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Contributions of Jeffrey Harborne and co-workers to the study of anthocyanins

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Abstract

Jeffrey Harborne and his co-workers have played a unique role in the over-all study of plant pigments and of anthocyanins in particular through their many publications and through Jeffrey's editorial work with *Phytochemistry*. Jeffrey has made important contributions to our understanding of the separation and structural identification of anthocyanins; to co-pigmentation; and to the role of anthocyanins in systematics and ecology in both reproductive and vegetative tissues. This work has had considerable influence on much of the current research on the genetics and regulation of anthocyanin biosynthesis.

Keywords: J.B. Harborne; Anthocyanins; Phytochemistry

1. Introduction

It was a great pleasure to be present at a meeting held at the Royal Botanic Gardens, Kew in July 1999 in honor of the retirement of Professor Jeffrey Harborne FRS (University of Reading) as Executive Editor of *Phytochemistry*. Throughout his working life, Jeffrey Harborne has made invaluable contributions to the field of phytochemistry focusing mainly on flavonoid compounds. Of special interest is his work on plant pigments and in particular his work on the anthocyanins.

Floral and fruit colors derive from a small group of pigments principally carotenoids, betacyanins (Centrospermae), anthocyanins and other flavonoids (flavones and flavonols). Anthocyanins are responsible for the wide range of colors in the petals of flowering plants and they may vary the color from salmon- pink, through scarlet, magenta and violet to deep blue (Haslam, 1995). They are present in flower petals to attract pollinators and in fruits and seeds as attractants for seed dispersers (Willson and Whelen, 1990). Structurally anthocyanins are glycosylated polyhydroxy/methoxy derivatives of 2-phenyl benzopyrilium (flavylium) salts. Six aglycones (anthocyanidins) dominate natural anthocyanin structures (Fig. 1) and most are substituted at the 3- and/or 5-hydroxy positions. The three main chromophores, pelargonidin, cyanidin and delphinidin occur usually singly or occasionally as mixtures and essentially all pink, scarlet and orange-red flowers contain pelargonidin, all crimson and magenta flowers cyanidin and all mauve and blue flowers delphinidin. Anthocyanins are hydrophilic and generally present in the plant cell vacuoles. Anthocyanins are also present in vegetative tissues, in leaves, stems and roots and in emergent seedlings. They are visible in the leaves of fall foliage, in rapidly developing shoots and on the undersurface of leaves of many tropical understory herbs.

In every aspect of current anthocyanin research, Jeffrey Harborne has acted as a pioneer — in the separation and structural identification of anthocyanins; in genetics and the inheritance of flower color; in plant systematics; in pollination ecology and in focusing attention on the ecological role of anthocyanins in vegetative tissues. This paper briefly discusses some of the work of Jeffrey Harborne and his many collaborators and serves to illustrate the influence that this research has had and is having on current research on anthocyanin pigments.

2. Chemical basis of flower color variation

2.1. Chemical identification

Some of the earliest work on anthocyanins, concerning the chemical and genetic basis of flower color variation, was carried out by Robert Robinson and his wife Gertrude,

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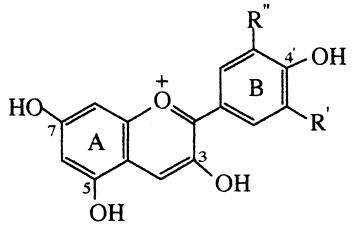

Fig. 1. Structure of the aglycone, anthocyanidin. The six most common anthocyanidins found in natural pigments are: pelargonidin R′ = H, R″ = H; cyanidin R′ = OH, R″ = H; peonidin R′ = OMe, R″ = H; delphinidin R′ = OH, R″ = OH; petunidin R′ = OMe, R″ = OH; and malvidin R′ = OMe, R″ = OMe.

at Oxford, and by Scott-Moncrieff at the John Innes Institute, England (Scott-Moncrieff, 1981). It was at this latter Institute that Jeffrey Harborne began his professional scientific career in 1955. Harborne did much of the early work on chemical identification of anthocyanins using both spectral (Harborne et al., 1953; Harborne, 1957a) and chromatographic methods (Harborne, 1959). Based on the chromatographic methods, first reported by Bate-Smith (1948) and Bate-Smith et al. (1953), Harborne developed suitable solvents for separating pigments with different glycosylation patterns (Harborne, 1957b). Thus, for the first time it became possible to identify plant anthocyanins and their sugar components on a micro-scale combining both spectral and chromatographic techniques (Harborne, 1962, 1963a, 1964a, 1965a,b).

In the early 1960's it was also shown that in many cases the sugars attached to the anthocyanidins are acylated with hydroxycinnamic or hydroxybenzoic acids (Harborne, 1964b). In addition to aromatic acylation, in the 1980's, new mild extraction techniques demonstrated the widespread occurrence of acylation with aliphatic dicarboxylic acids and anthocyanins occurring as zwitterions (Harborne and Boardley, 1985; Harborne, 1986a,b; Harborne et al., 1986a,b; Harborne and Self, 1987; Harborne et al., 1987; Harborne, 1988a; Harborne et al., 1989a; Harborne, 1990; Harborne et al., 1993). When plants in the Compositae (Asteraceae) which had earlier been screened for anthocyanins were reinvestigated, it was found that practically all species had malonyl or succinyl groups attached to the floral anthocyanins (Takeda et al., 1986). In angiosperms, malonylated residues linked to the glucosyl units are present in at least a third of all plant species analyzed.

