Flower Pigments within *Hemerocallis fulva* L. fm. *fulva*, fm. *rosea*, and fm. *disticha*

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Abstract. Various forms of *Hemerocallis fulva* differed in their relative anthocyanin : carotenoid ratios and the type of anthocyanin present. *Hemerocallis fulva* fm. *fulva* contained a single anthocyanin (cyanidin-3-rutinoside) and two carotenoids (zeaxanthin and lutein). *Hemerocallis fulva* fm. *rosea* contained a single anthocyanin (cyanidin-3-rutinoside) and traces of carotenoids. *Hemerocallis fulva* fm. *disticha* contained a single anthocyanin (delphinidin-3-rutinoside) and two carotenoids (zeaxanthin and lutein).

Flower colors are due to at least two types of pigments, the carotenoids and flavonoids. The flavonoids can be subdivided into copigments (e.g., flavonols and flavonones) and anthocyanins. In vivo, the anthocyanins and copigments occur as a complex held together through hydrogen bonding (Kondo et al., 1992). The color seen is the result of the physical exchange and interaction of electrons between the carbon rings of the individual anthocyanin and copigment molecules within this complex. The anthocyanin copigment complex is responsible for flower colors red through blue.

Flower colors yellow through orange are due to the presence of carotenoid pigments. Most flowers contain either anthocyanin or carotenoid pigments, not both. When the two pigment classes do occur together, new and unusual colors can be produced (Griesbach, 1984). For example, in orchids, red flowers could be either the result of a red anthocyanin–copigment complex and the absence of carotenoids or the result of a magenta anthocyanin–copigment complex and the presence of carotenoids.

Flowers of *Hemerocallis fulva* contain carotenoid and flavonoid pigments. Three forms of this species exist—fm. *fulva* with orange flowers [Royal Horticultural Society (RHS) 28 B (RHS, 1966)], fm. *rosea* with rose flowers (RHS 51 B), and fm. *disticha* with brown flowers (RHS 171 B). In this study, we determined the pigments responsible for the several color forms.

Materials and Methods

Plant material. Three color forms of *H. fulva* were obtained from the U.S. National Arboretum (USNA), Washington, D.C. *Hemerocallis fulva* fm. *fulva* is native to most of Eurasia. In addition, it has a long history of cultivation with many selected clones of unknown origin. The clone of *H. fulva* fm. *fulva* (USNA accession no. 37426) used in this study was collected in China by S-Y Hu of the Arnold Arboretum of Boston.

Hemerocallis fulva fm. *rosea* is restricted to a small area near Kuling, China. Arlow Stout of the New York Botanical Gardens selected the clone of *H. fulva* fm. *rosea* ‘Rosalind’ (USNA 24421) used in this study. He selected the ‘Rosalind’ daylily from a group of seedlings derived from self-pollination of a clone collected in 1924 by A.N. Stewart.

Hemerocallis fulva fm. *disticha* is native to subtropical China and first was collected in 1789. The clone of *H. fulva* fm. *disticha* (USNA 30365) used in this study was collected in China by M. Matsuoka of the Osaka Botanical Gardens, Japan.

Anthocyanin analysis. The anthocyanin pigments were characterized by high-pressure liquid chromatography (HPLC) as previously described for *Petunia hybrida* Juss. (Griesbach et al., 1991). The petals and sepals of individual flowers were removed and ground at high speed in a blender (Waring, New Hartford, Conn.) with 100 ml aqueous 10% acetonitrile, 15% acetic acid, and 1.5% phosphoric acid. The extract was filtered through no. 3 Whatman paper and reduced to dryness under vacuum at 40°C. The residue was then dissolved in 100 μ liter of the extraction solvent. The anthocyanins were resolved on a 7.8 $\times$ 300-mm Bondapak C18 column (Waters, Milford, Mass.) using a 30-min linear gradient of 0% to 10% acetonitrile in aqueous 15% acetic acid and 1.5% phosphoric acid, followed by a 10-min linear increase to 20% acetonitrile. Finally, they were held in 20% acetonitrile for an

additional 10 min. The flow rate was 1 ml·min⁻¹, and the eluant was monitored at 540 nm.

The anthocyanins were characterized by coelution, with purified known standards from *Hemerocallis* (Asen and Arisumi, 1968).

