

Painting the green canvas: how pigments produce flower colours

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Flowering plants are characterized by the production of striking flower colours and these colours are primarily caused by the accumulation of pigments in cells of the floral organs. The extraordinary array of colours displayed in flowers relies on four main pigment groups: chlorophylls, carotenoids, flavonoids and betalains. With thousands of different compounds, flavonoids are the most diverse and widespread pigment group. They include coloured anthocyanins, aurones and chalcones, as well as many flavonoid compounds such as flavones and flavonols that are invisible to humans, but visible to most pollinators since they absorb ultraviolet light (UV). Flowers may exhibit homogenous colours produced by only one type of pigment or extremely complex colour patterns caused by the accumulation of several types of pigments in the same or in different floral organs. Here, we review the ecological biochemistry of pigments affecting flower colour. We also present data of flower colour variation and provide future research directions guided by the physiological functions of floral pigments.

Introduction

The colours of flowers have revolutionized art, history and science alike. From the colour-inspired paintings of Claude Monet at Giverny to the Victorian-era Language of Flowers where flower colour was used to send stealth messages of love and political allegiance, the palette of the petals has played an influential role in many aspects of European history. In science, Mendel used flower colour to reveal the particulate laws of inheritance, Wright used flower colour as an empirical example in the development of population genetic theory and Fire and Mello borrowed an example of a flower colour change caused by small interfering RNA molecules that turn purple morning glories white when they pioneered RNAi in *Caenorhabditis elegans*.

Our fascination with flower colours is driven by the diversity of underlying pigments. Each of the four floral whorls can be coloured, from the calyx to the pistil. Although coloured nectar and pigmented pollen are botanical novelties, it is the painting of the petals that garners most of the attention. The vast range of petal colours contrast with the typically green background offering honest signals by the plant inviting pollinators in for a visit – a critical step in reproduction in most flowering plants.

Colour perception rests in the mind of the observer and is the product of the wavelengths of light reflected from the petal, the recipient's visual system and the light environment-specific context (distance, lightness and

visual background). Humans perceive only the part of the electromagnetic spectrum ranging from approximately 400 to 700 nm, which is called visible light. In this range we distinguish an array of colours from violet to red. Although the shape of petal cells may alter the wavelengths of reflected light, and thus the perceived colour (i.e., structural colour), flower colour is mainly controlled by the type and amount of various pigments; this is why flower colour is considered primarily pigmentary. Pigments are chemical compounds that, when exposed to light, can absorb certain wavelengths and reflect the remaining wavelengths. Ultimately, the specific wavelength that the pigments absorb depends on its chemical structure.

Extraordinary flower colour diversity produced by just four groups of pigments

Most of the pigments found in flowering plants belong to four major groups: chlorophylls, carotenoids, flavonoids and betalains (Figure 1). First, chlorophylls are the primary photosynthetic pigments and absorb blue and red wavelengths of light. The presence of chlorophyll in flowers is relatively rare and largely restricted to a few lineages (e.g., some species of *Aristolochia*, *Euphorbia*, *Fritillaria*, *Hedera* and *Ophrys*). Second, carotenoids are omnipresent in flowering plants. They are organic pigments of the isoprenoids group and absorb mainly blue wavelengths of