2.2. Co-pigmentation

The observation that the color of isolated anthocyanins could be varied by the presence of other substances, such as "tannin", was first made by Willstatter and Zollinger (1916) and Robinson and Robinson (1931) (in Haslam, 1993). Jeffrey Harborne's work and those of many others have clearly shown how anthocyanins with the same chromophoric structure can give rise to many different colors (Goodwin, 1988; Harborne, 1993).

Co-pigments are important as under very weakly acidic conditions, pH 4–6, as is typical in cell vacuoles, and in the absence of other substances, most anthocyanins exist substantially in stable colorless forms. Between pH 4 and 6, four anthocyanin structural types exist in equilibrium, namely the flavylium cation, the quinonoidal anhydro base, the colorless carbinol bases and the pale yellow "reversed" chalcones. Equilibration between the quinonoidal and carbinol bases occurs via the flavylium cation:

$$\text{quinonoidal anhydro base} \rightleftarrows \text{flavylium cation} \rightleftarrows \text{carbinol bases}$$

As the pH is raised, increasing amounts of the anhydrobase are formed. At neutral pH, the anionic form of the quinonoidal base (blue) is formed; in more acidic conditions the flavylium ion (red) predominates (Haslam, 1993, 1998).

Co-pigments may act either inter- or intramolecularly (Figueiredo et al., 1996). They have little or no color by themselves but probably stabilize and enhance the color of the anthocyanins by intermolecular hydrophobically reinforced π–π stacking of their aromatic nuclei with those of the pigment (Brouillard et al., 1991; Mistry et al., 1991). Such π–π interactions are thought to stabilize the flavylium-cation/anhydro-base forms which prevents the usual reaction with water to form the colorless carbinol-base forms.

Various molecules have been identified as good natural intermolecular co-pigments including the hydroxycinnamoyl esters (mentioned above) such as chlorogenic acid, galloyl esters (tannins) and flavone and flavonol glycosides (Figueiredo et al., 1996). When caffeine was tested, it was found to preferentially stabilize the quinonoidal base (Haslam, 1993). An anthocyanin in which the anhydro-base form is stabilized is the 'heavenly blue anthocyanin' from the blue petals of the Morning Glory (*Ipomoea tricolor*) in which two of the caffeyl ester groups are thought to be stacked intramolecularly with the anthocyanin nucleus (Mistry et al., 1991). Sugar attachment and methylation are also both probably important for pigment stability and generally have little effect on flower color per se. Methylation of one or more of the free hydroxyl groups also improves the stability of the anthocyanidin chromophore and is relatively common in the more highly specialized plant families (Harborne, 1993).

Also associated with co-pigmentation is the presence of metal ions (Goto et al., 1986). The presence of metal ions or metallo anthocyanins with magnesium and iron have been isolated in the blue pigment, commelinin, from the petals of *Commelina communis* and also from the cornflower, *Centaurea cyanus* while in *Hydrangea* flowers the metal present is aluminium.

2.3. Other factors affecting pigmentation

Additional factors which affect flower color are vacuolar pH. Both environment and genetic factors appear to be involved in the control of vacuolar pH. The pH of the vacuole generally varies between pH 4.5 and 5.5 but can range from pH 2.8 measured in a Begonia cultivar to pH 7.5 found in Morning Glory cv Heavenly Blue. The pH in epidermal cells from different parts of the same flower may also vary (Haslam, 1995). On aging, there is often an increase in the pH of the plant cell vacuole leading to an increase in the quinonoidal base which causes the aging or bluing effect. In petunia, seven genetic loci have been defined (pH 1–7) that, when mutated cause bluing of the flower. These mutations do not alter the anthocyanin composition but do increase the pH of the petal extracts (Mol et al., 1998). Mol et al. (1998) have recently emphasized that the shape of the epidermal cells is also an important factor in pigmentation. For example, whether the cell is conical or flat, influences the optical properties of anthocyanin and thereby the color that is perceived (Mol et al., 1998). Factors known to influence floral pigmentation are summarized in Table 1.

3. Distribution of anthocyanins

For more than 30 years, Jeffrey Harborne and coworkers have been working on the systematics of flower color (Harborne, 1963b, 1988b; Harborne and Turner, 1984). As anthocyanins are almost universal in flowering plants they are chemical compounds that are useful for systematic purposes both at the family and genus level. Systematic studies have been made using the distribution of anthocyanins present in the floral petals of many major plant groups including Poaceae (Harborne and Clifford, 1967); Umbelliferae (Harborne, 1976); Onagraceae (Harborne et al., 1976); Commelinaceae (Harborne and Stirton, 1980); the Araceae (Harborne et al., 1981); Lamiales (Harborne, 1992a); Leguminosae (Fabaceae) (Harborne et al., 1970), and the Compositae (Asteraceae) (Harborne, 1996), and at genus level in *Collomia* (Harborne et al., 1982) and *Patersonia* (Harborne et al., 1989b). Many family or genera have characteristic patterns- for example, substitution at the 3-,7- and 3′-positions is a characteristic feature in anthocyanins isolated from orchid flowers (Harborne and Williams, 1998) while anthocyanins acylated with *p*-hydroxybenzoic acid appear to occur characteristically in two unrelated plant families, the Campanulaceae and the Ranunculaceae (Harborne and Williams, 1995).

