## (2) **Hybridizing Concerns:** ***Color Inheritance, Polyploidy, and Sterility***

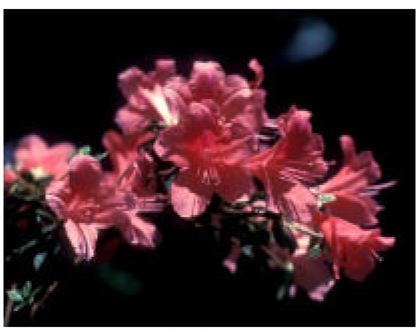
**by Donald W. Hyatt**

In any discussion of hybridizing, it is important to consider the science behind important aspects like flower color inheritance, polyploidy, and potential sterility of one or more of the parents. Attaining a hybridizer's goal is not always easy, but by understanding some of the genetics involved, one can make informed decisions as to which crosses might lead to success.

I am not sure when I made my first evergreen azalea cross, but I know I was hybridizing azaleas in the early 1960's when I was in high school. My first crosses were undoubtedly as informed as those made by bumblebees. If I admired one azalea flower I might take the pollen from it to put on another flower that caught my eye. After earning my degree in horticulture, I tried to be more scientific in my hybridizing since I had specific goals in mind. I am not sure it made a great deal of difference, though, but it did help me to understand what was happening.

### Color Inheritance

It was early 30 years ago when I crossed the orange-red species, *R. nakaharae*, with a dwarf white form of *R. kiusianum*. Expecting compact hybrids in shades of coral, pink, or white, I was shocked as each seedling bloomed and every one was purple! Neither parent had purple flowers, so why did I get purple seedlings? Obviously, azalea flower color inheritance was more complicated than I had imagined.



***R. nakaharae***

***R. kiusianum album***

**(*nakaharae* x *kiusianum album*)**

***Results of an Early Hyatt Cross***

The dominance of purple color in azalea hybridizing has been well documented and that trait has been very frustrating for many breeders who were seeking different azalea flower colors. One of Joe Gable's goals was to develop a hardy white azalea but it took 17 years to reach his goal, the superb creamy white he named, 'Rose Greeley.' [7] I was so pleased that it took "Best in Show" at the 2009 ASA Convention here in Northern Virginia, not for the fact that my plant won the award, but it brought attention to a wonderful azalea that people tend to overlook.

**Joe Gable's 17-year Quest: 'Rose Greeley'**

When I shared the confusing results of my *nakaharae* x *kiusinaum* hybrids with one of my mentors, the late Dr. August Kehr, he provided me with an article that described the action of the genes controlling flower color inheritance in evergreen azaleas. The original research paper by J. Heursel and W. Horn was written in German in and published in 1977. Fortunately, Augie provided me a translation in English.

The strong purple, orange, and red colors we see in evergreen azaleas are primarily due to the presence of pigments called anthocyanins. There are many variations of the anthocyanin molecule, but it is basically the same pigment that makes strawberries and roses red, blueberries and black berries deep purple, and delphinium flowers blue. The many different forms of anthocyanin are the result of other chemical groups attached at different locations on the basic molecule. Each form can be a slightly different color and that shade can further be modified by other factors including the acidity of the cell sap or presence of other pigments.

Heursel and Horn analyzed seedlings from thousands of crosses, and eventually established the existence of six gene pairs controlling flower color. [3] They identified four different forms of the basic anthocyanin molecule in evergreen azalea flowers, and those were contolled primarily by three of the genes. Depending upon which genes were active, an azalea could produce cyanidin which gives a blossom the color “geranium lake,” the familiar coral shade in evergreen azaleas like ‘Fashion’ or *R. nakaharae*. The anthocyanin molecule could also be of the form delphinidin, which has a blue to purple shade, but it could instead be the form peonidin, a carmine red shade. The fourth form the anthocyanin could take in azaleas is malvidin and its color is purple. A white azalea is one where there is no pigment produced.

The researchers identified the gene **W** (or **w**) as the one that produces the basic anthocyanin pigment in evergreen azaleas. The capital **W** means the trait is dominant and that form is the one that produces pigment. The lowercase **w** is called the recessive form of the trait, and in this case it keeps the plant from making any pigment. Since plants get one gene from each parent, there are several possibilities. They can have two genes of the dominant form **W**. Such plants are called homozygous (**WW**) and would produce anthocyanin pigment. They could have a gene of each type and that is called heterozygous (**Ww** or **wW**). Since they contain the dominant form of the trait **W** they would still produce pigment. The recessive gene **w** means that a plant must have both genes of that type for its action to be observed. Therefore, a plant must be homozygous (**ww**) with two genes for the recessive trait before it exhibits that factor, and in this case it produces no pigment and thus has white flowers. Figure #1 summarizes the possible combinations of the genes producing anthocyanin pigment in azaleas.

