclymenoides, prunifolium, prinophyllum, viscosum, flammeum.* Not shown: *R. alabamense, arborescens, canescens, eastmanii, occidentale.*

Rule No. 1

An individual plant in either the diploid clade or the tetraploid clade does not freely "self fertilize" to develop seeds. In those rare instances where selfing does occur, the offspring are seldom viable (if they germinate, they die at a young age).

Rule No. 2

Species within a given clade freely cross in both directions. The resulting offspring are normally viable and fertile. The offspring normally reflect characteristics that are intermediate between the two species involved.

Rule No. 3

Species in the diploid clade freely accept pollen from species in the tetraploid clade. The resulting offspring are often viable but usually sterile (bloom but do not produce seed) triploids. The offspring often reflect more characteristics of the tetraploid pollen parent. Offspring having pink- or salmon-colored flowers with a yellow blotch are not unusual. Deformed anthers, multiple petals, and color streaks in the corolla are also frequently seen.

Rule No. 4

Species in the tetraploid clade normally reject pollen from species in the diploid clade. Exceptions occur, but they are extremely rare.

Rule No. 5

Species in the diploid clade freely accept pollen from *R. molle.* The resulting offspring are often viable, but usually are sterile diploids.

Rule No. 6

Triploids resulting from interaction between the diploid and tetraploid clades are more likely to accept pollen from species in the tetraploid clade than from species in the diploid clade. This rule is much more preliminary than the others above.

Rule No. 7

There are no known instances of *R. vaseyi* successfully interacting in either direction with species in either clade.

Rule of Thumb

To maximize your opportunity for producing seed where you have the option of parents in either direction, always use the deciduous azalea of the lower ploidy or same ploidy as the seed parent.

These rules of engagement support Hall's finding that the two major clades of North American deciduous azaleas are divided such that species grouped in a given clade are much more closely related to other species in that clade than they are to species that are more similar in appearance in the other clade. In other words, *R. calendulaceum*, a tetraploid, is more closely related to the other tetraploid species *R. atlanticum, austrinum, colemanii, luteum*, and an austrinum-like pink azalea than to the similar- looking species *R. cumberlandense*, a diploid. The same is true for *R. colemanii*, a tetraploid, being closer to *R. calendulaceum, atlanticum, austrinum, luteum*, and an austrinum-like pink azalea than to the similar-looking species *R. alabamense*, a diploid. *R. atlanticum*, a tetraploid, is closer to other tetraploids than to the similar-looking *R. viscosum*, a diploid. The austrinum-like pink azalea, a tetraploid, is closer to other tetraploids than to the similar-looking *R. canescens*, a diploid.

The Evidence

Richard Jaynes showed that self-fertilized deciduous azaleas failed to produce seedpods in most instances and nonviable offspring resulted in those instances where seed was produced. We have failed in our attempts to self late-blooming deciduous azalea species.

Many of the late-blooming commercial hybrid azaleas produced by David Leach; George, Mary, and Jeff Beasley; Ed Mezitt; and Bob and Jan Carlson involve only species of the diploid clade combining *R. arbore-*

scens, *cumberlandense*, *prunifolium*, and *viscosum*. We have found that species in the diploid clade cross in both directions.

Tom Dodd, Earl Sommerville, and Gene Aromi produced several good-doer hybrid azaleas for the South involving primarily *R. calendulaceum* and *R. austrinum*, both tetraploids. Fred Galle produced 'Choice Cream' using *R. austrinum* x *R. atlanticum*, both tetraploids. Bob and Jan Carlson produced hybrids using *R. calendulaceum* x *R. luteum*, both tetraploids. Ian Donovan produced a cross of *R. atlanticum* x *R. calendulaceum*, both tetraploids. We found we could cross in both directions *R. calendulaceum* 'Cherokee', 'Marydel', and 'My Mary' with Donovan's *R. atlanticum* x *R. calendulaceum*. Both 'Marydel' and 'My Mary' were shown later to be tetraploids in the lab. Prior to the ploidy testing, many hybridizers were aware that *R. calendulaceum* accepted pollen more easily from *R. luteum*, *austrinum*, and *atlanticum* than from other deciduous azalea species.

Prior to the current information on the ploidy of North American azaleas, Frank Mossman, John Thornton, Anthony Waterer Sr., and the authors all noted that certain species crossed much more easily in one direction than the other. In retrospect, these hybridizers noticed that diploids normally accept pollen from tetraploids whereas tetraploids normally reject pollen from diploids.