Table 1
Modification of floral pigmentation

1. Variation in pigment class
2. Concentration of individual pigments
3. Structural variations within a pigment class
4. Modifications of anthocyanidin 3-glycosides:
(a) Further glycosylation
(b) Methylation
(c) Acylation with aromatic compounds (hydroxy cinnamoyl esters)
(d) Acylation with aliphatic dicarboxylic acids (malonate)
(e) Presence of flavonol or flavone co-pigment
(f) Metal chelation (magnesium and iron)
5. Changes in vacuolar pH
6. Cell shape

Although anthocyanins are almost universal in the roots, stems, leaves and flowers of angiosperms and are also common in both the vegetative and reproductive structures of many gymnosperms (Anderson, 1992; David Lee, personal communication), they are of sporadic occurrence in other groups of plants. Anthocyanin-like pigments have been detected in liverworts and mosses (Markham, 1988, 1990) and pelargonidin and cyanidin derivatives in the fern *Davallia divaricata* (Harborne, 1965c).

4. Ecological roles of anthocyanins

4.1. Role of anthocyanins in reproductive tissues

Anthocyanins play a vital role in reproductive tissues (Harborne, 1980, 1992b). Jeffrey Harborne was one of the first scientists to establish by chemical experimentation the direct relationship between anthocyanin type (flower color) and pollination ecology. He provided clear evidence that natural selection for particular colors in different environments depends upon the most active pollinators present. (Harborne, 1993). Harborne demonstrated how selection has worked in two directions: from cyanidin as the most basic or most primitive type of plant pigment, to flowers with scarlet orange colors (pelargonidin) arising by loss mutations. In the second direction, gain mutations have occurred in temperate climates producing the delphinidin colors common in bee pollinated families, such as the Primulaceae, Polemoniaceae, Hydrophyllaceae and Boraginaceae. The further loss mutation from pelargonidin to give the 3-desoxyanthocyanidin- luteolinidin and apigeninidin, only appears in very advanced tropical angiosperm families such as the Gesneriaceae and Bignoniaceae. Analysis of the anthocyanins present in 18 representative members of the Polemoniaceae showed a clear cut correlation between flower color, anthocyanidin type and pollinator (Harborne and Smith, 1978). A more extensive survey also demonstrated the correlation between anthocyanin type, pollinator and flower color in the Lamiaceae (Saito and Harborne, 1992).

Evolutionary changes in flower color can also be observed at the species level, and any change in color may have important evolutionary consequences (Weiss,

1991). Mutational inactivation of only a few structural genes may be sufficient to change the type of pollinators which in turn may result in genetic isolation and possibly speciation (Vickery, 1995). For example, species of *Mimulus* (monkey flower) are reproductively isolated owing to *M. lewisii* pollination preference by bumblebees and *M. cardinalis* by hummingbirds (Bradshaw et al., 1995).

4.2. Role of anthocyanins in vegetative tissues- protection from photoinhibition and photo-oxidation

The roles of anthocyanins in vegetative tissues have been less well studied. Jeffrey Harborne regards their function in vegetative organs as "an unraveling mystery" (D. Lee, personal communication). In vegetative tissues, anthocyanins are not present in the epidermis but in the vacuoles of cells in the spongy mesophyll. Young leaves of tropical plants, young, rapidly growing plants, autumnal leaves (Haslam, 1993) and leaves of plants under stress are often red in color.

Leaf undersurfaces of many tropical rainforest understory herbs are red because of the presence of anthocyanins (Benzing and Friedman, 1981). Their presence on the undersurface of the leaves is assumed to be selectively advantageous to plants growing in extreme shade perhaps because the presence of anthocyanins increases the amount of available light for photosynthesis (Lee et al., 1979). However, no mechanism has yet been discovered by which anthocyanins could backscatter radiation and increase light-capturing efficiency in the red wavelength. Gould et al. (1995) suggest an alternative role for anthocyanins, namely reducing susceptibility to photoinhibition. In the understory, plants can be subject to photodamage by brief flecks of sunlight or intermittent light. Anthocyanins absorb at the same wavelength as chlorophyll *b* in the 520/530 nm wavelength range. Comparing leaves with the red or green undersurface in two taxa native to rainforest understory shade, *Begonia pavonina* Ridl. (Begoniaceae), from Bukit Lanjang Forest Reserve, Malaysia and *Triolena hirsuta* Triana (Melastomaceae) from La Selva Research Station in Costa Rica, Gould et al. (1995) found that when anthocyanins are present not only is photoinhibition reduced, but photosynthesis is increased with higher chlorophyll concentrations. Anthocyanins reduce the effects of high light intensity by absorption (Gould et al., 1995).

Leaves of young, rapidly growing plants are often red in color. Under conditions of rapid cell wall elongation (growth) there is the potential for excess peroxide to diffuse into the vacuole and hydrogen peroxide and other oxygen radicals are also produced in times of plant stress. A function for anthocyanins in vegetative tissues is as antioxidants by reacting with free radicals and thus interrupting the propagation of new free radical species or by chelating metal ions such as Fe^{2+}. (Cooper-Driver and Bhattacharya, 1998). The production of anthocyanins- peroxidase system in the vacuoles may contribute to the overall mechanisms for protecting plants from oxidative damage (Yamasaki, 1997).