Carotenoid analysis. The carotenoid pigments were characterized by HPLC as previously described for *Ornithogalum* L. (Griesbach et al., 1993). The petals and sepals of individual flowers were removed and ground in 100 ml acetone and filtered through no. 3 Whatman paper. The solution was extracted three times with petroleum ether using 50 ml each time. The carotenoid-containing ether was reduced to dryness under vacuum, and the residue was dissolved in 100 μ liter 25% acetonitrile and 75% methanol. The carotenoids were resolved on a radially compressed, 8 $\times$ 100-mm Bondapak C18 column using a 20-min linear gradient of aqueous 75% to 100% acetonitrile : methanol (25:75). The solvent then was held at 100% for an additional 20 min. The flow rate was 1.5 ml·min⁻¹, and the eluant was monitored at 445 nm.

The carotenoids were characterized by coelution with known standards from spinach (*Spinacia oleracea* L.) (Thammasiri et al., 1986).

Fig. 1. High-pressure liquid chromatography anthocyanin profiles of *Hemerocallis fulva* (A) fm. *fulva*, (B) fm. *rosea*, and (C) fm. *disticha*. Cyanidin-3-rutinoside is peak 1 and delphinidin-3-rutinoside is peak 2.

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Results

The orange flowers of *H. fulva* fm. *fulva* contained a single anthocyanin (cyanidin-3-rutinoside) (Fig. 1A) and two carotenoids (zeaxanthin and lutein) (Fig. 2A). The rose flowers of *H. fulva* fm. *rosea* 'Rosalind' contained a single anthocyanin (cyanidin-3-rutinoside) (Fig. 1B) and little if any carotenoids (Fig. 2B). The brown flowers of *H.*

fulva fm. *disticha* contained a single anthocyanin (delphinidin-3-rutinoside) (Fig. 1C) and two carotenoids (zeaxanthin and lutein) (Fig. 2C). *Hemerocallis fulva* fm. *rosea* differed from the type species, *H. fulva* fm. *fulva*, because it contained a lower concentration of carotenoid pigments; *H. fulva* fm. *disticha* differed from the type species because it contained a different anthocyanin pigment.

Discussion

Hemerocallis breeding has begun only recently. The first artificially created hybrid ('Apricot') was named in 1892. Until 1930, all introduced cultivars were similar to the species progenitors in flower color (Arisumi, 1971). During the 1920s, Stout began a breeding program using new color forms of *H. fulva*. Stout's pioneering breeding program was responsible for creating cultivars with new flower colors (Eddison, 1992). Modern red cultivars contain predominantly cyanidin-3-rutinoside; modern purple cultivars contain predominantly delphinidin (Asen et al., 1968). These are the same pigments found within the various *H. fulva* forms.

We found that *H. fulva* fm. *rosea* differs from the type species by possessing a reduced carotenoid concentration. This reduced concentration was used by breeders to create pink flowers. Stout (1942) believed that the importance of *H. fulva* fm. *rosea* in breeding was due to a reduction in plastid pigmentation and reported that the clearest pink colorations were associated with the absence of plastid pigments. Stout (1938) also found that the unique color of *H. fulva* fm. *rosea* was recessively inherited. He released several pink cultivars, of which the most important to future breeding was 'Charmaine'. This cultivar is in the background of many of the modern pink cultivars.

Hemerocallis fulva fm. *disticha* differs from the type species by possessing a different anthocyanin (e.g., delphinidin). Although this form was first collected in 1749, it was not recognized formally as a valid botanical entity until recently (Hu, 1968). After observing the colored photographs and plates in Stout's articles, we believe that he used this form in breeding. Leonian (1939) obtained several of Stout's red cultivars and separated them into two groups, "those possessing purple pigment

and those without such a pigment." It seems that *H. fulva* fm. *disticha* is in the background of Stout's purple-reds. The *disticha* form most likely is in the background of Stout's most famous cultivar, 'Theron'. 'Theron' expressed a new color that Stout (1938) described as Mars violet. Many of our modern purple and black cultivars are descended from 'Theron'.

Historically, *H. fulva* fm. *fulva* contributed gene(s) for red pigmentation, *H. fulva* fm. *rosea* for reducing carotenoid pigmentation, and *H. fulva* fm. *disticha* for purple pigmentation. By mixing these genes in different combinations, the color forms of *H. fulva* are responsible for the wide range of new flower colors (e.g., pink, purple, and red) of the modern *Hemerocallis*.

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Fig. 2. High-pressure liquid chromatography carotenoid profiles of *Hemerocallis fulva* (A) fm. *fulva*, (B) fm. *rosea*, and (C) fm. *disticha*. Lutein is peak 1 and zeaxanthin is peak 2.