Plant Pigments and their Manipulation

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# Plant Pigments and their Manipulation

Edited by

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# Preface

It is difficult to overstate the importance of plant pigments in biology. Chlorophylls are arguably the most important organic compounds on earth, as they are required for photosynthesis. Carotenoids are also necessary for the survival of both plants and mammals, through their roles in photosynthesis and nutrition, respectively. The other plant pigment groups, such as flavonoids and betalains, also have important roles in both the biology of plants and the organisms with which plants interact. For example, the flavonoids (both pigmented and non-pigmented) serve biological functions in plants as diverse as signalling to micro-organisms, protecting against pathogens, ameliorating biotic and abiotic stress, influencing auxin transport, enabling plant fertility and providing visual signals to insects and animals for pollination and seed dispersal.

Human use of plant pigment extracts dates back 10 000 years, and it is thus not surprising that plant pigmentation is one of the oldest subjects in formal plant science. The conspicuousness of floral colour traits and the easy identification of mutants have led to many discoveries with impacts much wider than in the pigmentation field alone, from the studies of Mendel, Darwin, McClintock and others. The structural diversity of plant pigments has attracted chemists since the beginning of the twentieth century, and for carotenoids and anthocyanins well over 600 individual structures have now been defined for each class. Given the wealth of data on plant pigments, this volume of *Annual Plant Reviews* cannot hope to be exhaustive on the subject. Rather we have tried to provide an overview of pigment chemistry and biology, and an up-to-date account of pigment biosynthesis and the modification of their production using biotechnology.

Chapter 1, the introduction, presents an overview of pigmentation and some of the general functions of plant pigments. Following this are Chapters 2 to 6, covering the major plant pigment groups: chlorophylls, carotenoids, flavonoids (the coloured flavonoids of flowers and tannins) and betalains. Chapter 7 presents an overview of pigments outside these groups that are of particular significance in biology or commerce. In addition to these, there are three chapters focusing on research areas that are common across the pigment groups. Chapters 8 and 9 provide comprehensive reviews of two areas of particular current interest – the roles of plant pigments in human health and the amelioration of the effects of UV radiation, respectively. Chapter 10 provides an overview of modern techniques used for the extraction, separation, identification and quantification of the major pigment classes.

#### PREFACE

Together the chapters cover a wide scope of pigmentation research, from the importance of structural diversity in generating the range of colours seen in plants through to improving human health properties of crops by increasing pigment levels in transgenic plants. We hope that this volume will be of use to researchers, professionals and advanced students in both the academic and industry sectors. The contributors come from Australasia, Europe and the USA, and it is my pleasure as the Editor to thank them all for the time they have put into preparing chapters of a consistently high standard. I am also grateful to Graeme MacKintosh and David McDade at Blackwell Publishing for their assistance in preparing the manuscript. Finally, my thanks to all the present and past members of the Plant Pigments Group of Crop & Food Research for their hard work on flavonoid biosynthesis over many years, to our long-term collaborators Cathie Martin and Ken Markham for their encouragement of our group, and to Crop & Food Research for this book.

Kevin M. Davies

# 1 An introduction to plant pigments in biology and commerce

Kevin M. Davies

# 1.1 Introduction

This introductory chapter presents a general overview of plant pigmentation, together with some general functional and economic aspects not covered in detail in the chapters on specific pigment groups.

# 1.2 Plant pigmentation

# 1.2.1 The physical basis of pigmentation

Plant pigmentation is generated by the electronic structure of the pigment interacting with sunlight to alter the wavelengths that are either transmitted or reflected by the plant tissue. The specific colour perceived will depend on the abilities of the observer. Humans without colour blindness can detect wavelengths between approximately 380 and 730 nm, representing the visible spectrum of red, orange, yellow, green, blue, indigo and violet. So chlorophyll with peak absorbancies at 430 and 680 nm will leave wavelengths forming a green colour. Of course, often the colours are the result of a mix of residual wavelengths; for example, anthocyanins absorbing yellow-green light wavelengths of 520–530 nm will generate mauve colours formed by the reflection of a mix of orange, red and blue wavelengths. Thus the pigments can be described in two ways: the wavelength of maximum absorbance ( $\lambda_{max}$ ) and the colour perceived by humans. Further details of the generation of colours and the behaviour of light in plant tissues can be found in Hendry (1996) and Chapter 10 of this volume.