Many phenolic compounds are being sought for their antioxidant and antithrombotic properties, as potential agents against cancer and cardiac diseases. Antioxidant supplements can significantly improve certain immune responses. For example, the antioxidant and antiinflammatory activity of glycosides of cyanidin from tart cherries has recently been confirmed and these are being incorporated into meat products for improved nutritional qualities (Wang et al., 1999).

5. Genetic manipulation of flower color

Early in Jeffrey Harborne's career, he expressed an interest in the breeding of blue roses (Harborne and Rowley, 1958) but producing a blue rose has eluded traditional breeding techniques. However, an understanding of the biosynthetic pathway of anthocyanins and the enzymes involved, coupled with new DNA recombinant techniques has opened up a whole new chapter in manipulation of flower color.

5.1. Biosynthesis of anthocyanins

The synthesis of anthocyanins involves the enzyme, *chalcone synthase* (CS) which catalyzes the stepwise condensation of three malonyl-CoA units with *p*-coumaroyl-CoA to yield tetrahydroxychalcone (naringenin chalcone) (see Fig. 2). *Chalcone isomerase* (CI) then catalyzes the stereospecific isomerization of the yellow-colored tetrahydroxychalcone to the colorless flavanone, naringenin. Flavanones act as intermediates for the synthesis of flavones, isoflavones and 3-OH flavanones (dihydroflavonols). Narigenin is converted to dihydrokaempferol (DHK) or to a 3-0H flavanone by *flavanone 3-hydroxylase* (F3H).

The next stage is the conversion of the dihydroflavonol to the anthocyanin (see Fig. 3). DHK can be hydroxylated by the microsomal cytochrome P450 enzymes, *flavonoid 3′-hydroxylase* (F3′H) to produce dihydroquercetin (DHQ) or by *flavonoid 3′,5′-hydroxylase* (F3′5′H) to produce dihydromyricetin (DHM) (Holton and Cornish, 1995). The precise number of enzymes and their sequence of operation from the dihydroflavonol stage to the anthocyanin is still not entirely clear but it appears that three or more enzymes are involved. During the first stage, the dihydroflavonols are reduced to flavan-3,4-*cis*-diols (leucoanthocyanidins) by *dihydroflavonol 4-reductase* (DFR). Further oxidation, dehydration and glycosylation of the different leucoanthocyanidins by *anthocyanidin synthase* and *anthocyanin glucosyltransferase* produce the corresponding brick-red pelargonidin, red cyanidin and blue delphinidin glucosides.

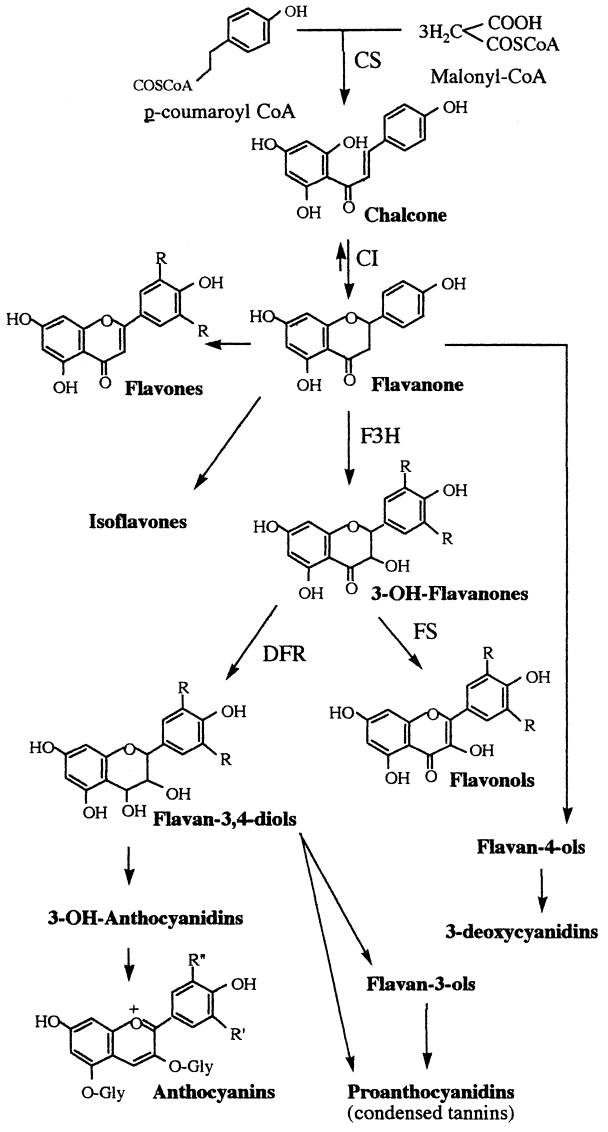

Fig. 2. Overall scheme for the biosynthesis of phenolic compounds. Abbreviations: enzymes CS: chalcone synthase; CI: chalcone isomerase; F3H: flavanone 3-hydroxylase; DRF: dihydroflavonol reductase: FS: flavonol synthase. Adapted from Cooper-Driver and Bhattercharya (1998).