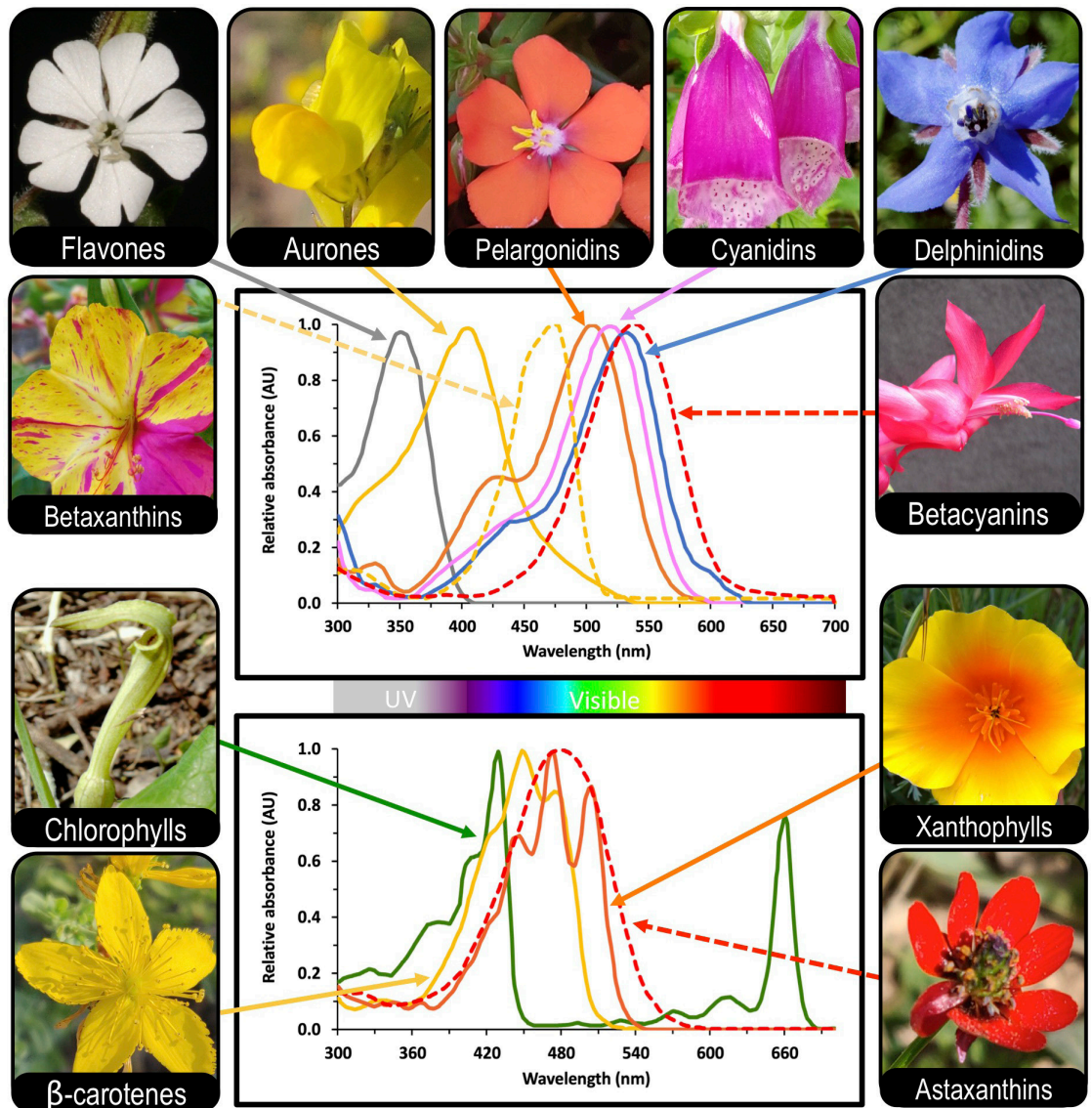


Figure 1. Absorbance spectra of the main groups of flower pigments and examples of flowers containing these pigments. Aurones and flavones are UV-absorbing flavonoids; pelargonidins, cyanidins and delphinidins are anthocyanins (flavonoids); betaxanthins and betacyanins are betalains; and xanthophylls, β -carotenes and astaxanthins are carotenoids. For chlorophylls, the absorbance spectrum of chlorophyll *a* is represented. The flower colour produced by each pigment is more diverse than those shown in these example species. Species names from top right to bottom left: *Silene littorea* (white-flowered morph, Caryophyllaceae), *Linaria spartea* (Plantaginaceae), *Lysimachia monelli* (Primulaceae), *Digitalis purpurea* (Scrophulariaceae), *Borago officinalis* (Boraginaceae), *Mirabilis jalapa* (Nyctaginaceae), *Schlumbergera x buckleyi* (Cactaceae), *Aristolochia paucinervis* (Aristolochiaceae), *Hypericum perforatum* (Hypericaceae), *Eschscholzia californica* (Papaveraceae) and *Adonis annua* (Ranunculaceae). AU, absorbance units.