The names of many common pigments convey little information to the general reader, as they tend to reflect historical discoveries rather than a set naming system. For example, carotene was first isolated from *Daucus carota* (carrot), violaxanthin from *Viola tricolor* (pansy), and the common anthocyanidins, pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin from *Pelargonium, Centaurea, Paeonia, Delphinium, Petunia* and *Malva*, respectively. However, these trivial names are often well-established, familiar to workers in the field and allow easy flow of text. Thus, the trivial names are used extensively in this book. More complete names, giving details of constitution and stereochemistry, have been developed for many compounds to meet the standards of the

International Union of Pure and Applied Chemistry (IUPAC) and International Union of Biochemistry (IUB) (Weedon & Moss, 1995). The details of such nomenclature, and lists of the IUPAC semi-systematic names, are available for some plant pigment groups in more specialised publications (Pfander, 1987; Harborne, 1988, 1994; Kull & Pfander, 1995; Weedon & Moss, 1995). Bohm (1998) gives a guide to relating the trivial flavonoid names to those in the *Chemical Abstracts*. In the same book he also provides a table listing the meanings of the trivial names often used for flavonoid di- and trisaccharides (e.g. sophoroside). The trivial names in this book are often accompanied by structural diagrams of the compounds, providing much of the information that would come from the full name. These diagrams commonly include basic representations of the stereochemistry, with a solid triangle for a bond representing above the plane of the paper and a dashed triangle for below the plane of the paper.

## 1.2.2 Structural variation of plant pigments

Plant pigments exist in many varied forms, some with highly complex and large structures. For example, over 600 naturally occurring carotenoid structures have been identified (Britton *et al.*, 1995) and over 7000 flavonoids, including over 500 anthocyanins (Chapter 10). The complexity of some pigments is well illustrated by the anthocyanin Ternatin A1, which consists of the base 15-carbon anthocyanin modified with seven molecules of glucose, four molecules of 4-coumaric acid and one molecule of malonic acid, corresponding to  $C_{96}H_{107}O_{53}^+$  (Terahara *et al.*, 1990). In this book we have grouped plant pigments on a common structural and biosynthetic basis into four major groups (Table 1.1), and Chapters 2–6 focus on these pigments. In addition to these major groups there is a great array of pigments that are of limited taxonomic occurrence, and often poorly characterised. Some of the more notable of these are covered in Chapter 7.

Pigment	Common types	Occurrence
Betalains	Betacyanins	The Caryophyllales and some fungi
	Betaxanthins	
Carotenoids	Carotenes	Photosynthetic plants and bacteria
	Xanthophylls	Retained from the diet by some birds, fish, and crustaceans
Chlorophylls	Chlorophyll	All photosynthetic plants
Flavonoids	Anthocyanins Aurones Chalcones Flavonols Proanthocyanidins	Widespread and common in plants, including angiosperms, gymnosperms, ferns, fern allies and bryophytes. Retained from the diet by some insects

Table 1.1 Major pigments of plants and their occurrence in other organisms

The most obvious and widespread pigments of plants are, of course, the chlorophylls. These are cyclic tetrapyrrole pigments chelated with magnesium, and they share structural features with the haem and bile pigments of animals. Also associated with photosynthesis, but additionally providing bright colours to flowers and fruits, are the carotenoids. Carotenoids are terpenoid pigments present in all photosynthetic plants and they also occur in photosynthetic bacteria such as *Erwinia* and *Rhodobacter*. Annual production of carotenoids by plants, algae and dinoflagellates has been estimated at 100 million tons (Britton et al., 1995). The flavonoids are phenylpropanoid compounds of widespread occurrence. There are several major classes of flavonoids; however, only a few of these provide pigments to plants, in particular the anthocyanins and proanthocyanidins (condensed tannins). Reviews of the biosynthesis and function of non-pigmented flavonoids can be found in earlier volumes in this Annual Plant Reviews series (Wink, 1999a, 1999b) and in Bohm (1998). The betalains are nitrogenous pigments that are the most taxonomically restricted of the major plant pigment groups, being found only in a few families of the order Caryophyllales and some fungi. Curiously, their occurrence is mutually exclusive to that of the anthocyanins.