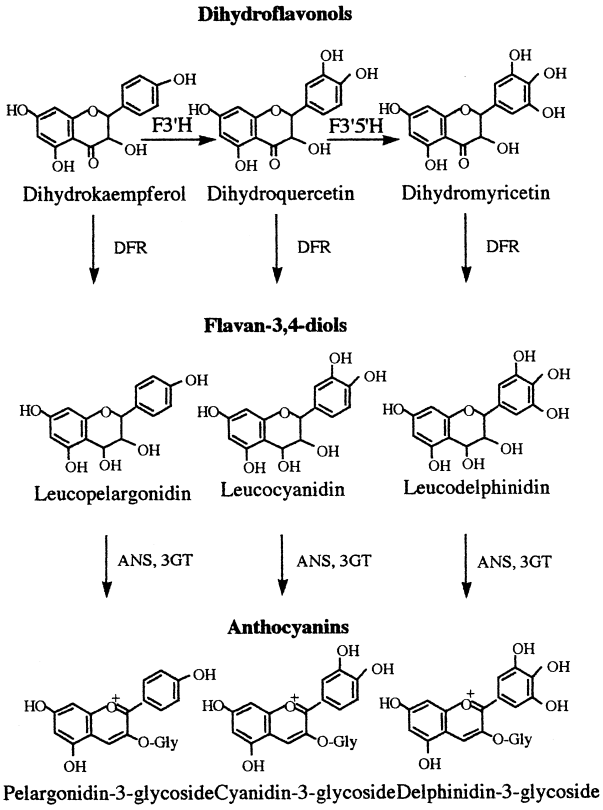

Fig. 3. Biosynthetic conversion of dihydroflavonols to anthocyanins. Abbreviations: enzymes F3′H: flavonoid 3′-hydroxylase; F3′5′H: flavonoid 3′,5′-hydroxylase; DFR: dihydroflavonol reductase: ANS: anthocyanidin synthase; 3GT: anthocyanin glucosyltransferase. Adapted from Holton and Cornish (1995).

Now that many of the genes encoding anthocyanin biosynthetic enzymes have been characterized and cloned from maize, snapdragon and petunia (i.e. chalcone synthase, chalcone isomerase, flavanone 3-hydroxylase, flavanoid 3′- hydroxylase, flavonoid 3′5′-hydroxylase, dihydroflavanol 4- reductase, anthocyanidin synthase, 3-glucosyltransferase, anthocyanin glucosyltransferases, and methyl transferases) transgenic approaches are now possible (Mol et al., 1998). This has allowed flower color to be altered in a highly directed fashion.

5.2. Creation of novel flower colors

The creation of novel flower colors in a number of commercially important species has been attempted by producing flowers with reduced pigmentation or conversely by increasing anthocyanin accumulation; introducing genes encoding novel enzyme activities or by the inactivation of endogenous genes.

5.2.1. Reduced pigmentation — antisense or co-suppression of CS genes

The key enzyme in the biosynthetic pathway that initiates the diversion of metabolic intermediates into pigment biosynthesis is chalcone synthase (CS) which catalyses the synthesis of narigenin from which all other flavonoids are derived. Antisense' or co-suppression of the CS genes have been described in petunia and in chrysanthemum (Courtney-Gutterson et al., 1994) resulting in flowers with reduced pigmentation.

5.2.2. Increased anthocyanin accumulation — co-suppression of an FS gene

As dihydroflavonols are the precursors of both anthocyanins and flavonols, the dihydroflavonols are potential substrates for the enzymes DFR, F3′H and F3′5′H, leading to pigment production, and/or for flavonol synthase (FS) leading to flavonol synthesis. Therefore competition between flavonoid-metabolizing

enzymes, FS and DFR for common dihydroflavonol substrates can shift the flavonol:anthocyanin ratio (Holton and Cornish, 1995). Co-suppression of the FS gene leads to increased anthocyanin accumulation.

5.2.3. New pigments — adding enzymes

In Petunias no pelargonidin derivatives are formed. This is due to the substrate specificity of the petunia dihydroflavonol 4-reductase (DFR) which is able to reduce dihydroquercetin and dihydromyricetin but is unable to reduce dihydrokaempferol. The same enzyme in *Zea mays* (maize) has a different substrate specificity since it is able to reduce the dihydrokaempferol substrate. A gene encoding the DFR from maize has been introduced into the petunia mutant — resulting in a change from white to strong brick-red coloration, due to the pelargonidin that is now being produced.

There is still a strong commercial interest in breeding stable blue flowers. Blue flowers are missing from a number of important ornamental plants, including carnations, chrysanthemums and roses (Holton and Tanaka, 1994). None of these plants is capable of synthesizing blue delphinidin pigments, presumably due to the absence of a gene encoding F3′5′H (see Fig. 4). A

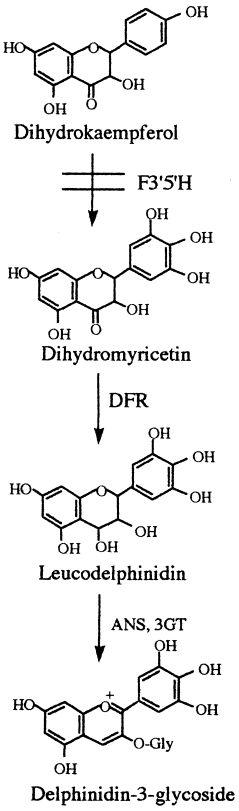

Fig. 4. Several commercially important plants lack the ability to synthesize delphinidin presumably due to an inability to convert dihydrokaempferol to dihydromyricetin. Abbreviations: enzymes as in Fig. 3.

possible novel way of breeding blue roses is suggested by Haslam (1993). As caffeine preferentially stabilizes the quinonoidal base form of anthocyanins, introducing the gene for caffeine synthesis clearly opens up another avenue for developing a blue rose and other blue flowers (Haslam, 1993). Blue flowers are of considerable interest as the presence of this color invariably involves some sort of unusual anthocyanin-copigment interaction and in several cases additional metallo-complexation (Bloor, 1999).

