REVIEW PAPER

Morphology and development of floral features recognised by pollinators

Heather M. Whitney · Beverley J. Glover

Abstract The diversity of angiosperm flowers is astounding. The conventional explanation for this diversity is that it represents the great variety of ways in which flowers have adapted to attract an even greater diversity of animal pollinators. Many animal behaviourists are therefore interested in how changes in floral morphology affect pollinator behaviour. The establishment of well-characterised model plant species has greatly furthered our understanding of how floral morphology is generated and varied. Many of these model species are pollinated by animals and attract their pollinators through the production of colour, shape, scent, size and rewards. An understanding of the developmental plasticity of floral morphology, and the constraints upon it, should inform research into animal responses to flowers. The use of genetically characterised model species, and the isogenic and near-isogenic lines available in them, will allow dissection of the different components of floral attraction and reward in natural systems.

Keywords Angiosperm · Floral reward · Flower colour · Flower development · Flower scent · Flower shape · Flower size · Flower symmetry · Pollination · Pollinator

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Introduction

Our rationale for this article is that many biologists studying pollinator behaviour are hard-pressed to keep up with developments in the rapidly expanding fields of floral developmental biology and its molecular genetic control. The recent review by Chittka and Raine (2006) of developments in neuroethology and psychophysics, and their consequences for flower recognition by pollinators, provided plant biologists with a clear summary of what bees can perceive in relation to floral characteristics. In this article we hope to provide behavioural scientists with an equally clear summary of the morphology and development of those floral characteristics. We have two main aims. First, we hope to provide behavioural biologists with an overview of the relative developmental lability of traits of interest, to aid them in the design of experiments which are biologically meaningful both with regard to what the animal can perceive and with regard to what is developmentally and evolutionarily possible for the plant. We hope that an understanding of how and why variation in flower colour, size and shape occurs will enable the design of experiments which test plausible shifts in stimuli. Second, we hope to stimulate behavioural biologists to consider using the flowers of model species in some experiments. In several model species it is possible to use genetically characterised lines which differ only in the trait of interest, resulting in a level of complexity that is little different from that obtained using artificial stimuli. The use of real flowers in such experiments carries the advantage of assessing the value of a trait in its natural context, rather than in isolation.

Model plant species

Plant molecular and developmental biologists, like their animal counterparts, have narrowed the majority of their

focus to study a number of model plant species, several of which are of potential interest to pollination biologists. The flowers of these are shown in Fig. 1.

In general, plant model species have been selected for small genome size, rapid generation time and prolific seed production. Most of the model species are also self-compatible and self-pollinate efficiently (Pang and Meyerowitz 1987; Endersby 2007). Their key features are described in Table 1. We only consider those model plant species that have animal pollinators, although there are several model species (such as maize and rice) that rely on wind pollination (Shimamoto and Kyozuka 2002).

The main advantage of using model species in behavioural experiments is that single genetic traits can be studied in isolation. It is possible to accurately determine the effect of single gene changes on plant morphology, and for a pollination biologist this provides the opportunity to look at the consequences for animal behaviour of a single, defined trait, present in a context which matches the real-world expectations of the animal.

As an example, model plant species, particularly Antirrhinum and Arabidopsis, were used to establish how the floral organs develop in the correct positions. According to the ABC model of flower development (Coen and Meyerowitz 1991) different combinations of the activities of four sets of genes are sufficient to induce the development of each of the floral organs (recently reviewed by Krizek and Fletcher (2005)). This model has provided the foundation on which all other studies of flower development are based, starting from its observation that all floral organs are simply modified leaves. The understanding of flower colour, size and shape is a matter of understanding the deviations from leaf development which occur in developing floral organs.

We will now consider the development and plasticity of various aspects of floral form. Flower size, shape, colour, texture, scent and reward have all been shown to play important roles in attracting pollinators.