light with a characteristic three-peaked absorbance spectrum (Figure 1). Carotenoids can paint flowers yellow to orange (and rarely red) and are the primary petal pigments in many yellow species of the Asteraceae and Leguminosae families. Third, the most diverse palette of pigments is the flavonoids, water-soluble polyphenols found in nearly all vascular plants. Anthocyanins primarily absorb green wavelengths of light and reflect shades of purple, blue, pink and red colours.

Other flavonoid groups such as catechins, flavonols, flavones, isoflavones and flavanones exclusively absorb in the UV part of the spectrum; thus, these 'UV-absorbing flavonoids' are invisible to humans but distinguished by many pollinator groups (bees, flies, butterflies and most birds). The flavonoids aurones and chalcones, and some glycosylated flavonols, absorb in the UV-blue range of the spectrum and produce tawny to yellow colours. Fourth, the betalains are

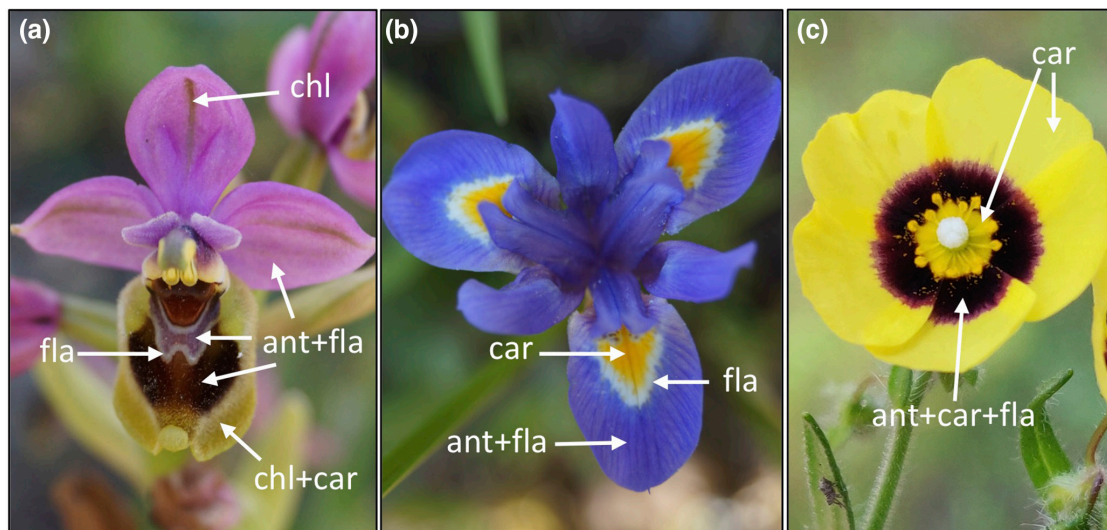


Figure 2. Examples of flowers with complex colour patterns accumulating several types of pigments. **(a)** *Ophrys tenthredinifera* (Orchidaceae), **(b)** *Moraea sisyrinchium* (Iridaceae) and **(c)** *Tuberaria guttata* (Cistaceae). Letters indicate the main pigment groups accumulated in each flower part listed in order of abundance. ant, anthocyanins; fla, UV-absorbing flavonoids; chl, chlorophylls; car, carotenoids.

nitrogen-containing compounds that produce yellow to pink and red colours and are restricted to some families of Caryophyllales, such as cactus (Cactaceae) and four o'clocks (*Mirabilis jalapa*). Interestingly, species producing betalains do not produce anthocyanins; this mutual exclusion of both pigments is probably caused by competition for the common substrate arogenate, among other factors. In addition to these major pigment groups, there are other compounds that are extremely rare in wild plants (e.g., quinochalcones in yellow and red flowers of *Carthamus tinctorius*, xanthones in some species of irises and gentians).