6. Concluding remarks

Currently there is a tremendous resurgence of interest in flavonoids generally and in anthocyanins in particular. Due to improved analytical techniques, namely HPLC, NMR and MS, many new anthocyanin structures are presently being recorded (Harborne and Williams, 1998). The majority of the new structures have both aliphatic (especially malonyl and acetyl) and aromatic (*p*-coumaroyl, feruloyl, caffeoyl and sinapoyl) substitution. A significant number of 3,7-disubstituted and 3,7,3′ trisubstituted pigments have also been described. The first macrocyclic anthocyanin has been isolated from flowers of two carnation cultivars (Bloor, 1998) a malic acid ester of cyanidin 3,5-diglucoside in which the malyl group is linked to both sugars through the 6-hydroxy position.

On the molecular side, current work is focusing on the genetics and regulation of anthocyanin biosynthesis, vacuolar transport mechanisms, the mechanisms by which regulatory genes determine tissue-specific patterns of pigmentation (Dooner et al., 1991), and ways to control vacuolar pH and co-pigmentation (Mol et al., 1998). Ecologically, we are only just beginning to recognize the important role anthocyanins play in growth, stress, and senescence.

Since the early 1950's, Jeffrey Harborne has made outstanding contributions to the study of anthocyanins (see selected references in this paper). Many of these papers have been published in the journal "*Phytochemistry*", thereby encouraging and shaping the work of future generations of phytochemists. We owe Jeffrey Harborne and all the other members of the Editorial Board of "*Phytochemistry*" a great debt for all the work they have done over the years in stimulating our interest in plants and their secondary metabolites. Perhaps for Jeffrey one day the dream of creating a blue rose may finally come true.

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References

Anderson, O.M., 1992. Anthocyanins from reproductive structures in Pinaceae. Biochem. Syst. and Ecol. 20, 145–148.

Bate-Smith, E.C., 1948. Paper chromatography of anthocyanins and related substances in plant extracts. Nature 161, 835–838.

Bate-Smith, E.C., Swain, T., Nordstrom, C.G., 1953. Chemistry and inheritance of flower colour in *Dahlia*. Nature 176, 1016–1018.

Benzing, D.H., Friedman, W.E., 1981. Patterns of foliar pigmentation in Bromeliaceae and their adaptive significance. Selbyana 5, 224–240.

Bloor, S.J., 1999. Novel pigments and co-pigmentation in the blue marguerite daisy. Phytochemistry 50, 1395–1399.

Bradshaw Jr., H.D., Wilbert, S.M., Otto, K.G., Schemske, D.W., 1995. Genetic mapping of floral traits associated with reproductive isolation in monkey flowers (*Mimulus*). Nature 376, 762–765.

Brouillard, R., Wigand, M.C., Dangles, O., Cheminat, A., 1991. PH and solvent effects on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine-derivatives. J. Chem. Soc., Perkin Trans. 2 (8), 1235–1241.

Cooper-Driver, G., Bhattacharya, M., 1998. Role of phenolics in plant evolution. Phytochemistry 49, 1165–1174.

Courtney-Gutterson, N., Napoli, C., Lemieux, C., Morgan, A., Firoozabady, E., Robinson, K., 1994. Modification of flower color in florists *Chrysanthemum*- production of a white- flowering variety through molecular-genetics. Biotechnology 12 (3), 268–271.

Dooner, H.K., Robbins, T.P., Jorgensen, R.A., 1991. Genetic and developmental control of anthocyanin biosynthesis. Ann. Rev. Genetics 25, 173–200.

Figueiredo, P., Elhabari, M., Toki, K., Saito, N., Dangles, O., Brouillard, R., 1996. New aspects of anthocyanin complexation. Intramolecular co-pigmentation as a means for colour loss? Phytochemistry 41 (1), 301–308.

Goodwin, T.W., 1988. Plant pigments. Academic Press, London.

Goto, T., Tamura, H., Kawai, T., Hoshino, T., Harada, N., Kondo, T., 1986. Chemistry of metalloanthocyanins. Annals of the New York Academy of Sciences 471, 155–173.

Gould, K.S., Kuhn, D.N., Lee, D.W., Oberbauer, S.F., 1995. Why leaves are sometimes red. Nature 378, 241–242.

Harborne, J.B., 1957a. Spectral methods for characterizing anthocyanins. Biochemical Journal 70, 22–28.

Harborne, J.B., 1957b. Variation in the glycosidic patterns of anthocyanins. Nature 179, 429.

Harborne, J.B., 1959. The chromatographic identification of anthocyanin pigments. Chromatographic Reviews 1, 209–224.

Harborne, J.B., 1962. Anthocyanins and their sugar components. Fortschritte Chemie Organ. Naturst. 20, 165–199.

Harborne, J.B., 1963a. The glycosidic pattern of anthocyanin pigments. Phytochemistry 2, 85–97.

Harborne, J.B., 1963b. Distribution of anthocyanins in higher plants. In: Swain, T. (Ed.), Chemical Plant Taxonomy. Academic Press, London, pp. 359–388.

