Paint colors sampled from the widely used artists colors from Winsor & Newton™.

# Out of the Blue

## Part 2

**By Jonathan Poulton, Ph.D.**
**Region 1, Iowa**

I still vividly remember the day that I got bitten by the daylily bug. My family and I were traveling in 2006 in Door County, Wisconsin, when we got totally lost. By some stroke of good fortune, we came upon an amazing field of daylilies. No, these were not the ditch lilies that commonly line Midwestern roads but daylilies of all shapes and sizes. Their owner, who I later discovered was Ron Mickelson (Sister Bay, Wis.), kindly showed us around and encouraged us to start hybridizing. I asked Ron whether hybridizing daylilies was easy. "Anyone can do it" he replied, adding the instructions: "Just cross pretty with pretty and see what you get!" I needed little encouragement. Within 24 hours of returning from vacation, I began crossing this with that, focusing predominantly upon bicolors and bitones. Yes, the daylily bug had struck again!

Perhaps my memory is faulty on this point, but I don't remember seeing much blue among the daylilies we saw that afternoon. It was not as though blue-faced daylilies hadn't been around for some time. A key step towards the Holy Grail of daylilies — obtaining a true blue daylily — was made in 1970 by Chicago hybridizer James Marsh when he introduced *Hemerocallis* 'Prairie Blue Eyes'. This cultivar achieved an AHS Honorable Mention in 1973 and won an AHS Award of Merit in 1976. By the mid-1990s, we had key diploids such as 'Crystal Blue Persuasion' (Salter, 1996) and 'Lavender Blue Baby' (Carpenter, 1996) — an AHS Stout Silver Medal winner in 2007 — that were to play significant roles in blue daylily programs either as dips or after tetraploid conversion. Ten years later, several blue-faced tetraploids of note became available including 'Bluegrass Music' (Grace-Smith, 2005) and 'Piece of Sky' (Moldovan, 2006). In the Winter 2008 edition of *The Daylily Journal*, Bob Faulkner noted that many well-known hybridizers had a blue program, including Pat and Grace Stamile, Jamie Gossard, the Shooters, Ludlow Lambertson, Gunda Abajian, Jack Carpenter, Elizabeth Salter, Linda Agin, Mort Morss, Phil Reilly, and Gerda Brooker. During the past five years, still others have joined in the blue daylily quest, and together they have succeeded in creating a wide range of blue-faced daylilies that Greg Crane reviewed in the Winter 2013 edition of *The Daylily Journal*. Thanks to the efforts of many hybridizers, there exists today a magnificent collection of daylilies with bluish eyes and with bluish eyes and edges.


'Prairie Blue Eyes'
(Marsh, 1970)
— Linda Sue Barnes photo


'Crystal Blue Persuasion'
(Salter-E.H., 1996)
— Julie Covington photo


'Lavender Blue Baby'
(Carpenter-J., 1996)
— Julie Covington photo

How might one — through conventional breeding or genetic engineering — arrive at a daylily with true blue coloration that extends over the entire petal and sepal surfaces? Hoping for straightforward answers, I posed this question to several of my plant geneticist colleagues but came away disappointed after learning that there is still much that we don't understand at the level of individual


Various bluish daylilies are shown with petals from an Asiatic dayflower (*Commelina communis*). Above from left: 'Blue Desire' (Gossard, 2007); 'Cosmic Odyssey' (Stamile, 2007); 'Lake Tahoe' (Smith-Harry-P., 2011); 'Bird Talk' (Lambertson, 2005); and 'Monday Morning Blues' (Petit, 2005). — Jonathan Poulton photos

'Bluegrass Music' (Grace-Smith, 2005) — Julie Covington photo

''Piece of Sky' (Moldovan, 2006) — Steve Moldovan photo courtesy of the Ohio Daylily Society

cells. For example, we know little about what regulates pigment production in daylilies and, more specifically, what controls the positioning and width of daylily eyes. Surprisingly, that lack of knowledge made the quest for the Holy Grail of daylilies all the more exciting and caused me to throw my hat in the ring. In fall 2012, I purchased about two dozen blue-faced daylilies as an early Christmas present to myself and, during this past summer, made many crosses of interest leading to pods aplenty! I wanted to document how blue these new purchases were in our Iowa City garden by photographing them against some color calibration chart. I considered purchasing the Royal Horticultural Society Colour Chart or the X-Rite ColorChecker Passport but decided against them because of cost and practicality under field conditions. Instead, I resorted to a much cheaper option. I employed petals of Asiatic dayflower (*Commelina communis*), a weed growing in our yard, to provide an unbiased color comparison on any given day and also between different days (see above). Using this side-by-side comparison, garden visitors concluded that under our growing conditions my purchases, while spectacular, did not exhibit true blue coloration but instead lavender, mauve, or purple. I'm sorry to say that I had to agree with them.