Within plants, the major pigment groups show wide occurrence in the different tissues. For example, flavonoids occur in almost all tissues, carotenoids in leaves, roots, tubers, seeds, fruits and flowers, and even chlorophylls occur in flowers and fruits as well as leaves. Within tissues, there is often distinct localisation of pigment types in different cell layers. For example, anthocyanins are typically found in epidermal cells in petals and sub-epidermally in leaves, and chlorophyll in the sub-epidermal photosynthetic cell layers of leaves. The subcellular localisation of the different pigment groups is also generally distinct. The chlorophylls and carotenoids are principally lipid-soluble, plastid-located pigments, although there are examples of water-soluble carotenoids, at least some of which are located in the vacuole via plastid-vacuole interactions (Bouvier et al., 2003a). The betalains are water-soluble and vacuolar-located. While flavonoids occur in many subcellular locations, as well as extracellularly, the coloured flavonoids are principally found in the vacuole (Bohm, 1998). For flavonoids, the subcellular localisation is just one of several factors that determine the behaviour of the pigment molecule in the cell and the colour generated from it (Brouillard & Dangles, 1993). The mechanisms by which pigments such as the flavonoids are directed to the correct subcellular compartment are poorly defined, although some of the steps for anthocyanins have been elucidated (Winefield, 2002). Interactions of plant pigments with other cellular compounds have been well defined for flavonoids and small molecules (Brouillard & Dangles, 1993), and it is known that both flavonoids and carotenoids interact with specific proteins in the cell (Vishnevetsky et al., 1999; Winefield, 2002).

Modern phytochemical techniques can enable the rapid identification of the class of pigment present in a plant tissue of interest, and with more extensive analysis the detailed structure of the compound may be elucidated. The techniques required for identification and analysis of pigments in a plant of interest can be daunting to newer researchers in the field. With this in mind, Chapter 10 provides

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an overview of the modern and most recent techniques used for extraction, separation, identification and quantification of the major pigment classes in plants, providing sufficient detail to support the practical application of such techniques.

## 1.2.3 The history of plant pigment research

Pigmentation is one of the oldest subjects in formal plant science and has lead to many discoveries with impacts much wider than those in the pigmentation field alone. Early studies included discovery of the purple pigment from Viola as a natural pH indicator (Boyle, 1664), comparisons of the solubility of different pigments (Nehemiah Grew in 1682, quoted extensively in Onslow, 1925 and cited in Bohm, 1998), and Mendel's studies on the genetics of flower and seed colour in *Pisum sativum* – pea (Bhattacharya & Bhattacharya, 2001). The first publications on carotenoids date from the early nineteenth century, and detail the basis of colours of common food colourants of the time, some of which are still in use today, e.g. saffron and annatto (Eugster, 1995). The term chlorophyll was first used by Pelletier and Caventou (1818), and the different chlorophyll pigments were first separated from each other by Stokes (1864), Sorby (1873) and Tswett (1906) (cited in Jackson, 1976 and Eugster, 1995). Tswett's research included the development of column chromatography, one of the basic methods of modern biochemistry, and allowed the ground-breaking work of Willstätter on the structure of carotenoids. The conspicuousness of the flower colour trait and the easy identification of mutants has established it as a favourite system for geneticists ever since Mendel, from pioneering studies such as those of Darwin (1868), Onslow/Whedale (1925/1907) through to transposon studies and recent breakthroughs such as those on transcriptional regulation in plants featured in Chapter 4. Studies on transposons in pigment genes of maize were part of the work that earned Barbara McClintock a Nobel Prize in 1983, and plant pigments have also featured in research earning Nobel prizes for Willstätter (1915), Fischer (1930, the chemistry of blood pigments and chlorophyll), Karrer (1937, work on the structures of plant pigments, in particular carotenoids) and Kuhn (1938, work on the structure of carotenoids and related vitamins). Studies on anthocyanins have been responsible for several important breakthroughs in plant science, including the isolation and identification of a plant transcription factor gene for the first time (Cone et al., 1986; Paz-Ares et al., 1986), the isolation of one of the first cDNAs for a plant cytochrome P450 enzyme (Holton et al., 1993), the first demonstration of antisense RNA technology in a transgenic plant (van der Krol et al., 1988) and the first description of transgene co-suppression (Napoli et al., 1990; van der Krol et al., 1990).

# 1.2.4 The biosynthesis of plant pigments

The biosynthetic pathways for the major pigments of plants are now well defined at the genetic and enzymatic level. Of course, some knowledge gaps do exist, but these are becoming fewer. In the case of flavonoids, much is also known on the transcriptional regulation of the pathway. However, little is known on gene regulation mechanisms for any of the other major pigmentation pathways. Changes in the transcription rate of pigment biosynthetic genes usually precede pigment production, and it is thought that transcriptional regulation is the major controlling step for the pigmentation pathways studied to date. However, translational and post-translational regulation may also be important in specific cases, particularly for the photosynthetic pigments. Indeed, the general emphasis on transcriptional regulation may in part be due to lack of appropriate studies to identify post-transcriptional control. This is illustrated by the recent use of genomics to show rhythmic expression of many *Arabidopsis thaliana* genes, including those of the phenylpropanoid and carotenoid pathways, with the potential involvement of changing RNA stability in generating the rhythms (Staiger, 2002).