Harborne, J.B., 1964a. The systematic distribution and origin of anthocyanins containing branched trisaccharides. Phytochemistry 3, 453–463.

Harborne, J.B., 1964b. The structure of acylated anthocyanins. Phytochemistry 3, 151–160.

Harborne, J.B., 1965a. Anthocyanins in ferns. Nature 207, 984.

Harborne, J.B., 1965b. Flavonoids: distribution and contribution to flower colour. In: Goodwin, T.W. (Ed.), Chemistry and Biochemistry of Plant Pigments. Academic Press, London, pp. 247–278.

Harborne, J.B., 1965c. Flavonoid pigments. In Bonner, J., Varner, R. (Eds), *Plant Biochemistry,* 2nd Edition. Academic press, London, pp. 618–640.

Harborne, J.B., 1976. A unique pattern of anthocyanins in *Daucus carota* and other Umbelliferae. Biochem. Syst. and Ecol. 4, 111–115.

Harborne, J.B., 1980. Flavonoid pigments. In: Rosenthal, G.A., Janzen, D.H. (Eds.), Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, pp. 619–655.

Harborne, J.B., 1986a. Identification and distribution of malonated anthocyanins in plants of the Compositae. Phytochemistry 25, 1337–1342.

Harborne, J.B., 1986b. The natural distribution in angiosperms of anthocyanins acylated with aliphatic dicarboxylic acids. Phytochemistry 25, 1887–1894.

Harborne, J.B., 1988a. Pelargonidin 3-(6''-succinyl glucoside)-5-glucoside from pink *Centaurea cyanus* flowers. Phytochemistry 27, 1228–1229.

Harborne, J.B., 1988b. The anthocyanins. In: Harborne, J.B. (Ed.), The flavonoids. Academic Press, London, pp. 1–20.

Harborne, J.B., 1990. Malonylated anthocyanins and related flavonoids. Proceedings of the 15th International Conference of the Groupes Polyphenols, pp. 15–24

Harborne, J.B., 1992a. Chemistry of flower colour in the Lamiales. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens, Kew, England, pp. 307–314.

Harborne, J.B., 1992b. Flavonoid pigments. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. 1, The chemical participants. Academic Press, New York, pp. 389–427.

Harborne, J.B., 1993. Biochemistry of plant pollination. Introduction to Ecological Biochemistry, 4th Edition. Academic Press, London, pp. 36–70.

Harborne, J.B., 1996. Chemotaxonomy of anthocyanins and phytoalexins in the Compositae. In: Hind, D.J.N., Beentje, H.J. (Eds.), Proceedings of the International Compositae Conference, Kew, 1994, Royal Botanic Gardens, Kew, vol. 1, pp. 207–218.

Harborne, J.B., Boardley, M., 1985. The widespread occurrence in nature of anthocyanins as zwitterions. Zeitschrift für Naturforschung 40, 305–308.

Harborne, J.B., Clifford, H.T., 1967. Anthocyanin composition of and distribution in the Poaceae (Gramineae). Proceedings of the Linnean Society of London 178, 125.

Harborne, J.B., Rowley, G.D., 1958. In quest of blue roses. Gardeners Chronicle, Gardening Illustrated 143, 427.

Harborne, J.B., Self, R., 1987. Malonated cyanidin 3-glycosides in *Zea mays* and other grasses. Phytochemistry 26, 2417–2418.

Harborne, J.B., Smith, D.M., 1978. Correlations between anthocyanin chemistry and pollination ecology in the Polemoniaceae. Biochem. Syst. and Ecol. 6, 127–130.

Harborne, J.B., Stirton, J.Z., 1980. Two distinctive anthocyanin patterns in the Commelinaceae. Biochem. Syst. and Ecol. 8, 285–287.

Harborne, J.B., Turner, B.L. 1984. Plant pigments. Plant Chemosystematics. Academic Press, London, pp. 128–179.

Harborne, J.B., Williams, C.A., 1995. Anthocyanins and other flavonoids. Natural Products Reports 12, 639–657.

Harborne, J.B., Williams, C.A., 1998. Anthocyanins and other flavonoids. Natural Products Reports 15, 631–652.