Asiatic dayflower (*Commelina communis*) — courtesy of Dr. Bill Hilton Jr., Hilton Pond Center for Piedmont Natural History, www.hiltonpond.org

For many years now, I've marveled at blue-flowered plants (e.g. gentians, delphiniums, and the Asiatic dayflower) both as an avid gardener and as a plant biochemist and wondered what manner of chemical wizardry goes on in such flowers. So, aided by several University of Iowa Biology Honors students, I searched the scientific literature, hoping to better understand the chemistry that underlies "blueness" in these flowers.

Four major classes of pigments confer color upon flowers. In addition to the green chlorophyll pigments, plants may possess betalains, carotenoids, and flavonoids. Whereas betalains are restricted to the plant order Caryophyllales (e.g. beetroot, bougainvilleas, and portulacas) and thus can be ignored here, carotenoids and flavonoids are broadly distributed floral pigments. Carotenoids confer

See Blue, page 42

*Delphinium elatum* 'Royal Aspirations' — Courtesy of Terry Dowdeswell, photographer, of Dowdeswell's Delphiniums Ltd

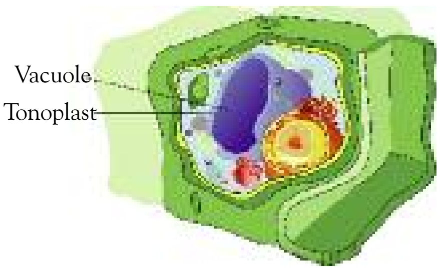
Gentian (Gentiana makinoi) — Courtesy of Trond Steen

**Blue**

**continued from page 41**

yellow, orange or red coloration. The best known flavonoids are the anthocyanins, which impart to flowers a wide range of colors from salmon and scarlet through red and purple to blue and blue-black.

How can these compounds exhibit such a broad range of colors? Over 1,000 different anthocyanins are found within the plant kingdom. Most are based on the three parental compounds — pelargonidin, cyanidin, and delphinidin — but slight differences in their chemical structures can result in quite dramatic differences in the color exhibited by these pigments. You should be aware that anthocyanins are housed within the outermost cells of flowers in membranous, water-filled bags called vacuoles. Also affecting the final color exhibited by an anthocyanin are its concentration, the pH (i.e. acidity) of the vacuole, and the presence of certain metal ions and copigments. If the vacuolar pH is more acidic, the anthocyanin will tend towards red. Alternatively, if the vacuolar pH is more alkaline, the pigment will tend towards blue. Finally, in a phenomenon called copigmentation, near colorless flavonoids (e.g. flavones and flavonols) form complexes with anthocyanins, thereby stabilizing them and shifting their color towards blue.




A vacuole is illustrated above.
— Courtesy of Mariana Ruiz and Wikipedia

Which anthocyanins provide the stunning blues seen in violas, gentians, and delphiniums? I contend that, if we better understood how these species achieve their spectacular hues, it might help us attain our goal of a true blue daylily.

A common misconception among garden enthusiasts is that flowers must accumulate delphinidin-based anthocyanins in order to have blue coloration. While it is true that the majority of violet and blue flowers contain delphinidin-based anthocyanins, some blue-flowered plants actually get their color from cyanidin-based anthocyanins. This was first demonstrated by Willstätter and Everest in 1913, when they showed that the same cyanidin-based anthocyanin is responsible for making roses red but cornflowers (*Centaurea cyanus*) blue! However, merely possessing cyanidin- and/or delphinidin-based anthocyanins doesn't guarantee blue flowers. Recent research has discovered that plants use rather complicated mechanisms, sometimes a combination thereof, to achieve those vibrant blues that we'd like to see in our daylilies (Yoshida et al., 2009a). They include the following:

**i) Formation of metalloanthocyanins:** In some species, stunning blue coloration is achieved by metalloanthocyanins. These are self-assembling complexes that possess anthocyanin molecules, copigment molecules (flavones), and metal ions in whole number ratios. For example, in blue cornflowers, the metalloanthocyanin contains six cyanidin-based anthocyanin molecules, six flavone molecules, and two metal ions (one ferric [$Fe^{3+}$] and one magnesium [$Mg^{2+}$]). In contrast, the metalloanthocyanin in the Asiatic dayflower that I used as blue standard in my photography has six delphinidin-based anthocyanin molecules, six flavone molecules, and two $Mg^{2+}$ ions.