Although the biosynthetic pathways of many plant pigments are now well defined, little is known of the turnover and degradation of most pigments. There is an obvious loss of chlorophylls during the autumn senescence of leaves, and some information is available on chlorophyll degradation. However, anthocyanin levels can also change rapidly, e.g. in flowers of the Yesterday, Today and Tomorrow plant (Brunfelsia calcina), which can turn from white to purple and back to white within three days. Simpson et al. (1976) provided a comprehensive review on the knowledge to that date on the metabolism of the various pigment groups. However, this earlier chemistry and biochemistry has not been followed by extensive findings on the molecular biology of the metabolism of pigments in plants. Indeed, the metabolism is perhaps better understood for the fate of pigments when absorbed from the diets of animals than it is for the plant tissues themselves. One gene has been identified that has a direct impact on colour fading of flowers, the Fading locus of Petunia hybrida (de Vlaming et al., 1982). Fully coloured flowers turn rapidly to white towards the end of flower development when the Fading gene is dominant and the genetic background is appropriate with regard to other genes that affect the type of anthocyanin present and the flower petal pH. Active degradation of the anthocyanins is thought to occur, rather than a change to a colourless form of anthocyanin (Schram et al., 1984).

### **1.3** The functions of pigments in plants

# 1.3.1 The function of pigments in vegetative tissues

Chlorophylls and carotenoids are required for photosynthesis, chlorophylls for the capture of light energy and as the primary electron donors and carotenoids as essential structural components of the photosynthetic apparatus, where they protect against photo-oxidation. The roles of the pigments in these processes are covered in Chapters 2 and 3. Plant pigments are also involved in other interactions of plants with light, in particular the response to UV radiation (described in detail

in Chapter 9), which is of growing concern with regard to changes in the global environment.

Anthocyanins also frequently occur in vegetative tissues. The most spectacular example is their contribution to autumn colours in leaves of many deciduous species, which they generate in combination with the retention of carotenoids and loss of chlorophyll (Matile, 2000; Hoch et al., 2001; Lee, 2002). In non-senescing tissues their occurrence is more sporadic. Some species accumulate them in significant amounts in healthy leaves, providing red or purple colours to the foliage. In other cases anthocyanin production is induced in leaves in response to stresses such as cold, high light levels, pest and pathogen attack or deficiency of nutrients such as phosphate and nitrogen. Anthocyanin colouration in leaves can vary with season, environment, between individuals of a population and between different leaves on a single plant. It is commonly thought that anthocvanins have a role in protecting the photosynthetic apparatus from damage in many of these situations, and those tissues that show more anthocyanin accumulation are often at greater photoinhibitory risk, e.g. during nutrient reabsorption in senescing leaves or in cold temperatures (Hoch et al., 2001). However, the details of how anthocyanins achieve this are not determined. One hypothesis is that anthocyanins help attenuate the light levels, modifying the quantity and quality of light incident on the chloroplasts and thus reducing excitation pressure (Gould et al., 2002a; Steyn et al., 2002). However, this would not account for their accumulation in other stress situations. An alternative is that anthocyanins are acting as both direct light screens under high light stress and general antioxidants against harmful reactive oxygen species in the various other stress situations in which they are prevalent (Gould et al., 2002a, 2002b, 2002c). Supporting data include observations that red-leafed morphs of some shade species have a significant antioxidant advantage over green morphs, that anthocyanins can enhance oxidative protection in species more directly exposed to the sun, and that anthocyanins can reduce photoinhibition and photobleaching of chlorophyll under light stress conditions (Gould et al., 2002c; Neill et al., 2002; Steyn et al., 2002). Recently, the role of anthocyanins in improving foliar nutrient reabsorption during senescence, through the shielding of the photosynthetic apparatus from excess light, was tested using wild-type and anthocyanin-deficient mutants of three deciduous woody species under varying environmental conditions (Hoch et al., 2003). Nitrogen reabsorption efficiencies of the mutants were significantly lower than the wild-type counterparts, supporting the protection hypothesis of anthocyanins in senescing leaves.