Harborne, J.B., Boulter, D., Turner, B.L. (Eds.). 1970. Chemotaxonomy of the Leguminosae. Academic Press, London, p. 612.
Harborne, J.B., Crowden, R.K., Wright, J., 1976. Anthocyanins of *Fuchsia* (Onagraceae). Phytochemistry 16, 400–402.
Harborne, J.B., Geissman, T.A., Jorgensen, E.C., 1953. The effect of aluminium chloride on absorption spectra of anthocyanins. Chem. and Ind. p.1389.
Harborne, J.B., Takeda, K., Self, R., 1986a. Identification of malonated anthocyanins in the Liliaceae and Labiatae. Phytochemistry 25, 2191–2192.
Harborne, J.B., Takeda, K., Waterman, P.G., 1993. Malonylated flavonoids and blue flower colour in Lupin. Phytochemistry 34, 421–423.
Harborne, J.B., Thomas-Barberan, F.A., Self, R., 1987. Dimalonated anthocyanins from the flowers of *Salvia splendens* and *S. coccinea*. Phytochemistry 26, 2759–2760.
Harborne, J.B., Wilken, D.H., Smith, D.H., 1982. Flavonoid and anthocyanin patterns and the systematic relationships in the *Collomia*. Biochem System and Ecol. 10, 239–243.
Harborne, J.B., Williams, C.A., Eagles, J., 1989b. Leaf flavonoid diversity in the Australian genus *Patersonia*. Phytochemistry 28, 1891–1896.
Harborne, J.B., Williams, C.A., Mayo, S.J., 1981. Anthocyanin pigment and leaf flavonoids in the family Araceae. Phytochemistry 20, 217–234.
Harborne, J.B., Takeda, K., Enoki, S., Eagles, J., 1989a. Malonated anthocyanins in Malvaceae: malonymalvin from *Malvia sylvestris*. Phytochemistry 28, 499–500.
Harborne, J.B., Terahara, N., Yamaguschi, M.A., Takeda, K., Self, R., 1986b. Anthocyanins acylated with malic acid in *Dianthus caryophyllus* and *D. deltoides*. Phytochemistry 25, 1715–1717.
Haslam, E. 1993. Nature's palette. Chemistry in Britain, October, 875–878.
Haslam, E., 1995. Fruit and floral pigmentation. Rev. Prog. Coloration 25, 18–28.
Haslam, E., 1998. Practical polyphenolics: from structure to molecular recognition and physiological action. Cambridge University Press, Cambridge.
Holton, T.A., Cornish, E.C., 1995. Genetics and biochemistry of anthocyanin biosynthesis. The Plant Cell 7, 1071–1083.
Holton, T.A., Tanaka, Y., 1994. Blue roses — a pigment of our imagination. Trends Biotechnol. 12, 40–42.
Lee, D.W., Lowry, J.B., Stone, B.C., 1979. Abaxial anthocyanin layer in leaves of tropical rain forest plants: enhancer of light capture in deep shade. Biotropica 11, 70–77.
Markham, K.R., 1988. Distribution of flavonoids in the lower plants and its evolutionary significance. In: Harborne, J.B. (Ed.), The flavonoids, advances in research since 1980. Academic Press, London, pp. 427–468.
Markham, K.R., 1990. Bryophyte flavonoids, their structures, distribution and evolutionary significance. Proc. Phytochem. Soc. Eur. 29, 143–159.
Mistry, T.V., Cai, Y., Lilley, T.H., Haslam, E., 1991. Polyphenol interactions. Part 5. Anthocyanin co-pigmentation. J. Chem. Soc. Perkin Trans. 2, 1287–1296.
Mol, J., Grotewold, E., Koes, R., 1998. How genes paint flowers and seeds. Trends in Plant Sciences 3, 212–217.
Saito, N., Harborne, J.B., 1992. Correlations between anthocyanin type, pollinator and flower colour in the Labiatae. Phytochemistry 31, 3009–3015.
Scott-Moncrieff, R., 1981. Notes Rec. Royal Society of London, 36, 125.
Takeda, K., Harborne, J.B., Self, R., 1986. Identification and distribution of malonated anthocyanins in plants of the Compositae. Phytochemistry 25, 1337–1342.
Vickery Jr., R.K., 1995. Speciation in *Mimulus*, or can a simple flower color mutant lead to species divergence? Great Basin Nat. 55, 177–180.
Wang, H., Nair, M.G., Strasburg, G.M., Chang, Y-C., Booren, A.M., Gray, J.I., DeWitt, D.L., 1999. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin from tart cherries. J. Nat. Prod. 62, 294–296.
Weiss, M.R., 1991. Floral changes as cues for pollinators. Nature 354, 227–229.
Willson, M.F., Whelen, C.J., 1990. The evolution of fruit color in fleshy-fruited plants. Am. Nat. 136, 790–809.
Yamasaki, H., 1997. A function of color. Trends in Plant Sciences 2, 7–9.

Gillian Cooper-Driver graduated from University College London in 1955 with a BSc Honours in botany and subsidiary chemistry. She subsequently completed an MSc in microbiology and moved to the USA to work with F.C. Steward at Cornell University, New York State, USA on carrot tissue culture. After a period spent raising a family, Gillian returned to botanical work with the Phytochemical Unit, Agricultural Research Council, at the Royal Botanic Gardens, Kew headed by the phytochemist, Tony Swain. While at Kew, she completed her PhD, University of London, working on the chemotaxonomy of ferns and the chemical ecology of bracken, focusing on phenolic compounds. During her time at Kew, she developed and consequently maintained close ties with Jeffrey Harborne and Christine Williams at Reading University. In 1976, Gillian moved to the USA to take up an academic position at Boston University. For the next 15 years, she continued to study the chemical ecology of ferns in nearby Natick, Massachusetts and in the White Mountains of New Hampshire funded by the National Science Foundation and by Earthwatch. Her research focused on environmental influences on chemical defense strategies in ferns against insect herbivores and fungal pathogens. She also continued to have a strong interest in the biochemical evolution of phenolic compounds. In 1992, Gillian was at last able to fulfill her life long ambition to spend some time in southern Africa and through Fulbright Fellowships was able to work both as a Visiting Professor at the National University of Lesotho and later at the National Botanical Research Institute in Namibia. During this period her research interests became focused on factors affecting fungal infection of the seeds of the unique gymnosperm, *Welwitschia mirabilis,* in the Namib desert. She is currently on a 2 year contract in southern Africa at the University of Lesotho and plans to spend summer and winter breaks continuing her *Welwitschia* research.