Secondary Metabolites: Attracting Pollinators

Secondary article

Article Contents

Jeffrey B Harborne, *University of Reading, Reading, UK*

When insects, bats, birds and other animals visit flowers to feed on the nectar and pollen, they usually pollinate the flowers in the process, so that both partners benefit from this mutualistic association. Secondary metabolites are involved primarily to provide either visual or olfactory attraction in terms of flower colour and flower scent. They may also be present in the nectar and pollen to interact with certain visitors.

Pollination Syndromes in Nature

Pollination is a key process in higher plant reproduction. Most angiosperms are self-incompatible and require pollen from one individual to be carried by an animal to the style of a second for successful fertilization. Even plants that are self-compatible benefit, too, from animal visitors to the flowers. As a result of coevolution between plants and animals, plants have evolved floral structures to fit an appropriate pollinator. For example, long narrow corollas are produced by humming bird pollinated plants, thus excluding visits by bees. At the same time, flower colour is modified to produce a colour, e.g. the scarlet pelargonidin, that attracts birds but not bees. In addition, special volatiles or scents may be released when the flower is ready for pollination. This may attract the pollinating animal, even from some distance.

In nature, there are a myriad different pollinating systems. Every plant will tend to produce different secondary metabolites – pigments and volatiles – to attract their favoured pollinator. It is the biochemistry of these chemical attractants, which determine the fidelity of a pollinating animal to visit a particular plant species, that is the main subject of this article.

Chemical Basis of Flower Colour

Flower colour is due to the presence of pigments present in chromoplasts or cell vacuoles of floral tissues. The most important group of flower pigments are the flavonoids, since they contribute cyanic colours (red to blue) as well as yellow and white. The other major group are the carotenoids, which provide yellow colours, with some oranges and reds. Other classes of less importance are chlorophylls, providing green colour in *Helleborus* flowers, and the betalain alkaloids, giving yellow, red and purple colour in *Cactus* flowers and other members of the plant order Caryophyllalles.

In the case of cyanic colour, the chemical basis is simple. There are three main pigments, known as anthocyanidins: pelargonidin (orange-red), cyanidin (magenta) and delphinidin (mauve). These differ in structure only in the number (one, two or three) of hydroxyl groups in the B-ring. These three chromophores occur, usually singly or sometimes as mixtures, in angiosperm flowers and provide a wide range of colours. Essentially, most pink, scarlet and orange red flowers contain pelargonidin, most crimson and magenta flowers cyanidin and most mauve and blue flowers delphinidin.

A number of other chemical factors modify the basic anthocyanidin colours. One modifying factor is methylation of one or more of the free hydroxyl groups, giving rise to peonidin (from cyanidin) and petunidin and malvidin (from delphinidin). This has a reddening effect on flower colour. All anthocyanidins occur *in vivo* as glycosides (anthocyanins), usually with sugars attached to the 3- or 3- and 5-positions. Sugar attachment is important to solubilize and stabilize the chromophores. It has little effect on colour *per se* since glycosylation is the rule rather than the exception.

Another chemical factor modifying colour is hydrogen bonding between anthocyanins and accompanying flavones or flavonols, which are called copigments. Copigmentation is important to stabilize the anthocyanin chromophore in the acid environment (pH about 4.5) of the flower cell sap. It also has the ability, when copigments are produced at high concentration, to shift flower colour from red or mauve to blue. This happens, for example, in blue lupins. An alternative strategy for shifting colour to blue is the presence of aromatic acyl groups attached through the sugar groups. This occurs in blue morning glory flowers. One final feature of the pigments of blue flowered plants needs mention: the presence of trace metal for stabilization of the pigment complex. Thus magnesium is present in the native pigment of blue *Commelina communis* flowers (Brouillard and Dangles, 1994).

Most yellow colours are due to carotenoid pigments present in the chromoplasts, where they may occur in bound form esterified with fatty acids. Deep orange flowers may have large amounts of β-carotene (*Narcissus*) or alternatively of lycopene (*Calendula*). Three groups of flavonoid pigment, yellow flavonols, chalcones and aurones, contribute occasionally to yellow flower colour, especially in the plant family Compositae. Such pigments can be detected *in vivo* by fuming petals with ammonia vapour, when the yellow colour turns to red. Both carotenoids and yellow flavonoids are found together in some plants of the same family, notably in *Rudbeckia hirta*. Here, the two classes of yellow pigment have different functions. The carotenoids provide visible colour, while the flavonoids absorb in the ultraviolet and provide 'invisible' honey guides to bee pollinators.