***Figure #1***

**Possible Gene Combinations**

| | W | w |
|---|---|---|
| W | W W | W w |
| w | w W | w w |

**Pigmented: WW, Ww, wW**
**Not Pigmented (white): ww**

Two other genes Heursel and Horn discovered interact with the production of the anthocyanin pigment and thereby change the form that is made and consequently the color of the flowers. With no interaction, the pigment is simple cyanidin which is the coral shade discussed before. However, the presence of the gene **O** (or **o**) controls oxidation of the anthocyanin and that can add an oxygen atom to the molecule and that produces a different form called delphinidin. The gene **P** (or **p**) controls the addition of a methyl group to the molecule and that will produce peonidin. When both O and P are present, the molecule gets both oxygen and methyl groups and that results in malvidin. These results are summarized in figure #2.

| Genotype | Anthocyanin Pigment | Color |
|---|---|---|
| **WWOOPP** or **WwOoPp** | malvidin | purple |
| **WWOOpp** or **WwOopp** | delphinidin | blue |
| **WWooPP** or **WwooPp** | peonidin | carmine |
| **WWoopp** or **Wwoopp** | cyanidin | geranium lake |

***Figure #2***

A secondary pigment called flavenol is also present in some azalea flowers and that can add a light yellowish caste to blossoms. However, flavenols also seem interact with anthocyanin making purple colors appear more intense. Knowing how these genes interact is helpful for hybridizers. I will summarize some of their findings concerning the other three genes but they are of less importance for flower color inheritance than the first three genes already discussed.

The researchers identified two genes involved in the production of the water-soluble flavenols. These pigments give that ivory or greenish yellow cast to certain azalea flowers like 'Puck' or 'Olga Niblett'. Gene **Q** (or **q**) controls flavenol production, but there was a secondary gene **M** (or **m**) that controls methylation of the flavenol. The researchers noted that high concentrations of flavenols seemed to intensify the purple color of anthocyanin pigments like malvidin.

The intense purple colors in azaleas like 'Dauntless' and 'Girard's Fuchsia' are probably due to the presence of flavenols with anthocyanin pigments. For instance, Joe Klimavicz crossed the tender bicolor 'Leopold-Astrid' with 'Girard's Fuchsia.' Instead of the hardy bicolor he was expecting, Joe found many buff-colored seedlings including the one he registered as 'Sandy Dandy.' If the brilliant purple of 'Girard's Fuchsia' is caused by flavenols intensifying the anthocyanin, when the purple pigment was not expressed in certain seedlings such as 'Sandy Dandy', the yellowish flavenols were still abundant and their presence gave the hybrid its unusual color.

**'Sandy Dandy'**

No matter how concentrated they become, flavenols by themselves are not strong enough to produce the deep yellow hues we admire in other flowers like daylilies, daffodils, and dandelions. Those colors are produced by pigments called carotenoids, and they are the same ones responsible for the deep yellow and orange colors found in deciduous azaleas like *R. calendulaceum* and *R. austrinum*. Carotenoids are not water soluble, but are contained in specialized protoplasmic bodies (plastids) and are not dissolved in the sap. Carotenoids are not naturally present in evergreen azaleas so getting a deep yellow evergreen azalea will require some other techniques to get them into the plant.

The sixth gene pair, **G** (or **g**), controls a process called the glycosidation of anthocyanin but apparently it does not influence flower color so I mention it only in passing.

These details from this research helped explain why my cross of *R. nakaharae* with the white *R. kiusianum* produced nothing but purple flowered seedlings in that initial cross, the "F1 generation." The white *R. kiusianum* probably had the genotype **wwOOPP.** It had two genes for the recessive trait (**ww**) and that meant that it could not produce any pigment. Hence, the flowers were white. Had either of those genes been a **W**, the plant would have produced pigment. Because the *kiusianum* also had the dominant genes **O** and **P**, had the azalea been able to produce pigment, the color would have been purple. It should be noted that the most typical forms of *kiusianum* are purple.

The orange-red *R. nakaharae* probably had the genotype **WWoopp**. Without the presence of either the dominant **O** or **P** genes, the color was not purple but the light orange-red, cyanidin. When crossed with the white *kiusianum*, though, the seedlings would get genes from both parents. They picked up the **W** from *nakaharae* that allowed the seedlings to produce pigment but they also picked up the genes **O** and **P** from *kiusianum* and that caused the pigment form to be malvidin. All seedlings had the likely genotype **WwOoPp** and that produces purple pigment.

Had I been more vigilant in my hybridizing efforts, I should have crossed some of those purple seedlings (the F1 generation) to produce a second generation, or an F2 cross. John Weagle of Nova Scotia made that same initial cross I made and saw all purple seedlings in that first generation. He did cross some of his F1 seedlings and that reshuffled the genes so he got all kinds of different combinations and ended up with a broad range of colors. [9]

**Sterility**

Another problem that azalea hybridizers often face is that some plants they would like to use in breeding can be sterile. There are several reasons for the problem, but it can be very frustrating when trying to breed for specific goals and one or more of the parents are infertile.

Some azaleas are sterile because flowers lack essential reproductive parts. They may be lacking anthers or pistils, or both. This is a common problem with flowers that are fully double. Double azaleas flowers like those found on 'Balsaminiflorum' or *R. yedoense* var. *yedoense* have neither pollen nor pistil, so they cannot be used in hybridizing.