Pigments in flowers: alone, mixed or layered?

A change in the specific type of pigment or in the mixing ratio of various pigments would affect the resulting colour (i.e., hue), whereas changes in the concentration of the compounds would mainly affect the colour intensity (i.e., colour saturation or purity). Flowers may show from simple, monochromatic colours to extremely complex colour patterning, as frequently occurs in species of orchids, irises, milkweeds or crowfoots (Figure 2). Complex colour patterns can be produced by the precise and orchestrated regulation of pigments biosynthesis in different parts of the petal (e.g., bullseye, stripes, picotee).

Similar flower colours can be generated by the accumulation of a unique type of pigment or by the combination of more than one pigment group. For instance, red coloration in petals may be due to the unique presence of red anthocyanins, red betalains or red carotenoids, or by the combination of purple anthocyanins with orange

carotenoids. Pigments are generally located in the epidermal and mesophyll layers of the petal's anatomy. The combination of different pigment types in the same cell may occur due to their accumulation in different cell organelles. Carotenoids and chlorophylls are lipid-soluble molecules and are stored in chromoplasts and chloroplasts of petal cells, respectively. Flavonoids and betalains are water-soluble molecules that mainly accumulate in vacuoles; thus, anthocyanins and other UV-absorbing flavonoids may be mixed in the same vacuoles. White petals typically reflect all wavelengths of visible light due to the absence of coloured pigments; however, these petals usually contain high concentrations of UV-absorbing flavonoids such as flavones or flavonols. Similarly, nectar guides (i.e., spatial cues to guide pollinators to rewards) are often invisible to humans, but visible to some insects because they are caused by an accumulation of UV-absorbing flavonols and flavones (Figure 3).

Anthocyanins as a model for studying flower colour *plein air*

Anthocyanins, from the Greek *anthos* (flower) and *kyanos* (blue), are without doubt the pigments that provide the greatest variety of floral hues. Anthocyanins can produce most colours of the visible spectrum, including some of the rarest colours such as turquoise (e.g., *Puya alpestris*) or black (e.g., *Streptanthus niger*). Anthocyanins are glycosylated forms of anthocyanidins, the core pigment structure. There are more than 600 anthocyanins described in vascular plants, and most of them are derived from six types of anthocyanidins: cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin (Figure 4). Most anthocyanins undergo subsequent