Animal Attraction to Flower Colour

Flower colour is first and foremost an advertisement for the floral tissues and provides a striking contrast with the uniform green background of plant vegetation. Any colour will do, the brighter the better. At night, of course, visibility becomes important so that most moth and bat pollinated plants will tend to have white flowers.

As a pollinator in daylight approaches the flower, it will discriminate between plant species on the basis of flower colour and floral structure. Some indications of pollinator colour preferences are provided in **Table 1**. This is only a general guide and there are well known exceptions. For example, humming birds are particularly catholic in their choice of suitable flowers and will visit plants with white blooms in Hawaiian rainforests. Evidence for visual discrimination can be seen in flowers that change colour after pollination. This happens in *Lantana camara*, with the colour going from yellow (carotenoid) to red (anthocyanin). The pollinator may be aware of the pollinated red flowers, which are retained on the flowerhead, from a distance but as it approaches it selectively visits only the nectar-rich yellow flowers (Weiss, 1991).

The selective advantage of blue flower colour for attracting bees can be seen in those plants that give rise to white mutant forms in natural populations. Such mutants are unable to maintain themselves, seed set and viability being poor, because they are discriminated against by their pollinators. In the larkspur, *Delphinium nelsonii*, the white mutants have inferior nectar guides and it takes the bees longer to locate the nectar. The pollinators thus experience lower net rates of energy intake and hence concentrate their attention on blue flowers (Waser and Price, 1983).

Some plant species produce more than one flower colour in order to attract pollinators. In *Pedicularis* (Scrophulariaceae), the scarlet corollas attract humming birds, while the magenta calyces and bracts, with high ultraviolet and reflective hairs, attract bumble bees. In the scarlet gilia, *Ipomopsis aggregata*, there are both red flowers to attract humming birds and white flowers to attract hawkmoths. In this remarkably successful plant, colour shifting occurs seasonally. Most of the flowers opening later in the summer are white rather than red. This coincides with the southern emigration of humming birds and the increasing pollination by the night-flying hawkmoths (Paige and Whitham, 1985).

Types of Floral Volatiles

The odour or scent of a flower plays a major role as attractant to pollinating animals in the angiosperms. Bees are especially responsive to fragrant or 'heady' scents, as typified in the garden violet, where the active volatile is the sesquiterpene α-irone. Odour is of especial importance in night-flying insects and other animals, where visual stimulus is lacking: bat-pollinated and moth-pollinated plants are generally strong smelling.

Because of the sensitivity of insects to small concentrations of volatile chemicals, flower odours are undoubtedly effective at relatively low concentrations. Maximum scent production is coordinated with the time when the pollen is ripe and the flower is ready for pollination. Floral scent can be an attractant for other purposes than food; for example, the blossoms may provide a suitable mating site for certain moth species.

Table 1 Colour preferences of different pollinators

Animal	Flower colour preferences
Bats	White or drab colours, e.g. greens and pale purples
Bees	Yellow and blue intense colours; sensitive to ultraviolet absorbing pigments
Beetles	Dull, cream or greenish
Birds	Vivid scarlets, also bicolours (red/yellow)
Butterflies	Vivid reds and purples
Flies	Dull brown, purple or green
Moths	Reds and purples, white or pale pink
Wasps	Browns

From the viewpoint of the human observer, flower scents broadly fall into two classes: those that are pleasant, fragrant or fruity; and those that are repellent. While we can make such a classification using the human nose, the pollinator concerned will be attracted irrespective of the quality of the odour. Pleasant odours are generally based on the presence of mono- and sesquiterpenoids. Limonene, for example, is a major constituent of citrus flowers, while geraniol is dominant in the geranium and the rose. There may be one or two major volatile constituents or else several compounds may act synergistically to provide a characteristic floral scent.

Other constituents of fragrant odours include volatile aromatic substances. One such compound is vanillin, used as a flavouring in ice cream, but actually present in vanilla and other orchid blooms. Simple aliphatic alcohols, ketones and esters also contribute to floral odours. Ethyl acetate, for example, with its peardrop odour, is dominant in the floral scent of *Zygogynum* species. It improves the effectiveness of pollination by making the moth pollinator drowsy.