Flower size

Flower size is extremely important in attracting pollinators and the literature indicates that larger targets are more attractive than smaller ones to most insects (Spaethe et al. 2001). Indeed, because of the poor resolving power of the compound eye, insect-pollinated flowers of larger sizes are much more visible to pollinators and should therefore be favoured by selection (Chittka and Raine 2006). However, although flower size differs greatly between different species, it is seldom very variable within a species (Bradshaw 1965). The lack of lability in flower size is often assumed to reflect the other adaptive consequences of a change in size. For example, larger flowers are more energetically costly to produce, and it is often assumed that plants have adapted to this by producing either few, large flowers or many, small flowers. This energetic and metabolic cost of flower production is not the only ecological variable which might operate to limit lability. Galen and Cuba (2001) showed that larger flowers of the alpine skypilot, *Polemonium viscosum*, were at a selective disadvantage when compared to smaller flowers because they were more frequently visited by nectar-robbing ants. However, ecological constraints are by no means the only reasons for the lack of intraspecific variability in flower size. Developmental genetic analysis has shown that flower size is constrained by a complex series of interactions between the genes and processes governing organ development.
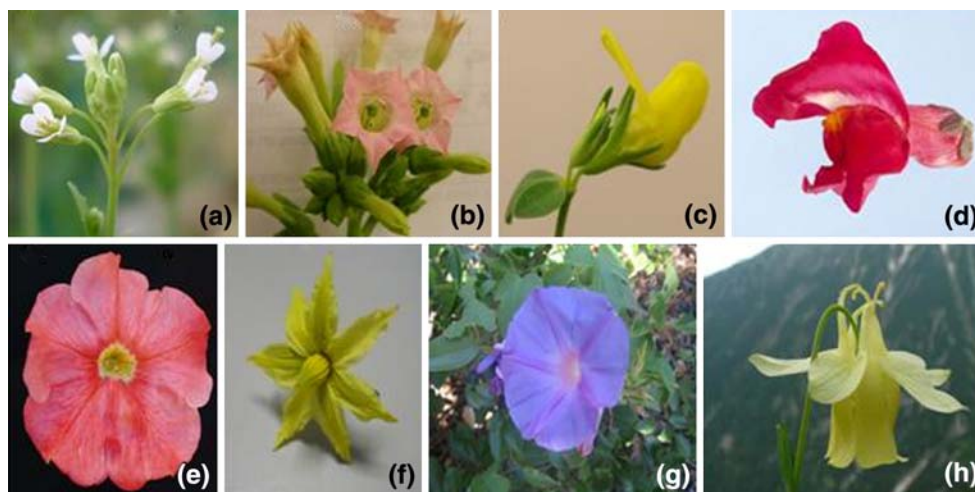
Fig. 1 Photographs illustrating the range of flowers produced by model plant systems. (a) *Arabidopsis thaliana*, (b) *Nicotiana tabacum*, (c) *Lotus japonicus*, (d) *Antirrhinum majus*, (e) *Petunia hybrida* (kindly provided by Ronald Koes), (f) *Solanum lycopersicum*, (g) *Ipomoea purpurea*, (h) *Aquilegia aurea* (kindly provided by Scott Hodges)

Table 1 Model plant species with animal-pollinated flowers

Model plant	Pollinator	Level of characterisation	Floral modifications available	References
Arabidopsis thaliana	Fly, but 98% self-pollinating in the wild	Genome sequenced. Easily transformed.	Colour (TG). Flowering time (SGM, NILs). Nectar (SGM). Size (SGM). Organ identity (SGM).	Stinchcombe et al. (2004) Bowman and Smyth (1999) Mizukami and Fischer (2000)
Antirrhinum majus	Bumblebee	Genetic and physical maps. ESTs.	Colour, pattern (SGM, NIL). Symmetry. Epidermal cell type (SGM). Nectar spur (SGM). Organ identity (SGM).	Schwinn et al. (2006) Whibley et al. (2006) Nath et al. (2003) Crawford et al. (2004) Noda et al. (1994) Golz et al. (2002)
Nicotiana tabacum	Primarily moth pollinated, though also visited by bees	Genetic maps. Easily transformed.	Colour (TG). Epidermal cell type (TG). Organ identity (TG).	Nishihara et al. (2005) Baumann et al. (2007)
Medicago truncatula	Honey bee	Genome currently being sequenced. Amenable to transformation. TILLING available.	Organ identity (SGM).	Benlloch et al. (2003)
Lotus japonicus	Bee pollinated	Integrated genetic map. Easily transformed. TILLING available.	Symmetry (SGM).	Feng et al. (2006)
Petunia hybrida (hybrid of *P. axillaris* and *P. integrifolia*)	*P. integrifolia* pollinated by solitary bees. *P. axillaris* pollinated by hawkmoths.	Genetic and physical maps. ESTs. Amenable to transformation.	Colour, scent, floral. Architecture, nectar quality and quantity (SGMs, QTLs). Epidermal cell shape (SGM). Volatile production (TG).	Stuurman et al. (2004) Baumann et al. (2007) Verdonk et al. (2005) Hoballah et al. (2007)
Mimulus spp.	Various depending on species, bee and hummingbird.	Significant mapping of genome.	Colour (NILs).	Bradshaw and Schemske (2003)
Ipomoea spp.	Various depending on species. *Ipomoea purpurea* is bee pollinated.	Mutant collections available. ESTs.	Colour (NILs, SGM).	Fukada-Tanaka et al. (2000)
Aquilegia spp.	Various depending on species, often bee or hummingbird.	Genetic map. QTL identification. Amenable to transformation.	Colour (NILs). Organ identity (SGM).	Clegg and Durbin (2003) Zufall and Rauscher (2004) Kramer et al. (2007)

A summary of the level of molecular genetic characterisation, the available lines with altered floral morphology, and key references, is provided for each species. TG—Transgenic lines, SGM—Single Gene mutant, QTLs—Quantitative Trait Loci, NILs—Near Isogenic Lines, ESTs—Expressed Sequence Tags

The idea that organ size is limited by internal plant architecture is at odds with the general hypothesis that natural selection should overwhelm developmental and genetic constraints. A number of authors have compared the variability in floral morphology to the variation in vegetative organ morphology, testing the hypothesis that plants with specialised pollination systems show greater decoupling of variation between floral and vegetative traits

than do plants with generalised pollination systems. However, such decoupling does not appear to be greater, or more common, in plants with specialised pollination systems, suggesting that genetic control of development does constrain morphology (Armbruster et al. 1999). The genetic constraints on organ size are surprisingly complex. A petal develops from a primordium of dividing cells, which arises on the floral meristem. The final size of the petals, and thus the corolla they form, is a factor of the combined amount of cell division and cell expansion that occurs throughout the development of each organ. In theory, then, an increase in cell division rate or the extent to which cells expand should result in larger petals, while a decrease in cell division rate or the extent of cell expansion should have the opposite effect.