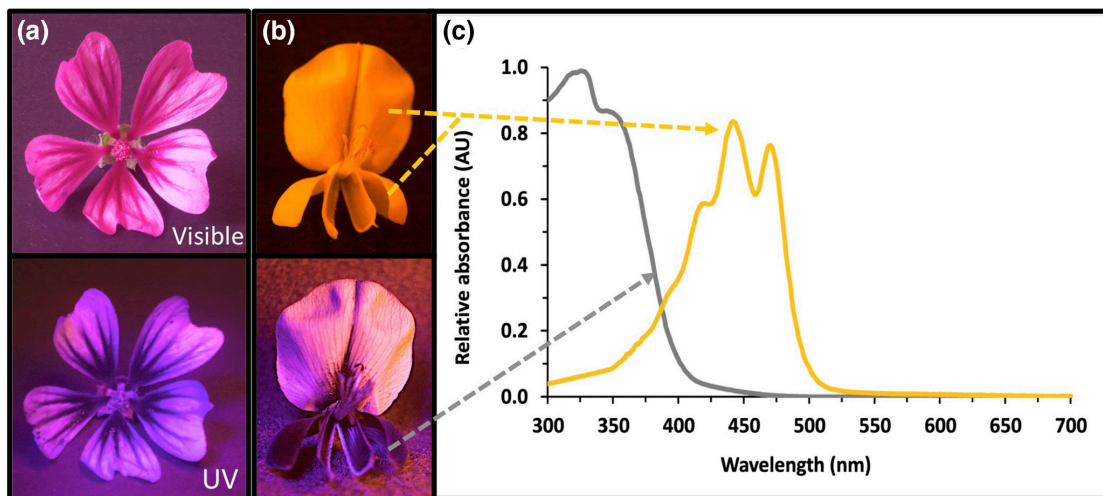


Figure 3. Examples of flowers with nectar guides. (a) *Malva sylvestris* (Malvaceae) shows dark pink radial lines that are visible for humans; in UV photography lines are also darker due to the presence of UV-absorbing flavonoids. (b) *Spartium junceum* (Fabaceae) shows yellow carotenoids in all petals; under UV photography, banner is UV-reflecting whereas keel and wings are UV-absorbing due to the presence of UV-absorbing flavonoids. (c) Absorbance spectra of banner (yellow line) and wings (grey line) extracts.

chemical decorations by adding a variety of glycosylations and acylations. Furthermore, metals or other flavonoids such as flavones and flavonols can act as copigments (weakly coloured substances that bind to anthocyanins to stabilize and reinforce their colour) further increasing the variety of colours – often enhancing the colouration. And, if that isn't enough modification, anthocyanin colour depends on the pH of the solution; in acid conditions the dominant flavylium cation provides red colour, but at higher pH the colour changes to blue due to quinonoidal forms. Thus, the pH of the cell can modify, at least theoretically, the chemical structure of the anthocyanins, producing drastic changes in colour. Finally, the epidermal cell shape through which the light enters and is reflected back to the observer can further affect coloration. Thus, there is an almost infinite palette of colour created by the variety of anthocyanin types in combination with multiple layers of decorations and the physical and chemical environment in which the pigments reside.

Genetically, most anthocyanin-based flower colours are extremely tractable to study. The anthocyanin biosynthetic pathway (ABP) is one of the most thoroughly studied and largely conserved biochemical pathways for secondary metabolites in plants. The ABP is one route through the broader set of flavonoid biosynthetic pathways and is fed from the phenylpropanoid pathway (producing lignin) by means of the first dedicated step involving chalcone synthase (Figure 4). At least six core enzymes are involved in anthocyanin biosynthesis and some of them also serve as branch points for the production of other flavonoids. There are three main routes down the ABP: directly producing pelargonidin or taking a side branch using

two additional enzymes (flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase) can add hydroxyl groups to one of the rings creating cyanidin and delphinidin. The other anthocyanins are created through methylation of the anthocyanin chemical structure. For example, peonidin is the methylated form of cyanidin producing pink to red pigments and petunidin and malvidin are methylated forms of delphinidin and create a variety of blue hues depending on their subsequent decorations. The activity of these six core enzymes is mainly controlled by tissue-specific expression conferred by a regulatory complex of three types of proteins: R2R3-MYB (MYB), basic helix-loop-helix (bHLH) and WD40. These transcription factors may regulate the activity of one or several structural genes, yet the exact steps in the ABP that are controlled by each protein can be cell- or tissue-specific.

The changing colours of flowers

Most angiosperm species exhibit a distinctive, constitutive flower colour that is stable within and among individuals regardless of the environment. However, sometimes flower colours of the same species vary across the landscape or even change colours over time. Thus, petal colours may change over a flower's lifespan, becoming darker or even changing the colour. For instance, flowers of *Brunfelsia pauciflora* (yesterday-today-and-tomorrow) change from dark purple to pure white caused by the active process of anthocyanin degradation (Figure 5A). In some studied cases, this colour change may be related to pollinator attraction (pinkifying of white petals after pollination in many redwood forest herbs