There are likely to be many other functions of pigments in plants that have not been determined as yet. A recent example of a new function for anthocyanins is that of protecting light-sensitive phototoxic plant defence compounds from degradation, which was described by Page and Towers (2002). Thiarubrines are phototoxic plant pigments that decompose to thiophenes when exposed to sunlight. In *Ambrosia chamissonis*, they occur in laticifers that are surrounded by anthocyanin-containing cells. Page and Towers (2002) were able to show that the anthocyanins around the laticifers functioned to photoprotect these defence compounds.

# 1.3.2 The function of pigments in reproductive tissues

The most obvious function of plant pigments, with the exception of chlorophyll, is to provide colour to flowers and fruit for attraction of pollinators and seeddispersal agents. These colours arise predominantly from flavonoid and carotenoid pigments, and a short guide to the likely pigments producing specific colours in flowers and fruits of plants is given in Table 1.2. There is, however, also a range of less common pigments that generate colours in specific species.

For many angiosperms, colour is key to attracting pollinators, whether they are bees, butterflies, other insects or birds, although it is frequently one of a number of factors, including fragrance, floral shape and nectar reward, which combine to determine pollinator choice. Flavonoids are the most common flower colour pigments and it is on these that most research has been done. The role of flavonoids in pollination was the subject of an extensive review by Harborne and Grayer (1994), in which the authors were able to identify many general trends with regard to pollinator preference for different colours. However, they also noted the shortage of detailed studies on specific pigments and pollinators. This has changed greatly in the last decade, with many studies determining pollinator preference with regard to individual colours (Melendez-Ackerman et al., 1997; Gumbert et al., 1999; Oberrath & Bohning-Gaese, 1999; Gigord et al., 2001; Johnson & Midgley, 2001; Jones & Reithel, 2001; Landeck, 2002), fragrances (Odell et al., 1999; Raguso & Willis, 2002) and even petal epidermal cell shape (Glover & Martin, 1998; Comba et al., 2000). These studies cover a mix of approaches, including observational field studies, laboratory studies using model flowers and studies of the frequency of natural colour variants or white-flowered mutants within a population. Table 1.3 presents some of the general colour preferences identified for different pollinators. Most beetle-pollinated flowers are cream, white or green, and it was thought that beetles were generally insensitive to anthocyanin colours, such as red and blue (Harborne & Grayer, 1994). However, beetle selection of specific cyanic colours has now been demonstrated in some studies, including that of Johnson and Midgley (2001), who found that monkey beetles (Scarabaeidae: Hopliini) preferred orange-coloured model flowers to red-, yellow- or blue-coloured ones.

For pollinators such as bees that can detect light in the UV spectrum, UVabsorbing pigments also influence flower selection. The main contributors to the UV absorbance of the flower are the chalcone- and flavonol-type flavonoids. Flavonols are very common in flowers, often being in greater abundance than the coloured pigments. The flavonoids may form UV-visible patterning in petals, often in combination with UV-reflective carotenoid pigments (Harborne & Grayer, 1994; Bohm, 1998).

Colour	Specific pigment type	Pigment group	Examples
Cream	Flavonols or flavones	Flavonoid	Most cream flowers
Pink to red		Carotenoid	Some red flowers and fruit, e.g. Lycopersicon esculentum (tomato) fruit
	Pelargonidin and/or cyanidin	Flavonoid	Most pink flowers and some fruit, e.g. <i>Eustoma</i> grandiflorum (lisianthus) flowers
	Pelargonidin and/or cyanidin	Flavonoid	Most red flowers and some fruit, e.g. <i>Malus</i> (apple) fruit
	Anthocyanin and carotenoid mix	Flavonoid and carotenoid	A few examples, e.g. Tulipa flowers
	Betacyanin	Betalain	A few examples in the Caryophyllales, e.g. <i>Bougainvillea</i> flowers
Orange		Carotenoid	Most orange flowers and fruit, e.g. <i>Tagetes erecta</i> (marigold) flowers
	Pelargonidin alone	Flavonoid	A few examples, e.g. Pelargonium flowers
	Anthocyanin and aurone mix	Flavonoid	Rare occurrence, e.g. Antirrhinum majus (snapdragon) flowers
	Anthocyanin and chalcone mix	Flavonoid	Rare occurrence, e.g. Dianthus (carnation) flowers
	Betacyanin	Betalain	A few examples in the Caryophyllales, e.g. <i>Portulaca</i> (purslane) flowers
Yellow		Carotenoid	Most yellow flowers and fruit
	Aurone	Flavonoid	Rare occurrence, e.g. Antirrhinum majus flowers
	Chalcone	Flavonoid	Rare occurrence, e.g. Dianthus flowers
	Flavonol	Flavonoid	Rare occurrence, e.g. Gossypium (cotton) flowers
	Betaxanthin	Betalain	A few examples in the Caryophyllales, e.g. <i>Portulaca</i> flowers
Green		Chlorophyll	All green flowers and fruit
Blue	Delphinidin	Flavonoid	Most blue flowers and fruit
	Cyanidin	Flavonoid	Rare occurrence, e.g. Ipomoea (morning glory) flowers
Purple		Carotenoid	Rare occurrence, e.g. Capsicum (pepper) fruit
	Cyanidin and/or delphinidin	Flavonoid	Most mauve flowers, e.g. <i>Petunia</i> and some purple fruit, e.g. <i>Solanum melongena</i> (eggplant)
		Flavonoid and carotenoid mix	Some flowers, e.g. Cymbidium orchids
Black	Delphinidin	Flavonoid and carotenoid mix	Some black flowers, e.g. Viola (pansy)