Several examples of floral volatile mixtures with pleasant fragrances and that attract bee, butterfly and moth pollinators are provided in **Table 2**. Some of the unpleasant chemicals, which are attractive to bats, beetles and dungflies, are also listed in this table. A dominant feature of 'distasteful' odours attractive to flies and certain beetles, is the faecal-smelling indole, sometimes accompanied by the even more foul-smelling methyl derivative, skatole. Both these compounds have been detected in the nauseating odour of the voodoo lily *Sauromatum guttatum* (Borg-Karlson *et al*., 1994).

The volatiles of the well-known aroid, lords and ladies, *Arum maculatum*, have been particularly well investigated and earlier reports of fishy-odoured amines in the scent have not been confirmed. Four other compounds dominate (**Table 2**). Of these, indole and *p*-cresol would seem to be the most important attractants, since these two volatiles are given off by the dung, which the dungfly pollinator normally feeds on. Their effectiveness in attracting the fly to the plant is enhanced by generation of heat in the spadix of the flower head, during volatile release (Kite, 1995).

Another source of 'bad' odours is the bat-pollinated flower. Here, various methyl di- and trisulfides have been characterized. These have been found not only in the plant *Crescentia cujeta*, but also in several other bat-pollinated plant species. Production of methyl sulfide floral volatiles is clearly a case of convergent evolution, since the various bat-pollinated plants that have them belong to several unrelated families (Knudsen and Tollsten, 1995).

Animal Attraction to Flower Scent

The floral volatiles may play a key role in attracting pollinators to the plant from far away. They may attract a bee, for example, from anything between 5 and 50 metres distant. As already mentioned (**Table 2**), fruity, distasteful odours are attractive to beetles, while sweet smells draw bees, moths and butterflies to the flower. Musty or fruity odours are produced to attract bats, while faecal odours are the province of dungflies.

What is particularly fascinating about investigations of animal responses to floral volatiles is the subsidiary role these volatiles may also play. Perhaps the most striking example is the distinctive pollination of orchid flowers in the genus *Ophrys* by male bees of the genus *Andrena*. Here, the floral volatiles have a pheromonal role, as well as being a pollen attractant. Pseudocopulation of the flower by the male bee (exclusively) depends on the orchid flower having the same shape, same colour and same scent as the female bee. In other words, there is a degree of matching between some of the floral constituents and the pheromonal volatiles of the female bee. In *Ophrys lutea*, for example, 1-octanol, decyl acetate and linalool are three constituents common to the floral volatiles and the pheromones of the appropriate bee species. In general, most *Ophrys* species that are male bee pollinated, produce particular pheromonal constituents that reciprocally attract different *Andrena* species (Borg-Karlson and Tengo, 1986).

Table 2 Floral volatiles of higher plants according to their pollinators

Floral volatile	Plant species	Pollinator
Dimethyl trisulfide, dimethyl disulfide, etc.	*Crescentia cujeta*	Bat
Geraniol, citral, farnesol, etc.	*Ophrys* spp.	*Andrena* male bee
Carvone oxide	*Catasetum maculatum*	*Eulaema* male bee
Linalool and its oxides	*Daphne mezereum*	*Colletes* bee
Indole, 1,2,4-trimethoxybenzene, cinnamaldehyde	*Cucurbita* spp.	Diabroticite beetle
Methyl anthranilate and isoeugenol	*Cimifuga simplex*	Butterfly
Methyl benzoate, linalool and geraniol	*Platanthera chlorantha*	Moth
trans-Ocimene and 1,8-cineole	*Brugmansia* × *candida*	Hawkmoth
2-Heptanone, indole, germacrene B and *p*-cresol	*Arum maculatum*	Dungfly

Another interesting example of the pheromonal use of floral scents by bees is taken from the bee-orchid literature of South and Central America. Here, the male euglossine bees are unusual in their mating behaviour. The males are brilliantly coloured and during mating congregate together in small swarms called leks to attract the females to them. While they are doing this, they release odour components, which they have previously collected while pollinating flowers, to attract conspecific males during lek formation. Orchid compounds used in this way include eugenol, vanillin, cineole, benzyl acetate and methyl cinnamate. Different bee species are differentially attracted by one or other of these odours. Thus, isolation mechanisms preventing different bee species from mating may be due to varying preferences for orchid scent compounds.