An increasing body of evidence indicates that the control of organ size is a rather more complex process than these basic mechanisms might suggest. In particular, it is becoming apparent that cell division and cell expansion are necessary to organ growth, but do not themselves control the final organ size. Instead, there appear to be mechanisms present that monitor organ growth and balance the number and size of cells to achieve the correct end point (Shpak et al. 2003). A number of studies have now shown that altering cell division or expansion through the use of transgenes or mutation does not necessarily lead to a change in organ size, because the other process adapts to compensate (Jones et al. 1998; Dewitte et al. 2003). For example, if cell division rate is reduced experimentally, cell expansion rate increases, so that the final organ is the same size as normal, but is composed of fewer, larger cells. Studies such as these suggest that an intrinsic mechanism coordinates cell division and cell expansion, to control overall organ growth.

Recent analysis of the *AINTEGUMENTA* (*ANT*) gene of Arabidopsis suggests that it might function as a checkpoint through which such control is exerted. Unlike the examples described above, changes in ANT function do result in changes in overall organ size. Loss of ANT function results in leaves and petals which are smaller than wild type, while increased *ANT* expression in transgenic plants increases the sizes of all organs (Mizukami and Fischer 2000). These alterations in organ size result from changes in total cell number that are not compensated for by changes in cell size. Further analysis of the *ANT* gene and related genes should allow us to understand how flower size can be altered under certain conditions but why it is so rarely variable in the wild. In the mean time, behavioural biologists might like to be aware that target size is not a realistic variable when considering the interactions between the flowers of one plant species and the animals that pollinate them, for developmental reasons as well as the more commonly discussed ecological ones.

Flower shape

Overall flower morphology is clearly under genetic constraint, such that it is usually possible to identify a plant by its flowers alone. Total flower shape is a factor of the size and shape of different organs, the symmetry of the flower and the additional embellishments such as nectar spurs. These component parts of overall flower shape vary in the extent to which they are labile, with petal shape and curvature strongly constrained. Floral symmetry is under the control of several genes, and can be perturbed relatively easily by mutation of any one factor. Embellishments such as nectar spurs are extremely labile in an evolutionary sense, being lost and gained multiple times in families such as the Orchidaceae (Bateman and DiMichele 2002; Rudall et al. 2003). Overall, analysis of pollinator responses to differences in shape of artificial targets can reasonably reflect the actual potential of flowers to change shape, as long as certain constraints on size and curvature are taken into consideration.

The shape of a petal is a result of the distribution of cell divisions and the distribution and direction of cell expansion which occur during its development. Studies in Antirrhinum have shown that petal growth rate is relatively constant, and that the asymmetric shape of the petal lobes results from changes in the direction of growth rather than local differences in the rate of growth (Rolland-Lagan et al. 2003).

Final petal shape is dependent upon the generation of surface curvature. Curvature of the petal surface is produced by differential growth of the margins relative to the central region of the tissue. In fact, many petals are essentially flat, showing no such curvature. However, it is very unlikely that flat petals arise by chance, implying that genetic mechanisms exist which regulate growth rate to ensure no curvature is produced (Nath et al. 2003). In some species petal curvature is an important part of corolla appearance, and in these cases it is likely that similar genetic mechanisms regulate relative growth differently to produce the final petal shape. In Antirrhinum, organ curvature is inhibited by the action of the *CINCINNATA* gene, which encodes a transcription factor from the TCP family (Nath et al. 2003; Crawford et al. 2004).

The petals of some species show distinct architectural features. The ventral petal of Antirrhinum, for example, has a distinctive hinge or lip region, which forms the landing platform for pollinating bees. While all Antirrhinum petals show differences between the basal portions (which fuse to form the corolla tube) and the distal regions, which remain separate as lobes, the division between the two is most marked in the ventral petal. Elaboration of landing platforms is a common developmental process in many animal-pollinated flowers, and one which involves a very clear fold

in a petal. A number of genes have been shown to play roles in landing platform development in Antirrhinum (Crawford et al. 2004; Perez-Rodriguez et al. 2005).

The flowers of many common species such as buttercup, rose and tulip are actinomorphic (radially symmetrical). An actinomorphic flower is the default form that develops through the activities of the ABC genes. However, many plants produce flowers that are zygomorphic (bilaterally symmetrical). Zygomorphic flowers most commonly have differences between the petals, or between the stamens, or both. Classic examples of zygomorphic flowers include Antirrhinum, sweet peas, and most orchid species.