In 1972, Frank Mossman wrote the following concerning his hybridization with *R. occidentale*, a diploid: "We have found that *Rhododendron occidentale* will cross with many other rhododendrons or azaleas if *Rhododendron occidentale* is the seed parent, but *R. occidentale* as a pollen parent produces few seed."

In 1974, Mossman added the following concerning his use of three diploid species as seed parents and *R. occidentale* as the pollen parent: "*Rhododendron prunifolium*, *cumberlandense*, or *viscosum* x *Rhododendron occidentale* will take."

Mossman stated concerning the work of Anthony Waterer, Sr.: "Anthony Waterer, Sr. of Knap Hill Nursery, England, was the first known hybridizer of *R. occidentale* in the 1860's and reportedly had little success for almost ten years. It is probable that he had pollen only, at first, and later had flower-producing plants to use for seed parents. His effort with the Ghent Azaleas plus *R. occidentale* was the beginning of the Knap Hill Azaleas and later the Exburys."

Photo John & Sally Perkins

▲ Figure 3 'Margaret Abbott' (Abbott).
Rule No. 3: *R. prinophyllum* (diploid) x *R. calendulaceum* (tetraploid)

In the 1990's, we found 'Marydel' and 'My Mary' rejected pollen from late -blooming deciduous azaleas such as *R. arborescens*, *cumberlandense*, *flammeum*, *prunifolium*, and *viscosum* but accepted pollen from each other and *R. calendulaceum*, a tetraploid.

John Thornton has found that *R. austrinum*, a tetraploid, normally rejects diploid pollen but, as a exception to Rule No. 4, was able to successfully cross *R. canescens* 'Crane Creek' onto *R. austrinum* on one occasion. The resulting seedlings were "sickly and sterile." *R. canescens* 'Crane Creek' is a lab-tested diploid.

Dick Cavender's cross of *R. calendulaceum* 'Colossus' x *occidentale* SM-30 and Jim Skonieczny's self-crossing of *R. calendulaceum* 'Colossus' x *occidentale* SM-189 are two possible additional exceptions of a tetraploid accepting pollen from a diploid. August Kehr was successful crossing an evergreen azalea onto *R. calendulaceum* 'Colossus'. Dick Jaynes in his work was only able to successfully cross evergreen azaleas onto diploids and not tetraploids. Carlson crossed *R. luteum*, a tetraploid, onto *R. calendulaceum* 'Colossus'. Britt Smith crossed *R. calendulaceum* 'Colossus' onto *R. occidentale*, a diploid, producing at least some fertile offspring. We have found no documentation of a member of the tetraploid clade accepting pollen from *R. calendulaceum* 'Colossus'. What is interesting to us is that *R. calendulaceum* 'Colossus', believed to be a *R. calendulaceum* by David Leach, August Kehr, and Clarence Towe, behaves, by the limited evidence above, as a member of the diploid clade. In other words, our rules suggest that *R. calendulaceum* 'Colossus' is more likely a *R. cumberlandense*. We have never used *R. calendulaceum* 'Colossus' in any of our crosses and are not personally familiar with the cultivar. We have shown that the cultivar 'Pumpkin', which is

Photo Illustration John & Sally Perkins

▲ Figure 4 Crosses onto *R. calendulaceum* 'Cherokee', a tetraploid.
Rule No. 4: Rejects pollen from diploids. Rule No. 2: Accepts pollen from other tetraploids.

a Carlson cross of *R. calendulaceum* 'Colossus' x *luteum*, accepts pollen from 'Snowbird', a lab-tested tetraploid.

Lab testing the ploidy of 'Colossus' would be informative; however, the more interesting question is how general is 'Colossus' in violating Rule No. 4 above. Are the crosses mentioned above repeatable? Does 'Colossus' accept pollen from most or all *R. occidentale*? This is doubtful, since Frank Mossman used 'Colossus' successfully only as the pollen parent in his hybridization program involving *R. occidentale*. Does 'Colossus' accept pollen from other diploid species besides *R. occidentale*? Does 'Colossus' accept pollen from tetraploids?

Frank Abbott of Saxtons River, Vermont, produced 'Margaret Abbott' using *R. prinophyllum*, a diploid, x *R. calendulaceum*, a tetraploid (see Figure 3). In a personal letter from Joseph Gable written in the 1940's, Frank was reminded to always put the seed parent first in listing the cross as this convention had not always been followed in the past and led to confusion.