It is clear that in many other cases as well as the two outlined above, insects 'learn' to recognize the smell of individual flowers and it is this factor, more than any other, that is responsible for the phenomenon of flower constancy. Here, insects limit their attention to few or only one plant species. Is it possible that plants produce substances in their scents, upon which the insects become 'hooked'? It has been suggested that some especially attractive compound is released from flowers of *Brugmansia* × *candida* and is present in the nectar to affect the hawkmoth pollinator. It has been observed that the hawkmoth becomes erratic in flight after visiting the flowers and shows all the signs of being 'hooked' by the plant. Unfortunately, history does not relate which of the two major scent volatiles (**Table 2**) is involved or whether some tropane alkaloid may be secreted in the nectar instead.

Chemistry of Nectar and Pollen

The majority of nectars consist simply of solutions of sugars, an important nutritional source to the animal pollinator. Most nectars are very sweet to taste and vary in sugar content from 15 to 75% by weight. The three common sugars of plant metabolism, glucose, fructose and sucrose, are present. Oligosaccharides may also be found in nectars, especially the trisaccharide raffinose. There are differences in the amounts of the main sugars. Indeed, angiosperm nectars can be divided into three groups; those in which sucrose is dominant (e.g. *Berberis*); those in which all three sugars occur in about equal amount (e.g. *Abutilon*); and those in which glucose and fructose are dominant (most crucifers). There would appear to be an evolutionary trend from nectars with mainly sucrose to those with mainly glucose and fructose. The advantage to the pollinator would be a more readily assimilable sugar mixture. Although the enzyme invertase, which converts sucrose to glucose and fructose, has been detected in nectars, it does not seem to be functional, since sugar patterns in nectars are not subject to diurnal or seasonal variation.

Another nutritional feature of plant nectars is the presence of protein amino acids. These are very minor components, but nevertheless provide a useful supply of nitrogen to those pollinators (e.g. butterflies) that lack any other nitrogen source. All the twenty common protein amino acids have been detected in nectars, but usually there are up to 12 that can be readily analysed. Again, they vary both quantitatively and qualitatively from species to species according to which pollinator is involved.

On a weight basis, lipids are more energy rich than sugars, so it is not surprising to find that some plant families, especially the Scrophulariaceae, substitute lipid bodies for sugar in the nectar. Such plants are bee-pollinated and the oil is mainly used by the bees to feed their young. The chemistry of these lipid bodies is unusual and a number of special fatty acids have been detected.

More and more frequently, trace amounts of plant toxins have been recognized in nectars. Their origin is obscure, but presumably they are derived from other parts of the flower. Typically, they are the same secondary metabolites that occur in greater amount in other tissues. For example, bees feeding on the ragwort *Senecio jacobaea* are known to produce honey contaminated with the pyrrolizidine alkaloids that are present. Analysis of the nectars of several plant species has confirmed that alkaloids in particular can be present. Thus nicotine has been detected in traces in tobacco plant nectar, while atropine occurs to the extent of 273 $\mu g\ g^{-1}$ fresh weight in the nectar of the deadly nightshade, *Atropa belladonna*. Other toxin classes detected in nectars include phenolics, iridoids and diterpenoids.

Pollen is usually more accessible than the nectar and is collected by many flower visitors, especially beetles and bees. Nutritionally, it is a rich source of food with 16–30% protein, 1–7% starch, 0–15% free sugar and 3–10% fat. Trace amounts of vitamins and inorganic salts also occur. There are varying amounts of secondary substances. Pollen is usually coloured, especially by carotenoid but also by flavonoids. The carotenoids of pollen are commonly α- and β-carotene, lutein, zeaxanthin and various epoxides. The flavonoids are commonly flavonol glycosides, and substances such as kaempferol and quercetin 3-sophorosides may be regular constituents. The flavonols of plant pollens do vary somewhat from species to species, so that it is sometimes possible to use pollen flavonols to identify the plant origin of a particular honey. This is because the pollens are collected by bees to feed to their young as 'bee pollen' and some of this is inevitably present in the honey produced by those bees.

Finally, it should be noted that pollen from different plant species have characteristic odours, which can be perceived by both humans and insects. The compounds responsible for these odours are similar in chemistry to the odour principles of the flower. Nevertheless, there may be significant differences in relative amounts and also in actual constituents. Such differences allow the bee to distinguish between the flowers and the pollen of a given plant species.