The key to producing a zygomorphic flower is to provide it with a signal which marks a region of the developing floral meristem as top or bottom. Within Antirrhinum four mutant lines, *cycloidea* (*cyc*), *dichotoma* (*dich*), *radialis* (*rad*) and *divaricata* (*div*), are lacking various aspects of this signal (Luo et al. 1996; Almeida et al. 1997; Corley et al. 2005). The lateral petals of the *cyc* mutant are converted into ventral petals, and the dorsal petals are morphologically intermediate between dorsal and lateral petals. The *dich* mutant produces flowers with a similar phenotype to those of *cyc*. However, if the two mutant lines are crossed together to create a *cyc/dich* double mutant then the flowers that are produced are fully radially symmetrical, with all the petals converted to the ventral form (Luo et al. 1996). This led to the conclusion that *CYC* and *DICH*, both of which encode TCP family transcription factors, provide dorsalising signals to the developing floral meristem (Luo et al. 1996).

DIVARICATA, by contrast, acts to provide a ventralising signal to the floral meristem, and the *div* mutant has an abnormal ventral petal with little or no hinge and lip region. *DIV* encodes a transcription factor from the MYB family (Galego and Almeida 2002), as does *RAD*, which prevents any ventralising signal invading the dorsal part of the flower (Corley et al. 2005).

Zygomorphy has evolved multiple times from actinomorphy. There are also examples of reversion from a zygomorphic form to an actinomorphic form (Endress 2001). Current work in the field is focussed on assessing whether evolution of zygomorphy involves repeated recruitment of the same molecular components to direct similar developmental programmes, or whether different developmental pathways apply in different taxa (Cubas 2003). Within the Leguminosae, recent reports suggest that *CYC*-like genes have been recruited to the development of zygomorphy in *Lotus japonicus* and *Lupinus nanus* (Feng et al. 2006; Citerne et al. 2006).

Many flowers have embellishments to one or more organs, which alter the total flower shape. For example, the flowers of many species, such as *Linaria vulgaris* (toadflax), produce a nectar spur on the ventral petal, while some species, such as

Aquilegia vulgaris, produce nectar spurs on all petals. The spur contains secreted nectar, only accessible to particular pollinators. *L. vulgaris* itself is primarily pollinated by long-tongued bumblebees (Newman and Thomson 2005). Petal spurs require elaborate outgrowth of a small region of the petal, and their development is not well understood. An actinomorphic mutant of *L. vulgaris* with all petals converted to the ventral form and making spurs was described by Linnaeus and has been reported many times since. More recently, two mutants of *Antirrhinum majus* which produced ectopic nectar spurs on the ventral petal were identified. Antirrhinum, unusually among its close relatives, does not normally produce a nectar spur. Both mutants were shown to contain transposon insertions in genes encoding homeodomain transcription factors, causing the genes to be expressed in abnormal places (Golz et al. 2002). This discovery provides an opportunity to assess whether the nectar spurs of species such as *L. vulgaris* and many orchids are produced through petal-specific expression of similar genes. If they are, then analysis of the regulation of such genes should provide a functional explanation for the extraordinary evolutionary lability of nectar spurs in the Orchidaceae, a situation for which pollinator behavioural explanations are also currently lacking.

Flower colour

Biological colours can be produced in two different ways. "Structural" colours are caused by the refraction of light from complex physical surfaces, and are found in many birds and insects and a few flowers that mimic animal pollinators (such as the iridescent speculum on the labellum (elaborated lower petal) of many *Ophrys* species). However, plants usually produce colour by synthesising pigments which absorb subsets of the visible spectrum, reflecting back only what they do not absorb and causing the tissue to be perceived as the reflected colour (reviewed in Grotewold 2006). The final colour of a flower is relatively easily altered, and provides a good system in which assessment of pollinator responses can provide useful data on likely evolutionary trajectories. Final flower colour can be modified by changes to the genes encoding enzymes which make pigments, the genes encoding proteins which regulate expression of pigment structural genes and thus pigment pattern, or to genes determining epidermal cell pH, metal content or shape.

Plant pigments can be divided into three chemical classes: the flavonoids, the betalains and the carotenoids. The flavonoids are the major floral pigments in angiosperms, and give rise to ivory and cream colours (flavonols and flavones), yellow and orange colours (aurones and chalcones) and the red-pink-purple-blue range (the

anthocyanins). They are water soluble and accumulate in cell vacuoles. The betalains are a group of pigments found exclusively in the Caryophyllales. They give the red colour to beetroot and also to some flowers. The carotenoids are much more widespread, although less significant as floral pigments than the anthocyanins. They are lipid-soluble and are found in plastids throughout the plant. Carotenoids give yellow and orange colour to flowers.

Plant pigment synthesis has been extensively reviewed by Grotewold (2006). The biochemistry of pigment synthesis is well described for all three groups of floral pigments. At the molecular genetic level it is the anthocyanins which are best understood, with the genes encoding most of the enzymes of the anthocyanin synthetic pathway sequenced from both *Petunia* and *Antirrhinum* (Martin and Gerats 1993). In these model plants mutants are available with lesions in many of these genes, producing flowers with modified colours as the pathway terminates early or forms a novel endpoint (Martin and Gerats 1993). From studies of these plants it has become clear that it is easier for a blue flower to change colour towards red than for a red flower to become bluer, and that it is easier for a red/orange flower to change to yellow/ white than the other way around, because such changes involve the loss of enzyme activities rather than their gain (Grotewold 2006). These mutants provide the potential for analysis of pollinator responses to particular pigments. For example, using isogenic lines differing only in the single gene of interest, it is possible to assess the role of individual enzymes of flavonoid synthesis in the pollination success of the plant. Pollinator responses to naturally occurring shades of ivory, cream, yellow, white, pale pink, magenta, red and purple can be analysed, and conclusions drawn about the relative attractiveness and visibility of these common pigments in real flowers. These data would allow the generation of hypotheses concerning the most "successful" flower colours with respect to certain pollinators, and those hypotheses would allow the identification of natural situations where factors other than pollinator preference and plant genetic lability are determining flower colour.

