# MEDAL REVIEW

# Floral symmetry

## Enrico S.Coen

Genetics Department, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

### Introduction

Monstrous flowers are curiously attractive. For years, gardeners have sought and maintained plants with abnormal numbers, types or arrangements of floral organs. In some varieties, flowers have even lost their sexual function as their reproductive parts have been modified or replaced. The scientific implications of floral monstrosities have not gone unnoticed. In 1744, Linnaeus devoted a dissertation to an aberrant plant of toadflax, Linaria vulgaris, that had flowers with radial symmetry rather than the normal bilateral symmetry. The aberrant form, which he called Peloria (greek for monster), caused Linnaeus to question whether species were as fixed and immutable as was generally assumed at that time (Linnaeus, 1744; Gustaffson, 1979). Abnormal flowers also played a major part in Goethe's theory that the different types of plant organs, such as leaves, petals and stamens, were simply variations on a common underlying theme (Goethe, 1790). He cited abnormal flowers, in which one type of organ could apparently be converted or replaced by another type, as confirming the fundamental equivalence between organs.

Interest in floral aberrations gradually declined, however, towards the end of the 19th and during most of this century, as plant biologists concentrated on physiological, cellular and molecular processes. Abnormal flowers began to be thought of as rather uninformative teratologies in which the developmental system had been given a nonspecific jolt, making it veer off in a peculiar direction. They were unruly freaks of nature that would not repay further study. It has only been during the last 10 years, through the combined use of molecular and genetic approaches, that floral aberrations have started to regain the attention of biologists.

My first scientific encounter with monstrous flowers occurred in 1983, when I was being interviewed for a position at the John Innes Institute, Norwich. During my visit, Brian Harrison and Rosemary Carpenter took me around the fields and glasshouses to show me some of their mutant *Antirrhinum* plants (snapdragons). Brian had been working on unstable *Antirrhinum* mutants with variegated flowers at the John Innes since the late 1950s, and was about to retire. Rosemary had joined Brian in the early 1960s and over the years they had built up an extraordinarily rich collection of genetic material. They delighted in showing me *Antirrhinum* flowers with all sorts of colours and patterns. I asked whether they had any examples of mutants with altered flower morphology. As if by magic, they produced two remarkable plants. One had small green flowers, with no obvious petals and a column of tissue projecting from the centre. They called this mutant 'coy' because of the rather modest appearance of its flowers (it turned out to be an allele of the *globosa* gene, described below). The second plant was even more dramatic: it had perfect radially symmetrical flowers instead of the normal bilaterally symmetrical type. What really struck me about these mutants was how such profound alterations in the basic plan of the flower could be produced without any apparent effect on the rest of the plant. The mutations were clearly affecting genes with very specific and fundamental roles in flower development.

I had previously done a Ph.D on Drosophila with Gabby Dover, on the evolution and genetic behaviour of ribosomal DNA (Coen et al., 1982; Coen and Dover, 1983). Through this, I had become interested in applying genetic and molecular methods to the study of development and evolution. One problem that had caught my attention was the genetic control of floral development in Primula vulgaris. Individuals of this species are of two sexually incompatible types with distinct floral morphologies. These differences are controlled by a complex genetic locus and I was interested in trying to isolate and study this at the molecular level. I obtained a postdoctoral fellowship to work on the problem and Dick Flavell kindly agreed to let me carry out the work in his lab at the Plant Breeding Institute, Cambridge. After working on this for a while, it became clear to me that trying to develop the required molecular and genetic tools in the Primula system from scratch was over-ambitious.

The Antirrhinum material seemed to offer a much better opportunity to follow up the interests I had now developed in plants. There was already a clear possibility of applying molecular analysis to the system because the group of Heinz Saedler and Hans Sommer at the Max-Planck Institute, Cologne had just shown that some of the Antirrhinum variegated flower mutants, sent to them by Brian Harrison, were caused by transposon insertions (Wienand et al., 1982; Bonas et al., 1984a,b). Transposon behaviour and the way it influenced gene expression was itself a fascinating problem that had now become open to molecular and genetic analysis. In the longer term, I felt that the availability of cloned transposons might also provide a way to study the flower developmental mutants that had so struck me. These were some of the main reasons for my wanting to pursue the Antirrhinum system in Norwich. There was, however, another important reason. Over the years, Rosemary Carpenter had acquired unrivalled experience in Antirrhinum genetics. She had systematically maintained records and genetic pedigrees going back 20 years and was very enthusiastic about continuing with her work and collaborating with others. Having had little experience in plant genetics myself, working with Rosemary seemed to have the makings of a perfect



**Fig. 1.** Pigmentation patterns in flower petals of *Antirrhinum*. (a) wild type showing most pigmentation in petal lobes. (b) *pal* allele conferring stronger pigmentation in tube than lobes. (c) *pal* allele conferring weak pigmentation mainly confined to the tube. (d) *pal* allele with pigment only at the base of the tube. (e) *del* mutant showing pigment only in petal lobes (adapted from Almeida *et al.*, 1989).

collaboration. I was completely hooked and was fortunate enough to get a position to join the Institute in 1984.