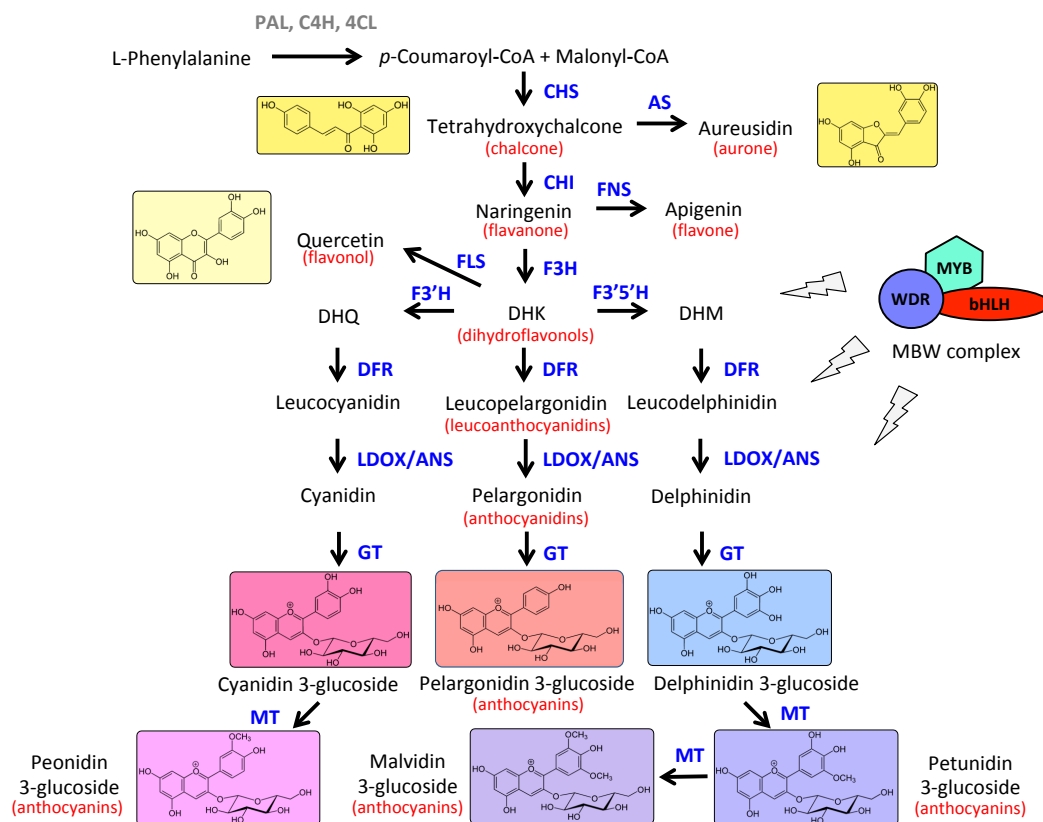


Figure 4. Simplified flavonoid biosynthetic pathway leading to the biosynthesis of anthocyanins and other flavonoids groups acting as flower pigments. Main enzymatic activities (blue capital letters next to arrows), flavonoid subclasses (red letters) and metabolic products are indicated. In the boxes, molecular structures of flavonoids and the approximate colours in flowers are shown. CHS, chalcone synthase; AS, aureusidin synthase; CHI, chalcone isomerase; FNS, flavone synthase; F3H, flavanone-3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LDOX, leucoanthocyanidin dioxygenase; GT, glucosyl transferase; MT, methyl transferase. The MBW regulatory complex consists of a basic helix–loop–helix protein (bHLH), WD Repeats (WDR) and R2R3-MYB domains (MYB). This regulatory complex is the most common in angiosperms.

like *Trillium ovatum*). Instead of a single flower changing colour, *Moricandia arvensis* produces UV-reflecting lilac flowers in spring, but switches to UV-absorbing white flowers in summer.