The majority of petal pigmentation patterns are specified by the expression patterns of regulatory genes encoding transcription factors which control the activity of the pigment biosynthetic genes (Mol et al. 1998; Ramsay and Glover 2005). Use of mutants in these regulatory genes allows us to add variations in pigment patterning (such as ivory corolla tube but magenta petal lobes, or magenta cells overlying petal veins in a white background) to the colour traits we can investigate with regard to pollinator response and preference.

Interactions between floral pigments and metal ions alter the final colour of the petals. For example, the bright blue colour of cornflowers stems from an interaction between a magenta anthocyanin and ions of iron, magnesium and calcium. The combination of the pigment with the metals results in a molecule with its absorption spectrum shifted towards blue (Shiono et al. 2005).

The pH of petal cells can also affect the final colour of the flower, as pH determines anthocyanin structure and absorption spectrum. For example, the light blue petals of *Ipomoea tricolor*, Morning Glory, owe their colour to the effect of a high petal pH on their anthocyanin. The closed buds of these flowers are purplish red and their cells have a pH of 6.6. However, when the flowers open the petal cell pH increases to 7.7, and the pigment changes colour to sky blue (Yoshida et al. 1995). Yoshida et al. (2005) showed that the increased pH is due to active transport of Na^+ and/ or K^+ from the cytosol to the vacuoles. Further support for this mechanism comes from the characterisation of a mutant of *Ipomoea nil*, which fails to undergo colour change on maturity and remains purple as a result of constant pH (Fukada-Tanaka et al. 2000). Yamaguchi et al. (2001) isolated the gene perturbed in the purple mutant and confirmed that it encoded a protein with similarity to Arabidopsis and rice vacuolar Na^+/H^+ exchangers.

Petal pH has been shown to be under the control of genetic factors in a number of other species, including *Petunia* from which seven loci have been identified which determine petal cell pH (de Vlaming et al. 1983; Mol et al. 1998). In *Petunia* these loci work to maintain the acid status of the vacuole and thus the red colour of the anthocyanin, and their influence in pollinator attraction can again be tested using isogenic mutant lines.

The genetic control of floral pigment production, and its association with different pollinators, has been mapped in *Petunia* (Hoballah et al. 2007), *Aquilegia* (Whittall et al. 2006) and *Mimulus* (Bradshaw and Schemske 2003). Bradshaw and Schemske (2003) provided clear evidence that both bumblebees and hummingbirds distinguish between different coloured forms of *Mimulus*, using near isogenic lines (NILs). *Mimulus lewisii* is normally pink, as a result of anthocyanin deposition, and is primarily pollinated by bumblebees. *Mimulus cardinalis* is normally orange/red, as a result of both anthocyanin and carotenoid deposition, and is primarily pollinated by hummingbirds. Bradshaw and Schemske (2003) introgressed the *YUP* locus, responsible for carotenoid deposition, from each species into the other background, through 4 generations, ensuring 97% genetic identity between the new lines and their most similar parent. This resulted in orange coloured *M. lewisii* flowers and deep pink *M. cardinalis* flowers. Pollinator visits to mixed plots were recorded, and revealed that orange-flowered *M. lewisii* received 68-fold more visits from hummingbirds than the wild type pink, but a significant reduction in bumblebee visits. Similarly, the

pink flowered *M. cardinalis* received 74-fold more visits from bumblebees than the wild type orange (although little reduction in hummingbird visits). These experiments show that both bumblebees and hummingbirds exhibit strong discrimination on the basis of petal colour. The near iso-genic nature of the lines used in this elegant study makes it likely, although not certain, that colour is the only signif-icant factor in the choices made by pollinators.

In *Aquilegia*, Whittall et al. (2006) investigated expression of genes encoding enzymes necessary for anthocyanin biosynthesis in a range of species. They found that flowers lacking anthocyanins, and therefore coloured white or yellow, usually showed reduced expression of genes encoding enzymes acting late in the biosynthetic pathway. Lack of expression of late stage genes will result in a build up of white, cream or yellow flavonoids, and the species in which this occurred were often found to be hawkmoth pollinated, compared to the bee and humming-bird pollination usual in anthocyanin-containing species.

In *Petunia* Hoballah et al. (2007) demonstrated that white-flowered *P. axillaris* lines show multiple losses of function of the *AN2* gene, encoding a transcription factor that regulates the enzymes of anthocyanin synthesis. Transgenic introduction of a functional copy of *AN2* from pink-flowered *P. integrifolia* resulted in pink flowers which were signifi-cantly less attractive to the usual pollinators, hawkmoths, in

controlled conditions. However, the transgenic flowers did receive more visits from bumblebees than did wild type white flowers, confirming that this particular locus plays a significant role in pollinator discrimination between closely related *Petunia* species (Hoballah et al. 2007).