Fortunately, not all double flowered azaleas are sterile. Upon careful inspection, one may notice that some doubles will have occasional stamens tucked among the petals. Sometimes, stray anthers with viable pollen will be attached to deformed petals and those can often be used for as a pollen source. Some doubles will have deformed pistils and those usually do not accept pollen well. Other flowers on the same plant may have a normal pistil and those are more likely to set seed. If interested in breeding for double flowers, it is helpful to look closely at those blossoms in order to find all the essential parts.

Flowers that are hose-in-hose, or having the appearance of two sets of petals, are usually female sterile. In other words, they will not set seed. Even though the flowers often have a normal looking pistil, there must be some structural problem that keeps the pollen tubes from reaching the ovaries and no mater how much pollen is piled on the pistil, they just never set seeds. "Never" is a strong word, though, and there are occasionally exceptions. In rare instances, a hose-in-hose flower can produce a chance seedpod. That implies that one flower must be deformed in some way to allow pollination but unfortunately there is no way for a hybridizer to tell which of the thousands of flowers on a plant might be the fertile one. The late George Ring found a stray seedpod on ‘H. H. Hume’ and seeds from that produced ‘Ring’s True.’ Both are hose-in-hose and white. The reality is that if a hybridizer wants to use a hose-in-hose plant for breeding, it is best to use that plant as the pollen source.

**Polyploidy**

Most plants and animals are called “diploid.” They have two sets of chromosomes containing their genes, and one set comes from each parent. The actual genes, many thousands of them, are on those chromosomes and they are composed of DNA. The DNA controls what type of organism an individual cell will become and it is remarkably similar from the simplest one-celled amoebas to evergreen azaleas to humans. Most evergreen azaleas are naturally diploid with 13 pairs of chromosomes, or a total of 26 chromosomes. Humans have 23 pairs or 46 chromosomes, and more than 30,000 genes.

Scientists refer to a normal diploid organism as “2n” with “n” being the number of pairs of chromosomes. Tetraploid organisms have double the normal number of chromosomes, and they are listed as “4n.” Tetraploids are rare in the animal kingdom but are much more common with plants. Plants with the extra chromosomes are often desired in breeding since they can have some very desirable characteristics, like heavier textured flowers or more robust growth habit

There are some naturally occurring tetraploid evergreen azaleas like ‘Banka’ and ‘Taihei’ as well as several deciduous azalea species including *R. calendulaceum* and *R. austrinum*. How they became tetraploid is not clear. It is also possible to artificially convert a normal azalea into a polyploid but the techniques are often complicated and use toxic chemicals that are best applied under carefully controlled laboratory conditions. [2][4][5]

Tetraploids are promising in certain breeding efforts, but they can also cause difficulties. For instance, a tetraploid crossed with a diploid produces a triploid (3n) but triploids are usually sterile and cannot be used in further breeding. The azalea ‘Redwings’ is triploid, which explains why it has not been used in hybridizing. [4] There are also other polyploid possibilities with plants including hexaploids (6n) and even octaploids (8n), both of which are usually fertile. They can provide some interesting options when used in hybridizing.

There was an interesting scientific study about bicolor flowers that showed the blossoms have both 2n and 4n cells. [1] The researchers showed that ‘Leopold-Astrid,’ a stunning florist azalea that has double white flowers with the petals edged in red, has both diploid and tetraploid cells. The plant itself is basically a normal diploid and the white center of the flowers is diploid tissue, too. However, the contrasting red edge is tetraploid and that causes the color change. This research would imply that tetraploid azaleas might not be useful when breeding for bicolor blossoms.

**‘Leopold-Astrid’ (Red edge is 4n)**

Knowing some of the science controlling evergreen azalea flower color inheritance is useful when seeking specific hybridizing goals, but there is still a lot of chance involved. Having all the desired characteristics come together in a seedling is really quite similar to playing cards. Each seedling will be genetically different from its siblings, and the assortment of genes it receives is comparable to dealing a hand in a card game. One shouldn't expect a Royal Flush in poker or Seven No Trump in bridge on every deal! The reality is that most of the seedlings will be average and sometimes even inferior to the parent plants. Only in rare cases will all the desired qualities to come together in a single plant.

There is a humorous story about the brilliant playwright George Bernard Shaw that parallels this dilemma in hybridizing. Apparently, an attractive socialite once approached Shaw with the following proposition. She noted that with his great mind and her beautiful body, they would produce the most perfect child. Shaw replied, "But Madame, what if the child inherits my body and your brains?" He declined her offer.

And so it is with hybridizing. The probability of all desired characteristics coming together in a single seedling is usually quite low, so it is important for the hybridizer to raise a many plants from a promising cross instead of just a few. No matter how many seedlings are raised, there is still no guarantee of success but understanding some of the science behind the cross can greatly improve the odds. Of course, if we never make the cross, there is no chance that we will get that winning new hybrid. When the azaleas are in bloom, start spreading around that pollen and give luck with a little bit of science thought a chance!

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