Table 1.2 The most common pigment types associated with flower and fruit colours in plants. (The terms pelargonidin, cyanidin and delphinidin are used to refer to pelargonidin, cyanidin or delphinidin derived anthocyanins.)

Pollinator	Flower colour preference
Bees	Blue, yellow and UV-absorbing pigments
Birds	Bright red and scarlet
Beetles	White, cream, green and occasionally orange and red
Butterflies	Strong pinks, reds and mauves
Flies	White, green, dark brown and purple
Moths	White, cream and occasionally red
Wasps	Purple and blue

Table 1.3 Flower colour preferences of some pollinators, presented in terms of colour perceived by humans

In some cases, colour combinations and floral patterning may help attract a range of pollinators, including organisms as varied as hummingbirds and bumble bees, or provide more specific signals within the flower (discussed extensively in Harborne & Grayer, 1994 and particularly Bohm, 1998). For example, distinctive spots on the flower lip or pigment lines in the flower tube may act as a nectar guide to bees. This is well illustrated by the yellow face and two yellow throat stripes of aurone pigment in Antirrhinum majus (snapdragon) flowers. There are a few cases of elegant plant-animal co-evolution in which colour patterning has been shown to be part of floral mimicry. In particular, flowers of the orchid genus Ophrys use scent, shape and colour to mimic female bees, causing the male bee to attempt copulation, thus achieving pollination (Schiestl et al., 1999; Paxton & Tengo, 2001; Ayasse et al., 2003). The relationship between different orchid species and different bee species can be highly specific, and it is likely based on the pattern of scent compounds produced by the flower (Schiestl et al., 1999). There have been recent breakthroughs in understanding the genetic basis of colour patterning in flowers, discussed briefly in Chapter 4.

Change in flower colour during the later stages of flower development or in response to pollination has been recognised for many years. Indeed, as early as the late nineteenth century, Müller described the colour change of *Lantana* flowers from yellow to purple over an ageing period of three days, and the preference of pollinators for the younger, yellow flowers (see Weiss, 1991). That the yellow flowers were fertile, offered nectar and pollen that older flowers did not, and were preferred by the butterfly pollinators was later confirmed for *Lantana* species by Weiss (1991). The same study also offered a possible reason for maintenance of the older flowers – larger inflorescences were more successful in attracting butterflies. In most cases, a change in colour is likely to be associated with a change in nectar and pollen availability (Weiss, 1991, 1995; Harborne & Grayer, 1994; Bohm, 1998; Oberrath & Bohning-Gaese, 1999). At least 200 other plant genera contain species that show colour change during flower development and interact with a wide range of pollinator species, making it a common occurrence in plant reproduction (Weiss, 1991).

The specific studies on individual plant and pollinator species are supported at a higher level by general evolutionary and ecological trends (Harborne & Grayer, 1994; Gumbert *et al.*, 1999). In particular, blue flower colours are more common in the temperate ecosystems, in which bees are key pollinators, while bright red colours are more prevalent in tropical ecosystems, in which other insects and birds are more important.

Carotenoids and flavonoids also commonly colour pollen, although their functions in pollen are not well elucidated. They have been shown to have a role in signalling to pollinators (Lunau, 2000), and it is possible they also have protective activities against various stresses. Colourless flavonoids are known to be involved in plant fertility in some species (Taylor & Jorgensen, 1992; Jorgensen *et al.*, 2002), but this has not been shown for coloured flavonoids. The role of pigments in fruit is an obvious one, of signalling the ripeness of the fruit to seed-dispersal agents. Both carotenoids and flavonoids commonly provide fruit colours.