Flower texture

Flowers are very rarely smooth. A single petal may contain multiple types of epidermal cells, each providing different textural effects and potentially altering the visual appear-ance of the petal.

Most work has focussed on conical-papillate epidermal cells, which are present on a large subset of Angiosperm petals (Kay et al. 1981). In many species these cells are further ornamented by ridges of thickened cuticle. In Antirrhinum, the conical-papillate cells are found only on the adaxial epidermis of the petal lobes (where they will be seen by potential pollinators as they approach the flower). The *mixta* mutant of Antirrhinum fails to develop conical-papillate petal cells (Noda et al. 1994).

By comparing the ability of epidermal cells to focus light in the wild-type and *mixta* mutant lines, conical-papillate cells have been shown to enhance visible pig-mentation (Fig. 2). The difference in flower colour can be

Fig. 2 Petal texture influences flower colour. (**a**) Scanning electron micrograph of wild-type Antirrhinum petal epidermis, showing conical cells. (**b**) Scanning electron micrograph of Antirrhinum *mixta* mutant flat petal epidermal cells. (**c**) Photograph illustrating the colour difference between these flowers
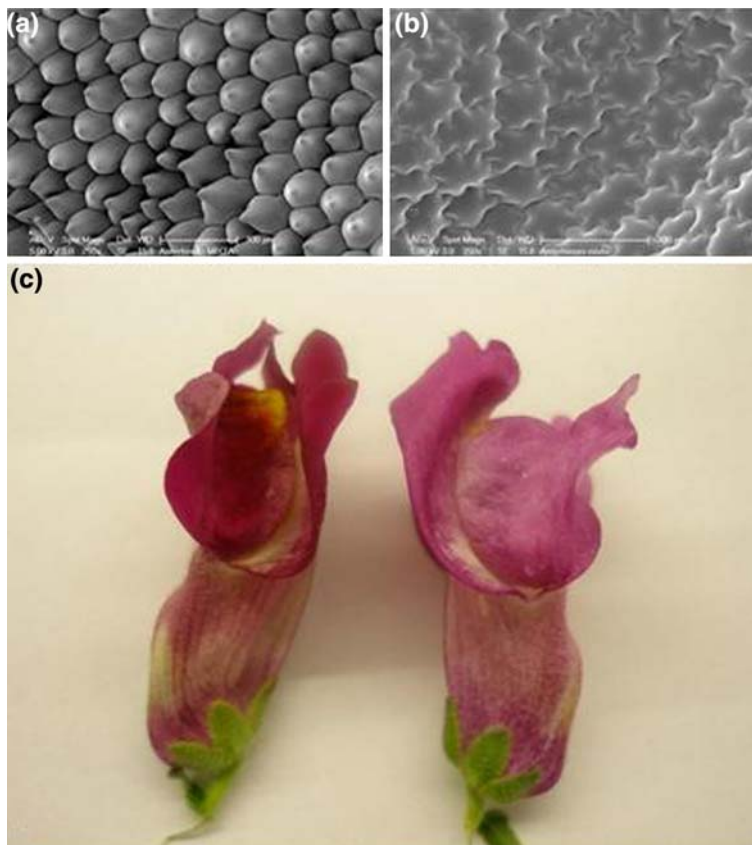
attributed to the focusing of the light onto the light-absorbing pigments in the epidermal cells, and to the reduction in reflection of light at low angles of incidence, resulting in the greater intensity of colour of wild-type conical-celled flowers (Gorton and Vogelmann 1996). It is likely that this trick is used by many plant species to enhance petal colour without incurring the energetic and chemical expense of synthesising extra pigments.

The texture of a flower may also provide tactile guides to pollinator position on the flower, or may be used as a cue by insects in their discrimination between particular flowers. Bees have been trained to recognise the petals of different species by the shape of the cells. Bees were provided with a food reward when they touched epidermal layers composed of certain cell types, but no reward when they touched other shaped cells. They learned very quickly only to search for food when presented with the epidermal tissue which usually accompanied the reward (Kevan and Lane 1985). To investigate the influence of the texture of epidermal tissues on pollinator behaviour the response of bumblebees to the wild type Antirrhinum and *mixta* mutant lines was analysed in a field experiment. Analysis of bee behaviour indicated that bees distinguished between the two genotypes both before and after landing (Glover and Martin 1998; Comba et al. 2000). It is therefore unlikely that epidermal cell shape is used solely as a tactile cue since cell shape evidently provides information that is available before landing (perhaps through depth of colour or other effects on light reflection and scattering). However, this does not exclude the possibility that cell shape is also used by pollinators as a tactile cue post-landing, and recent experiments with resin casts of wild type and *mixta* mutant Antirrhinum petals have confirmed that bees can discriminate between them using touch alone (HW, Lars Chittka and BJG, unpublished).

Floral scent

Floral scents are, in most flowers, a broad and complex mixture, containing anything up to several hundred different volatile organic compounds (Knudsen et al. 1993). The volatiles produced by a flower can vary over its life-span, in different environmental conditions, following circadian rhythms and pre- and post-pollination.