Another phenomenon is flower colour variation among individuals within a population or among populations. This variation may be discrete, creating two or more distinct colour morphs (aka a flower colour polymorphism like in *Lysimachia arvensis*) or the variation could be more of a continuous gradient (such as that found in *Silene littorea* and *Parrya nudicaulis*). In terms of pigment production, flower colour polymorphisms can be caused by changes in pigment concentration, leading to a gradient of flower colour intensity or total lack of pigments (e.g., dark pink through light pink to white petals of *Silene littorea*). Flower colour polymorphisms can also be caused by variation in the layers of multiple pigment types, producing flowers with different colours. For example, in *Raphanus sativus*, petal colour is determined by the presence or absence of

anthocyanins and carotenoids. Since each pigment group is controlled independently, the mixing and matching of anthocyanins and carotenoids produce four main colour morphs: bronze (anthocyanins and carotenoids), pink (only anthocyanins), yellow (only carotenoids) and white (absence of both pigments; Figure 5B). Interestingly, not all types of flower colour polymorphisms are equally frequent. In the flora of the Iberian Peninsula and the British Isles, flower colour polymorphisms involving the loss of pigments (i.e., white colours) are more frequent than those caused by changes in pigment type.

Future prospects: more than meets the eye

In many cases, flower colours have clearly been painted by the preferences of their animal pollinators. However, this is not always the case and may be the exception rather than the