Flavonoids and carotenoids can also colour seeds, e.g. the yellow carotenoids and purple flavonoids of maize kernels. Such pigmentation may be related to timing of seed germination or plant defence. In some cases the pigments may reach very high levels. In the resinous seed coating of *Bixa orellana* (Fig. 1.1), which is the source of the commercial food colourant annatto, levels of the apocarotenoid bixin can reach 10% dry weight (Britton, 1996).

# 1.3.3 The roles of plant pigments in non-plant organisms

Many organisms absorb plant pigments from their diet, and may sequester them until they reach high levels. Flavonoid uptake has been demonstrated for a range of insects. In particular, butterflies and grasshoppers have been shown to take up the colourless or weakly pigmented flavonols and flavones, reaching levels of 2% dry weight of the wings of some butterflies. The sequestered compounds possibly act as visual attractants to mates (Harborne & Grayer, 1994; Bohm, 1998). Uptake of the more strongly pigmented flavonoids, such as anthocyanins, to the level of providing pigmentation to the insect has been implied by observation of insect colour on different plant food sources but it has not been characterised in detail. Flavonoids are taken up into the bloodstream in much smaller amounts by mammals, including humans, and their role in human health is a subject of much current research, which is covered in detail in Chapter 8. The polyphenolic tannins, reviewed in Chapter 5, also impact on animal health through the amelioration of bloat in ruminant animals.

Plant-derived carotenoids feature as key pigments in such familiar animal tissues as flamingo feathers (principally ketocarotenoids), shrimp and lobster shells, beetle shells, egg yolks and fish flesh (such as astaxanthin in goldfish) (Britton *et al.*, 1995; Britton, 1996). In marine invertebrates, the carotenoids can occur as 'carotenoprotein' complexes. These can generate vivid colours, including blues, greens, reds and purples. Two examples of varying carotenoid–protein interactions in lobster are the blue astaxanthin-protein compound crustacyanin of



Fig. 1.1 The red-coloured flower, seed pods and seed (see inset) of the tropical bush *Bixa orellana*, which is the source of the natural food colourant annatto. (Photograph by the author.)

the carapace and the green astaxanthin-lipoglycoprotein compound ovoverdin of the ovaries (Zagalsky, 1995; Britton *et al.*, 1997). The effect of the protein on the carotenoid colour in invertebrates can often be observed upon cooking of the animal, as the heat can lead to the breakdown of the complex and a dramatic colour change (Britton *et al.*, 1995). Carotenoid occurrence in bird plumage has also been extensively studied in relation to the specific chemical structures that occur, which can be modified by the bird's metabolism (McGraw *et al.*, 2002), and the impact of the colours on mating behaviour (Olson & Owens, 1998). Like flavonoids, carotenoids are also of much interest with regard to their healthpromoting effects in the human diet, a subject also discussed in Chapter 8.

An extreme example of the use of ingested pigments by animals, in this case from algae in the diet, is shown by photosynthetic molluscan sea slugs, in particular *Elysia chlortica* (Rumpho *et al.*, 2000). In these organisms, chloroplasts are taken up intact from the food source and are maintained intracellularly in specific cells that line the highly branched digestive system. The chloroplasts remain functional for at least nine months, providing both camouflage for the mollusc and a supply of carbon. The mollusc has been shown in the laboratory to be able to sustain itself in the absence of food by photoautotrophic  $CO_2$  fixation. The uptake of chloroplasts is widespread in the molluscan order Ascoglossa, but in some cases this may represent use only for camouflage and not the remarkable interaction described for *E. chlortica*.

# 1.4 Economic aspects of plant pigments

Human use of plant pigment extracts dates back as long as recorded history. The most obvious use has been the application of pigments such as henna for tattooing and carthamin, indigo and other pigments to generate bright colours in clothing. For example, anthraquinone, indigoid and flavonoid pigments have been identified in the fourth century AD Egyptian textiles (Orska-Gawrys *et al.*, 2003). Further back than this the use of carthamin extract (from safflower, *Carthamus tinctorius*) to dye the wrappings of mummies has been reported, and there is written evidence from 4600 years ago documenting human use of indigo (Gilbert & Cooke, 2001). Furthermore, a key part of garment manufacture since the time of early human societies has been the tanning of animal leathers with polymeric phenols, with record use dating back 10 000 years (Bohm, 1998). Today there are still tanneries carrying out leather treatment and dyeing with plant pigments in much the same manner as they have been doing for centuries (Fig. 1.2).