Blue cornflower (*Centaurea cyanus*) — Courtesy of the website http://commons.wikimedia.org/

Himalayan blue poppy (*Meconopsis grandis*) — Courtesy of the website http://commons.wikimedia.org/

Blue iris (*Iris ensata*) — Courtesy of Serena Graham-Dwyer

**ii) Formation of "fuzzy metal complex pigments":** Found in many blue flowers, these pigment complexes also contain anthocyanin molecules, copigment molecules, and metal ions, but not in whole number ratios. For example, the sky-blue coloration of the Himalayan blue poppy's petals is due to its cyanidin-based anthocyanin complexing with iron ($Fe^{3+}$) ions and flavonols. In contrast, blue hydrangea flowers get their color from a delphinidin-based anthocyanin that complexes with aluminum ($Al^{3+}$) ions and acylquinic acids.

**iii) Blueing mechanisms without contributions from metal ions:** In other species, blue coloration arises when anthocyanins complex with suitable copigments; no metal ions are involved here. Excellent examples are *Geranium* 'Johnson's Blue', blue iris (*Iris ensata*), and *Heliophila coronopifolia*.

*Hydrangea macrophylla* 'Glory Blue' — Courtesy of the website https://www.gardenerdirect.com

*Geranium* 'Johnson's Blue' — Courtesy of the website http://commons.wikimedia.org/

**iv) Stacking of polyacylated anthocyanins:** Anthocyanins tend to be unstable at the pHs usually found in plant vacuoles (pH 3-6) and require stabilization by one of the mechanisms described above. Notable exceptions are polyacylated anthocyanins that are able to stack, making them more stable and intensifying their blue hue. These anthocyanins cause the blue colors of gentians (e.g. *Gentiana makinoi*), Chinese bellflower (*Platycodon grandiflorum*), and butterfly pea (*Clitoria ternatea*).

Chinese bellflower (*Platycodon grandiflorum*) — Courtesy of the website http://commons.wikimedia.org/

Butterfly pea (*Clitoria ternatea*) — Courtesy of the website http://commons.wikimedia.org

**v) Elevating vacuolar pH:** The blue morning glory (*Ipomoea tricolor* 'Heavenly Blue') also produces a polyacylated anthocyanin but it increases its blueness by elevating vacuolar pH (Yoshida et al., 2009b). During the 24 hours required for the morning glory flower to open, the vacuolar pH increases from pH 6.6 (slightly acidic) to pH 7.7 (slightly alkaline), causing this flower's cyanidin-based anthocyanin to turn from a

**See Blue, page 44**

**Blue**
**continued from page 42**

Blue morning glory (*Ipomoea tricolor* 'Heavenly Blue') — Courtesy of My Virtual Maryland Garden

dull purple to that brilliant blue that we all enjoy.

Having learned what leads to blue flowers in other plant species, let us now return to daylilies and ask the question: "Which anthocyanins have been found in *Hemerocallis*?" Surprisingly, the scientific literature contains few anthocyanin analyses of daylily flowers. Undoubtedly the most comprehensive study was undertaken by Katherine Bisset (1976) during her doctoral research at Florida State University. Noting the absence of blue-faced daylilies at that time, Bisset wished to elucidate any restrictions to blueness in *Hemerocallis* and to undertake studies that would facilitate creation of a blue-flowered cultivar by hybridization. One of her experimental approaches was to measure the anthocyanin content of 32 selected daylily cultivars within three different color ranges: orange/gold, red/scarlet, and lavender/purple. Bisset found that none contained pelargonidin derivatives. As Table 1 indicates, one cultivar ('Burning Daylight' [Fischer-H.A., 1957]) lacked anthocyanins, obtaining its orange hue from its carotenoids. The remaining cultivars contained either cyanidin derivatives, delphinidin derivatives, or both. As expected, the lavender-purple daylilies were more likely to accumulate delphinidin-based anthocyanins. While none of Bisset's selected cultivars exhibited any blue hue in their flowers, it is important to acknowledge that, by having either cyanidin derivatives and/or delphinidin derivatives, all but the cultivar 'Burning Daylight' had the potential to display such coloration. That they didn't do so indicates that these cultivars lacked the sophisticated mechanisms required for blueness that, for example, gentians, hydrangeas, and delphiniums possess.