Animal Attraction to Nectar and Pollen

The principal purpose of nectar and pollen is to reward the pollinator for unwittingly pollinating the flower. In many cases, the reward is a significant food source for that pollinator and indeed the survival of the animal may depend on the nourishment provided by the plant. In exceptional cases, there may be little reward; this is true for the male *Andrena* bee pseudocopulating with an *Ophrys* flower, since there is no significant nectar provided.

What is remarkable about the chemistry of nectar in particular is that the amounts of sugars and amino acids present in particular nectars are related to the needs of particular pollinators. Thus, nectars with high levels of sucrose, compared to fructose and glucose, are present in plants pollinated by large bees, humming birds and Lepidoptera. By contrast, nectars with low sucrose are produced by plants fed upon by small bees, passerine birds and neotropical bats.

The same trends in amounts of amino acids in nectars have been observed. Primitive woody plant families tend to produce nectars low in amino acid. These are pollinated by bees, insects that can forage for nitrogen from other sources. By contrast, highly evolved plant families produce nectar with nearly three times more amino acid present. These are pollinated by butterflies and moths, which lack other sources of nitrogen. It thus appears as if plants have evolved to produce larger amounts of nitrogen in the nectar in response to the nutritional needs of their chosen pollinators (Baker and Baker, 1990).

How far the secondary constituents of nectars are involved in plant–pollinator relationships is not yet clear. However, there are two well-known examples where the pollinator benefits from the secondary metabolites present. This is true of the adult ithomiine butterflies in South America and the danaid butterflies of North America, which have a dietary requirement for pyrrolizidine alkaloid. These are imbibed by the adult and used for defence by both sexes against bird predation but also for pheromone production by the male. The alkaloids can be obtained by sucking withered borage leaves but a much more accessible source are the nectars of *Eupatorium* and *Senecio* species, which secrete the same alkaloids.

The purpose of toxin accumulation in nectars in more general circumstances is still uncertain. A defensive role against herbivory or against an undesirable animal visitor is certainly possible. The production of iridoids such as catalpol in the nectar of *Catalpa speciosa* is apparently to protect the plant from ants, which are well-known nectar thieves.

References

Baker HG and Baker I (1990) The predictive value of nectar chemistry to the recognition of pollinator type. *Israel Journal of Botany* **39**: 157–166.

Borg-Karlson AK and Tengo J (1986) Odour mimetism? Key substances in *Ophrys lutea-Andrena* pollination. *Journal of Chemical Ecology* **12**: 1927–1941.

Borg-Karlson AK, England FB and Unelius CR (1994) Major volatiles released from *Saromatum guttatum*. *Phytochemistry* **35**: 321–323.

Brouillard R and Dangles O (1994) Flavonoids and flower colour. In: Harborne JB (ed.) *The Flavonoids, Advances in Flavonoid Research since 1986*, pp. 565–588. London: Chapman and Hall.

Kite GC (1995) The floral odour of *Arum maculatum*. *Biochemical Systematics and Ecology* **23**: 343–354.

Knudsen JT and Tollsten L (1995) Floral scent in bat pollinated plants: a case of convergent evolution. *Botanical Journal of the Linnean Society* **119**: 45–57.

Paige KN and Whitham TG (1985) Individual and population shifts in flower colour by scarlet Gilia. *Science* **227**: 315–317.

Waser NM and Price MV (1983) Pollination behaviour and natural selection for flower colour in *Delphinium nelsonii*. *Nature* **302**: 422–424.

Weiss MR (1991) Floral colour changes as cues for pollinators. *Nature* **354**: 227–229.

Further Reading

Bergstrom G (1978) Role of volatile chemicals in *Ophrys*–pollinator interactions. In: Harborne JB (ed.) *Biochemical Aspects of Plant and Animal Coevolution*, pp. 207–232. London: Academic Press.

Harborne JB (1993) *Introduction to Ecological Biochemistry*, 4th edn. London: Academic Press.

Kullenberg B and Bergström G (1975) Chemical communication between living organisms. *Endeavour* **30**: 59–66.

Proctor M, Yeo P and Lack A (1996) *The Natural History of Pollination*. London: Harper Collins.

Real L (ed.) (1983) *Pollination Biology*. Orlando, FL: Academic Press.

Stanley G and Linskens HF (1979) *Pollen: Biology, Biochemistry and Management*. Berlin: Springer-Verlag.