The majority of floral volatiles are either terpenoids or benzenoids, but alcohols, ketones, fatty acids and esters can also be present. Different compounds are produced by flowers attracting different pollinators, such as ammonia and amines in the case of carrion-fly attracting flowers (Procter et al. 1996).

Due to its importance in the perfume industry, volatile production has been well studied, particularly in *Petunia*,

Antirrhinum and *Clarkia breweri*. The genes encoding the enzymes responsible for the synthesis of many monoterpenes, sesquiterpenes and phenylpropanoids have been isolated (Bohlmann et al. 1998; Dudareva et al. 1998; Bushue et al. 1999). However, what is less well understood is the extent to which individual pollinators can identify and are attracted to the various components of floral scent. Guerrieri et al. (2005) tested the ability of bees to distinguish between a range of arbitrarily chosen odors, and observed that alcohols, aldehydes and ketones were the most important chemicals in bee scent perception, with the majority of odour receptors focussed on discriminating between molecules of these types. These compounds are not all commonly released by flowers, and so pollinator discrimination of floral scents may differ somewhat from this general model. The situation is likely to be much more complex in many animals that do not use visual signals to the same degree, such as some beetles, moths and bats. The use of mutant or transgenic lines of *Petunia* or Antirrhinum would allow fascinating insight into this aspect of pollinator attraction. Preliminary analysis of scent production in Antirrhinum suggests that the scents produced vary with flower colour polymorphisms, and it is known that pollinator visitation also varies with flower colour (Odell et al. 1999). The available scent and colour isogenic mutants should allow detailed dissection of these interactions.

Floral rewards

Insect pollination is thought to have originated with insects consuming nutrient-rich pollen and inadvertently transporting some of the excess. Preserved gymnosperm pollen has been found in the guts of insects from as far back as the Permian, suggesting that this mutualism started well before the appearance of the angiosperms (Grimaldi 1999). However, it is also likely that some specific adaptations to animal pollination also arose before the angiosperms, with several gymnosperm species, notably members of the Gnetales, known to produce nectar (Endress 1996). Although pollen and nectar are the most common floral rewards, resin, oil, heat or brood sites are also offered by some flowers. We will consider pollen, nectar and heat in detail here.

Pollen

Pollen is a very nutritious resource, particularly rich in nitrogen, containing 3–10% fat, 1–7% starch and 16–30% protein by dry-weight (Harborne 1993). However, this does mean that pollen is an expensive reward for the plant to produce. Some plants that produce just pollen as a reward

have evolved ways of making pollination more efficient, such as buzz (or vibratile) pollination (Buchmann 1983, 1986). Buzz pollination occurs when pollen is contained within anthers which dehisce through pores at the tip, rather than through longitudinal slits running the length of the anther. Such poricidal anthers release their pollen when vibrated, usually by the flight muscles of bees. Pollen flies out of the pores and is attracted to the bee's body by electrostatic forces. The plant provides no nectar reward, and the small size of the anther pore ensures no pollen is wasted by release into the air. This example demonstrates that pollen quantity and pollen presentation are both relatively flexible in evolutionary time.

Nectar

Nectar is thought to have evolved as a reward somewhat later than pollen, probably sometime before the late Jurassic and therefore before the advent of the angiosperms (Endress 1996; Thien et al. 2000). It is logical that nectar did not appear until after animals had been recruited, by the presence of nutritious pollen, to serve as pollen vectors. Nectar is an aqueous solution of three main sugars (sucrose, glucose and fructose) ranging in total concentration from 15 to 75%. Relatively little is known about the heritability of nectar production as variation due to heredity is swamped by variation caused by the environment (Mitchell 2004). This not only makes analysis of nectar production difficult but also means that analysis of pollinator responses to differing concentrations and volumes of nectar may in fact provide more information about plant reproductive success in certain habitats than about plant reproductive success as a consequence of nectar synthetic programmes. However, recent analyses suggest that at least two QTLs are involved in controlling the amount of nectar produced in Petunia, while another QTL corresponds to the hexose:sucrose ratio (Stuurman et al. 2004).

In different species nectaries can occur on almost any part of the flower. In Arabidopsis, the *crabs claw* mutant was found to be completely lacking nectar due to the need for activity of this gene during growth and maturation of nectaries (Bowman and Smyth 1999). Although nectaries develop on different organs in different species, the *CRABS CLAW* gene appears to be generally necessary for nectary development (Lee et al. 2005). Variability of *CRABS CLAW* expression pattern is presumed responsible for variation in nectary position, and since this trait is relatively labile in evolutionary time it is of interest to assess pollinator responses to nectary positioning within the flower.

Heat

Flowers that are warmer than ambient temperature occur for a number of adaptive reasons, only two of which are directly related to reward. Maintaining their flowers at a temperature that is higher than ambient confers a fitness benefit on plants, particularly when conditions are cool. A warmer temperature speeds up the development of floral organs and of seed. In addition, a warmer flower might experience increased volatilisation of scent compounds, advertising to animals across a wider geographic range and thus attracting increased pollinator attention. As a reward, heat can function in two ways. The simplest of these is by providing a direct metabolic reward to pollinators. A more complex situation might occur where a pollinator's sucrose receptors are poorly buffered for temperature, causing the animal to perceive a warmer sucrose solution as sweeter than a cooler solution of the same concentration. In this instance floral warmth does not actually provide a reward, but is perceived by the animal as doing so. The relative importance of these two scenarios for animals feeding from "ordinary" nectar-rewarding species (as opposed to extreme thermogenic species offering heat as their only reward) is unknown.