**Table 1: Anthocyanin content of selected daylily cultivars**

| Flower color | Cultivars lacking anthocyanins | Cultivars containing cyanidin derivatives only | Cultivars containing derivatives of both cyanidin and delphinidin | Cultivars containing delphinidin derivatives only |
|---|---|---|---|---|
| Orange-Gold | 1 | 4 | 1 | 0 |
| Red-Scarlet | 0 | 6 | 5 | 0 |
| Lavender-Purple | 0 | 1 | 9 | 5 |

(Source: Katherine E. Bisset, Ph.D. Thesis, Florida State University, 1976)

Let's now fast forward to 2014! As Greg Crane's excellent Part 1 in this series clearly demonstrated, much has been achieved by hybridization during the past 40 years. Today, there are many daylily cultivars that display tantalizing hints of blue in their eyes and edges. In addition, we are also aware of other blue-flowered species in the daylily family such as *Dianella* and *Stypandra* species. Unfortunately, we have absolutely no clue how these blues come about chemically in any of these plants. I contend that, by analyzing a handful of blue-faced daylily cultivars, we may find valuable information that could facilitate creation of a true blue self by either conventional breeding or genetic engineering. Nowadays, scientists have highly accurate methods for the analysis of anthocyanins, colorless flavonoids, vacuolar pH, and metal ions in small amounts of plant tissue. Thus, for example, a well-equipped laboratory could measure these components in tiny tissue samples taken along a transect in Faulkner's 2013 introduction 'Rhapsody in Blue' (Faulkner, 2013). These studies may provide evidence for one or more of the five strategies employed by other blue-flowered species. Alternatively, it may reveal that other, as yet unidentified, mechanisms are responsible for the blueness seen in today's blue-faced daylilies.

*Hemerocallis* 'Rhapsody in Blue' (Faulkner, 2013) with transect illustration — Phil Reilly photo with Jonathan Poulton illustration

Given the amazing progress that has been made towards the Holy Grail of daylilies during the last decade, I am optimistic that we will in due course achieve this lofty goal by conventional breeding, but it may require some of those "lucky mutations" that my geneticist colleagues hinted at when I visited them. Such mutations might promote blueness by providing appropriate vacuolar conditions for formation of blue complexes (e.g. by raising delphinidin levels, obtaining the correct anthocyanin/copigment ratio, elevating vacuolar pH, modulating concentrations of certain metal ions, etc.). Should hybridization attempts fail, genetic engineering could provide an alternative pathway to our goal.

Ten years ago, scientists at Tennessee State University successfully established methods for transferring foreign genes into *Hemerocallis* (Aziz at al., 2003). Two candidate genes already suggest themselves. These genes encode: (i) the morning glory vacuolar proton transporter that causes the increase in vacuolar alkalinity during flower opening (Yamaguchi et al., 2001), and (ii) a protein that transports $Fe^{3+}$ ions into the vacuoles of cornflower petals (Yoshida and Negishi, 2013).

Having closed my lab upon retirement from the University of Iowa in 2010, I am no longer able to undertake the aforementioned analyses or genetic engineering. Instead, I shall continue the quest for a true blue daylily by conventional breeding, hoping that I encounter some of those "lucky mutations"! ■

**References:**

Aziz AN, Sauve RJ, and Zhou S (2003) Genetic transformation of Stella De Oro daylily by particle bombardment. Canadian Journal of Plant Science 83: 873-876

Bisset KE (1976) Spectrophotometry, chromatography and genetics *of Hemerocallis* pigments. Ph.D. Thesis. Florida State University, Tallahassee, FL

Royal Horticultural Society Color Chart, URL: http://www.rhsshop.co.uk/productdetails.aspx?id=10000006&item-no=MARK0008

X-Rite ColorChecker Passport, URL: http://xritephoto.com/ph_product_overview.aspx?ID=1257

Yamaguchi T, Fukada-Tanaka S, Inagaki Y, Saito N, Yonekura-Sakakibara K, Tanaka Y, Kusumi T, and Iida S (2001) Genes encoding the vacuolar $Na^+/H^+$ exchanger and flower coloration. Plant Cell Physiology 42: 451-461

Yoshida K, Mori M and Kondo T (2009a) Blue flower color development by anthocyanins: from chemical structure to cell physiology. Natural Product Reports 26: 884-915

Yoshida K, Miki N, Momonoi K, Kawachi M, Katou K, Okazaki Y, Uozumi N, Maeshima M, and Kondo T (2009b) Synchrony between flower opening and petal-color change from red to blue in morning glory, *Ipomoea tricolor* cv. Heavenly Blue. Proc Jpn Acad Ser B Phys Biol Sci. 85: 187-197

Yoshida K and Negishi T (2013) The identification of a vacuolar iron transporter involved in the blue coloration of cornflower petals. Phytochemistry 94: 60-67