Ron Rabideau of Rarefind Nursery grew two orange-flowered seedlings from seed he collected from a native *R. prinophyllum*, a diploid, on his parents' property in Ashburnham, Massachusetts. This *R. prinophyllum* was growing near an orange Exbury azalea, most likely R. 'Gibraltar', which is a lab-tested tetraploid. This interaction provides support for the possibility of diploid x tetraploid occurring in nature. Tom Ranney and Clarence Towe have documented natural occurring triploids. Ploidy testing of Ghent hybrids by T.G.R. Eeckhaut, L.W.H. Leus, A.C. De Raedt, and E.J. Van Bockstaele showed a mixture of triploids and tetraploids.

Research by Ernest Henry Wilson and Alfred Rehder indicates that three of the earliest (1830) English deciduous hybrids where the pollen parent is known were each diploid x tetraploid, namely *R. viscosum* x *R. luteum*, *R. flammeum* x *R. luteum*, and *R. periclymenoides* x *R. calendulaceum*. In this timeframe many native deciduous species were grouped under *Azalea viscosa* and *nudiflora* (R. periclymenoides).

An examination of ARS seed exchange lists from 1990 to 2008 indicates that for crosses involving an azalea from each of two clades, the crosses where the diploid is the seed parent far outnumber those where the seed parent is the tetraploid. Moreover, most of the interclade crosses where the tetraploid is the seed parent, *R. calendulaceum* is listed as the seed parent. It is interesting to point out that *Rhododendron cumberlandense*, a diploid, is often misidentified even by experts as *R. calendulaceum*, a tetraploid.

Jukka Kallijarvi wrote the following in an email conversation comparing azaleas to rose hybrids: "Rules No. 3 and 4 are, in fact, a rule of thumb in rose hybridization. Pollen from tetraploids works on diploids, but not vice versa. Also, tetraploid roses are generally much easier to hybridize than diploids."

In 2008 we performed 18 tests using *R. calendulaceum* 'Cherokee', a tetraploid, as a seed parent. In each instance, pollen from the five different tetraploids produced seedpods. In each instance, pollen from the 13 different diploids failed to produce seedpods (see Figure 4). In the same year, pollen from 12 of these diploids produced seedpods when applied to other diploids. In 2009 we placed pollen from *R. colemanii* and an *austrinum*-like pink azalea, both tetraploids, onto several tetraploids, producing seedpods in each instance. In total over the years, we have done 50 crosses placing diploid pollen onto tetraploids and all have failed to produce seedpods.

In 2009, we performed 42 tests placing tetraploid pollen on fertile diploids. Forty of these crosses produced seedpods. Our experience indicates that, for the same diploid seed parent, pollen from a tetraploid produces larger seedpods than pollen from a diploid (see Figure 5). In some cases, much larger seedpods result. Hans Eiberg states that the size of a seedpod is determined by the number of seeds and the amount of DNA in each seed, so for a fixed number of seeds the seedpod would increase in size depending on whether the resulting seed was diploid, triploid, or tetraploid.

In 2009 we placed *R. molle* pollen on eight diploids producing, seedpods in each instance. Frank Abbott produced 'Jane Abbott' using *R. prinophyllum* x 'Miss Louisa Hunnewell' where

'Miss Louisa Hunnewell' is a cross between the Japanese and Chinese forms of *molle*. Ed Mezitt and Harold Pellett produced hybrids using *R. prinophyllum* x *R. molle* hybrids. Felix and Dijkhuis produced hybrids using *R. viscosum* x *molle*.

There are no documented interspecies crosses involving *R. vaseyi*. All of our attempts at crossing other species onto *R. vaseyi* have failed.

Caution about Historical Documentation

Tetraploid x diploid crosses of deciduous azaleas are mentioned in the literature. The reader when reviewing such crosses has to be mindful of three things:

1. Many deciduous azaleas documented as "natural hybrids" of tetraploid x diploid species are selections found in the wild. The parentage is based on the conjecture of knowing both the physical characteristics and the distribution of the species in the immediate area. Which species is the seed parent is unknown.

2. The Botanical Code recommends placing the names in a hybrid formula in alphabetical order. However, it permits listing the seed parent first and pollen parent second.