Mechanisms to warm flowers are believed to have evolved early in angiosperm history. Various degrees of floral thermogenesis are widely distributed throughout the extant basal angiosperms and some basal monocots, as well as in more derived species. Such mechanisms include heliotropism of the flower, colours that increase floral temperature through heat capture, floral structures that minimize convective heat loss and conical petal epidermal cells, which focus solar radiation into the absorptive floral pigments.

The influence of conical petal epidermal cells on pollinator behaviour has been tested in isogenic lines of Antirrhinum. Comba et al. (2000) showed both that the *mixta* line of flowers (described above) was cooler than the wild-type, and also that the mutant line attracted fewer pollinators. Heat may attract pollinators by dispersing volatile compounds further, but it has also been shown that bumblebees prefer warmer artificial flowers to cooler ones, suggesting that heat functions as a reward in this system (Dyer et al. 2006). Whether this is by providing a direct metabolic benefit or by deceiving the bee into believing that the nectar is more concentrated in the warmer flower, remains to be tested. The use of model species differing in temperature through mutations affecting petal cell shape and heat-absorbing pigment synthesis should allow a detailed analysis of how floral warmth influences pollinator behaviour in flowers both with and without nectar rewards.

Conclusions

The use of a collection of model plant species, each with its own distinctive floral morphology, has allowed the molecular genetic dissection of the development of a number of floral traits. Flower size, of crucial importance in pollinator visual discrimination, is controlled by an extremely robust set of developmental processes, and is not very variable within a species. Flower shape, in particular flower symmetry, has been extensively studied in Antirrhinum and also in legumes, where it has evolved independently through recruitment of similar genes. Flower colour is extremely well characterised, particularly with reference to the biochemistry of pigment production but also with regard to the effects of metal ions and pH. Petal texture has been shown to affect a surprisingly large number of floral traits, and the relative importance of these will likely differ between species. Floral scent and floral reward remain the least well-characterised floral traits, although work in Petunia is beginning to shed light on the production and regulation of floral scent compounds (Dudareva and Pichersky 2000; Verdonk et al. 2005).

The information provided by these developmental genetic studies is of use to behavioural biologists in two main ways. First, it provides information on the flexibility of a trait of interest within a species, indicating what the degree of flexibility is and in what directions it occurs. This information can be used to inform the design of behavioural experiments, to ensure that parameters tested are relevant to real-world situations. Thus, flower size is constrained very tightly by complex sets of interacting genes, and, while it may be interesting to conclude that a large increase in flower size will make flowers more attractive to pollinators, such a situation is not feasible within the developmental constraints of the plant's genetic makeup. On the other hand, flower colour is extremely labile, and experiments to test animal responses to different artificial colours might be very informative. However, within a species it is much more usual for the direction of flexibility to be from blue to red or from red to yellow or white, rather than in the other directions, as a result of the frequent loss of genes encoding key enzymes of pigment synthesis. Comparatively, it is extremely difficult for a yellow flower to become redder, or for a red flower to become bluer, and this information may be useful in designing choice tests.

Second, the range of morphological variants available in model species provides a potential toolbox for behavioural biologists interested in animal interactions with flowers. By selecting isogenic (mutant) or near isogenic lines varying in the trait of interest from one of the model species described, it is possible to dissect the interaction between floral morphology and animal behaviour. The beauty of these systems is that they provide realistic examples of animal-attracting flowers, but the isogenic and near isogenic lines available mean that the complexity and variability of wild plant populations is removed, and only the trait under analysis is variable. Using such lines, the role of yellow pigment in attracting bees and hummingbirds to otherwise pink Mimulus flowers has been determined (Bradshaw and Schemske 2003) and the role of petal epidermal cell shape in attracting bumblebees to Antirrhinum is under investigation (Comba et al. 2000; Dyer et al. 2007). We hope that this review will stimulate more pollination biologists and behavioural scientists to consider using the same toolbox to enrich their own research programmes.

Glossary

ESTs	expressed sequence tags, a collection of sequences of active genes
Genetic map	a map of the positions of genes within a genome
Homeodomain	a structural feature of a family of transcription factors that control tissue growth
Isogenic lines	lines differing at only a single genetic locus
Mutant	genetic line containing an alteration to one or more genes
MYB family	a family of genes encoding transcription factors that control petal cell shape and pigment synthesis
Near isogenic lines	lines differing at only a few genetic loci
QTL	quantitative trait locus, a gene contributing to a quantitative trait such as height
TCP family	a family of genes encoding transcription factors which control floral symmetry (among other things)
TILLING	targeted induced local lesions in genomes, a method of identifying mutations in genes of interest
Transposon	piece of mobile DNA which can insert within genes, causing mutation. Can be a useful tag to help molecular cloning of a gene.
Transcription factor	protein (encoded by a gene) that regulates the expression of another gene or genes
Transgene	piece of DNA inserted into a genome through genetic engineering
Transgenic	a plant line containing a transgene

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