From an economic perspective, putting to one side the vital role of chlorophylls and carotenoids in photosynthesis, the most obvious contribution of plant pigments to agriculture is with regard to consumer choice of fresh fruit, vegetables and floriculture products. However, the impact of the non-photosynthetic pigments is often much wider. For example, they are also of economic importance as



**Fig. 1.2** Traditional tanning and dyeing of leather, including the use of many plant-derived compounds, in the Moroccan city of Fez. (Photograph by the author, taken in 2002.)

flavour and colour components of teas, wine and other beverages, as natural food colourants, for the health of ruminant animals, as plant defence agents and for amelioration of damaging UV light. The great range of non-coloured flavonoids and alkaloids produced by plants is often key to plant defence, and the biosynthesis of these compounds, and their importance to plant defence, agriculture and the pharmaceutical industries, have been extensively reviewed in previous Annual Plant Reviews (Wink, 1999a, 1999b).

# 1.4.1 Natural food colourants

Food quality is first assessed by its visual characteristics such as colour. Fresh food is often highly coloured by the major plant pigment groups, e.g. carotenoids and anthocyanins in fruit and chlorophylls in green vegetables. However, for processed foods the pigmentation is often lost during manufacturing, and the visual appeal of the final product is enhanced using added colourants. Until the discovery of synthetic dyes in the mid-nineteenth century, the food industry was solely reliant on natural food colourants. Although their use in many applications was superseded by synthetic dyes, in recent years there has been a return to the use of natural colourants, and an increased interest in new sources and improving their performance in food applications. Four plant pigment types are widely used as food

colourants: annatto, anthocyanins, betalains (beetroot pigment) and curcumin (the main pigment of turmeric spice). Together with the insect-derived pigment cochineal, they account for over 90% of the market for natural food colourants (Hendry, 1996). Use of chlorophyll as a food colourant is very limited in comparison to these pigments, principally because of its poor stability during food processing or in response to light or acid conditions in the final food product. Plant pigments used as colourants in smaller amounts include the carotenoids xanthophyll and lutein, carthamus yellow from *C. tinctorius* petals, iridoids derived from *Gardenia* fruit and the carotenoid derivatives crocin and crocetin from *Crocus sativus* (saffron). One of the most important natural colourants is caramel, which is used extensively in both the food and beverage industries, particularly in soft drinks. However, as it is not a true plant pigment, being derived directly from sugar by processing, it is not considered here. Plant pigments are also key components of spices sold in large amounts, in particular paprika (containing a mix of carotenoids), turmeric and saffron.

In addition to their use as food colourants, there is extensive use of pigment extracts as animal feed supplements. For example, carotenoids such as lutein are used in poultry/egg production and aquaculture. Furthermore, large amounts of nature-identical synthetic pigments are used, in particular  $\beta$ -carotene. The types of pigments used in different food applications are determined by their solubility, and for the water-soluble pigments, their behaviour in response to pH. The anthocyanins and betalains are water-soluble, and the chlorophylls, curcumin and carotenoids typically oil-soluble.

Annatto is one of the oldest known dyes used for foods, textiles and cosmetics. It is extracted from the resinous coating on the seeds of the tropical bush *B. orellana*. The species occurs in the wild in tropical North America and was used by Native Americans in pre-Columbian times as a source of pigment. Today, around 7000 tonnes of seed are processed annually by the pigment industry. The main pigment of annatto is *cis*-bixin, a monomethyl ester of the diapocarotenoic acid norbixin (Fig. 1.3), and supplies yellow to orange colours. It is sparingly soluble in oil and is principally used in dairy and fat-based foods. Present in smaller quantities in the pigment extract is a water-soluble carotenoid, *cis*-norbixin, which can also be generated by alkaline treatment of bixin (Britton, 1996).

Curcumin (Fig. 1.3) is the principal pigment in the spice turmeric, which is extracted from the rhizomes of *Curcuma longa*, a perennial member of the ginger family (Zingiberaceae) that has been cultivated in Asia for many centuries. It supplies strong yellow colours and is generally oil-soluble. Turmeric has traditionally been used for colouring and flavouring meals; it is still used in large quantities for this purpose as well as extensively in a wide range of processed foods. Around 300 000 tonnes are produced annually in India, mostly for spice with a small amount for preparation of pure curcumin (Francis, 1996).

Anthocyanins are widely used as food colourants (Jackman & Smith, 1996). First described as pH indicators, the colours of anthocyanin vary greatly on the pH of the food, but generally are used only in acidic foods and provide red to blue