3. Our native deciduous azaleas are often misidentified even by experts.

Labels at arboretums and display gardens can be wrong by neglect, malicious or benign label switching, and incorrect identification by the initial source. In fact, an examination of Galle's wonderful tome, *Azaleas,* shows that oncenatural hybrids are excluded and one discounts 'Galle's Choice' (documented as *R. calendulaceum* x *R. alabamense* but very likely *R. calendulaceum* x *R. colemanii*); there is not a single occurrence of a documented cross with tetraploid deciduous azalea species as the seed parent and a diploid native deciduous azalea species as the pollen parent.

Worthy of mention is that no matter how carefully one performs the multiple steps involved in producing a named hybrid, including accurate identification of parents; proper hybridization techniques; proper seed handling; and proper labeling of seed, seedlings, and transplants; one unintentional mistake may result in an inaccurate documentation of the parentage. We believe that reproducibility, using multiple crosses on the same parent and the same pollen across many different parents, and the distribution of seeds to the seed exchanges will address some of these issues.

Home Tests for Ploidy

Prepare unopened flower buds from known tetraploid and known diploid deciduous azaleas for hybridization by removing the corolla and immature stamens, then wait one to two days allowing their styles to straighten and stigmas to become receptive. For clarification, the use of the terms pollen and seed fertile are based on prior experience. "Pollen fertile" refers to a deciduous azalea's pollen that has been used previously to successfully produce seed from hand crosses. "Seed fertile" refers to a deciduous azalea that has produced seed from either hand crosses or is known to set open-pollinated seed freely.

▲ Figure 5 Bigger than normal diploid seedpod on *Rhododendron arborescens* var. *rubra*, a diploid blooming next to 'Marydel', a tetraploid.

To increase confidence, perform both the pollen and the seed home tests for multiple parents of known ploidy. For conclusive knowledge, send the azalea to a lab for testing.

Rarely does a deciduous azalea that accepts pollen from both diploids and tetraploids also have the capacity to produce pollen that takes on both diploids and tetraploids. There is speculation that a fertile triploid might act similarly to a tetraploid.

Pollen Parent Test

One can test the likely ploidy of a "pollen fertile" deciduous azalea as follows. Place the pollen of the deciduous azalea of unknown ploidy onto stigmas of known tetraploid and known diploid from the two clades mentioned above. If the known tetraploid(s) x unknown ploidy produces seedpods, then the unknown ploidy is very likely a tetraploid. If the known tetraploid(s) x unknown ploidy fails to produce seedpods but the known diploid(s) x unknown ploidy produces seedpods, then the unknown ploidy is very likely a diploid.

If no seedpods are produced from either known set of seed parents, then no conclusion is reached.

Seed Parent Test

One can test the likely ploidy of a "seed fertile" deciduous azalea as follows. Place the pollen of known tetraploids and known diploids from the two clades mentioned above onto different flowers of the deciduous azalea of unknown ploidy. If the unknown ploidy x known diploid(s) produces seedpods, then the unknown ploidy is very likely a diploid. If the unknown ploidy x known diploid(s) fails to produce seedpods but the unknown x tetraploid(s) produces seedpods, then the unknown ploidy is very likely a tetraploid. If no seedpod is produced for either known set of pollen parents, then no conclusion is reached.

Conclusion

Hall's clade work and Ranney's ploidy work divides the deciduous azaleas into a six-species tetraploid clade and an 11-species diploid clade where *R. canadense*, *molle*, and *vaseyi* are excluded. Their work caused a paradigm shift in how we think about our deciduous azaleas and how we approach doing hand crosses involving these azaleas. The rules of engagement address this shift in our thinking and have dramatically increased our ability to predict the possibility of producing seedpods.

Our evidence suggests that the ability to produce seedpods is clade dependent but is not species dependent with respect to the two clades. In other words, in general, species within a clade behave similarly with respect to accepting pollen from within the clade and between the two clades. All species within a given clade accept pollen from one another. All species in the tetraploid clade reject pollen from all the species in the diploid clade. All species in the diploid clade accept pollen from all the species in the tetraploid clade.

It is highly likely that individual plants exist, such as *R. calendulaceum* 'Colossus' and *R. canescens* 'Crane Creek', that fall outside these rules of engagement (especially Rule No. 4 above). The questions are as follows:

- Are the exceptions repeatable? Were viable offspring produced? Were fertile offspring produced?
- Are there more such plants? How do such plants get identified and documented?
- Are such plants associated with certain species?
- What is the actual lab-tested ploidy of these plants? Are most such plants diploids, tetraploids, or possibly triploids?
- Do such plants fall outside Rule No. 4 when crossed with only a few plants in the other clade, or for an entire species in the other clade, or for several or all the species in the other clade?

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