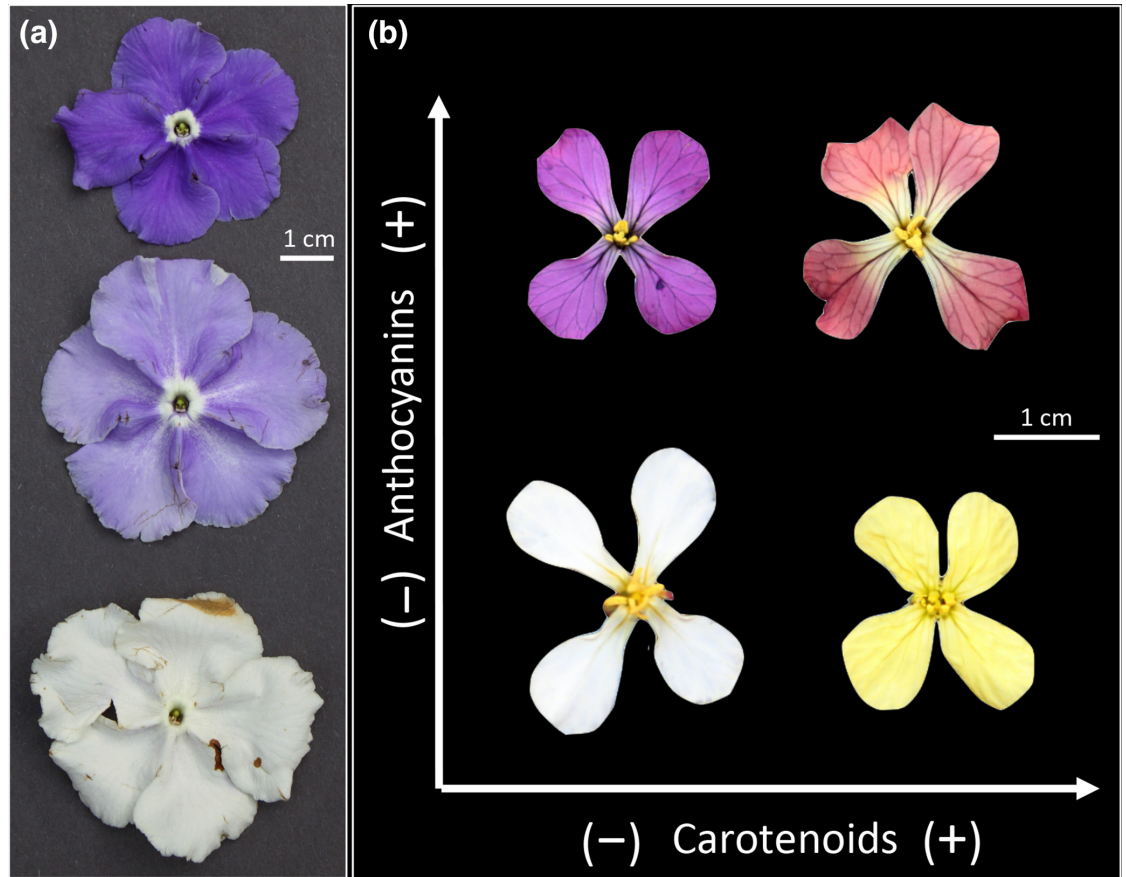


Figure 5. Examples of species showing variation in petal colour. (a) *Brunfelsia pauciflora* (Solanaceae) changes petal colour during the flower's lifespan from dark purple to lilac to pure white (yesterday-today-and-tomorrow). (b) *Raphanus sativus* (Brassicaceae) exhibits a petal colour polymorphism, with plants producing one of the four possible types of flowers depending on the independent presence (+) or absence (-) of anthocyanins and carotenoids.

rule. We now know that there is a balance (or tug-of-war) between pollinator attraction and the physiology of these metabolic sinks (petals do not produce photosynthate, but still respire). For example, carotenoids, betalains and flavonoids all have antioxidant activities, which help cells prevent cellular damage. The contrasting roles of the underlying pigments of flower colours are captured by the anthocyanins and UV-absorbing flavonoids. Both of these pigments have been demonstrated to confer tolerance to a wide range of abiotic and biotic stresses such as cold, UV radiation, drought, salinity, herbivory and pathogens. Furthermore, small changes in anthocyanin decoration not only stabilize and intensify the colour, but simultaneously provide physiological protection from numerous environmental stresses that plants face. Although most studies addressing the non-pollinator functions of flavonoid pigments focus on vegetative tissues, evidence is mounting that pigments and their precursors play similar roles in floral tissues which although ephemeral are still exposed to stressful conditions both physiologically and ecologically.

Here, we have summarized only a piece of the huge puzzle on the ecological biochemistry of floral pigments. Although the propriety of flower pigments to paint the green canvas has unquestionably dazzled both pollinators and humans, it is only recently that we are beginning to understand some of the overlooked effects of pigments to cope with environmental stresses. With the help of new molecular, biochemical and data analysis techniques, we are beginning to unravel processes that have long concerned early plant biochemists. For example, in 1916, Muriel Wheldale remarked “The function of anthocyanins in rendering the corolla, perianth, bracts and other parts of the inflorescence attractive to insects may be regarded as their biological significance... Several physiological functions have been assigned to anthocyanins... Though each hypothesis has been closely criticised by the various investigators of the problem, the final issue is far from being complete and satisfactory” (*The Anthocyanin Pigments of Plants*, 1916). ■

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José Carlos del Valle is a postdoctoral researcher from the Vegetal Biology and Ecology Department at the University of Seville (Spain). His research is mainly framed in the field of evolutionary biology. In particular, his studies were mainly focused on the study of the accumulation patterns of flavonoids in reproductive and vegetative tissues of plants, as well as the plasticity of this phenotypic response and their genetic bases. He has been conducting other research in distinct topics in the field of evolutionary biology, including the study of flower colour polymorphism, population genetic and phylogeographic studies. Email: jvgarcia@us.es



Justen B. Whittall is a professor in the Department of Biology at Santa Clara University in Silicon Valley, California, USA. There, he studies flower colour from many angles – ecological, evolutionary, genetic and biochemical – with the help of his undergraduate research assistants. His flower colour studies have included examples from *Aquilegia*, *Raphanus*, *Parrya* and *Streptanthus* along with collaborations with Drs Del Valle and Narbona on *Silene littorea* and *Lysimachia arvensis*. Email: jwhittall@scu.edu