Before going to Norwich, I decided to visit a few labs in the USA to find out more about plant transposons. My most memorable experience was talking with Barbara McClintock at Cold Spring Harbor about her work on maize transposable elements. She had a deeply lined, almost walnut-like face that scrutinized me with a pair of bright piercing eyes (she was in her eighties at the time). Talking with her was very stimulating, although I found it difficult to keep up as she darted from one subject to the next. I mentioned some of the work I was intending to do on Antirrhinum but she thought I might be wasting my time and advised me to think again. I left awestruck by the boundless energy and child-like enthusiasm of someone more than three times my age. About 10 years later I met her again and to my great surprise she greeted me with 'Sure, I remember you. I gave you some bum advice several years ago'.

### **Pigments and prepatterns**

In the 1950s, Curt Stern proposed a general way that genes could determine patterns (Stern, 1954). Based on the analysis of *Drosophila* mutants with altered distribution of bristles, he suggested that the fly contained a hidden prepattern of regional differences. Genes could interpret this prepattern in various ways, accounting for many different observable phenotypic patterns. However, the details of what these prepatterns were or how genes could interpret them were entirely unclear.

This problem formed the basis of the first project I started working on when I arrived in Norwich, being concerned with how patterns of flower colour might be determined. A series of alleles at the *pallida* (*pal*) locus had been described that gave different patterns and intensities of flower colour in *Antirrhinum* (Figure 1a–d; Baur, 1924; Fincham and Harrison, 1967). The patterns varied from flowers having pigmentation only at the base, through to flowers that were almost as fully pigmented as wild type. The alleles had all been derived from an unstable allele, *pal<sup>rec</sup>*, that gave variegated flowers with red sectors on an unpigmented background. The problem was how a

to isolate the *pal* gene. Based on its variegated phenotype and unstable genetic behaviour, it seemed very likely that  $pal^{rec}$  was caused by a transposon insertion. The red sites

 $pal^{rec}$  was caused by a transposon insertion. The red sites on the flowers could be explained by somatic excision of the transposon from the *pal* gene during development, restoring sectors of gene activity and hence red flower colour. If the transposon responsible for *pal<sup>rec</sup>* could be identified, it should be possible to use it as a tag to isolate *pal*. Fortunately, a good candidate for the transposon, called Tam3, had just been isolated by Hans Sommer's group in Cologne (Sommer *et al.*, 1985). Cathie and I therefore set off to spend a few months in Cologne to try and test whether *pal<sup>rec</sup>* was caused by a Tam3 insertion. The trip proved successful and we returned clutching several clones of the *pal* gene (Martin *et al.*, 1985).

single unstable allele of this kind could generate such a

wide range of alleles conferring different colour patterns.

Cathie Martin, who had also just been appointed at Norwich, Rosemary Carpenter and I embarked on trying

We were now in a position to study the origin and structure of the *pal* alleles that gave different flower colour patterns. It became clear from biochemical studies and structural comparisons that the pal gene encoded an enzyme involved in flower pigment biosynthesis. The patterns of colour might therefore be more easily explained if they were due to changes in the regulation of *pal* rather than to alterations in the protein product. We showed that all the alleles were regulatory mutants that had arisen from *pal<sup>rec</sup>* by imprecise transposon excisions or rearrangements (Coen et al., 1986; Robbins et al., 1989; Hudson et al., 1990). In most cases, plant transposons excise imprecisely, leaving small DNA alterations or deletions, possibly due to variation in the way that DNA hairpins are resolved at the excision site (Coen and Carpenter, 1988; Coen et al., 1989). The transposon responsible for palrec was located within the promoter region, so imprecise excision had produced a series of overlapping promoter deletions, altering the pattern of *pal* expression and hence flower colour (Almeida et al., 1989).

Our results indicated that the flower patterns resulted from the way the *pal* promoter interpreted an underlying pattern. The flower seemed to contain a prepattern which could be revealed in various aspects when particular parts



**Fig. 2.** Floral diagrams of the *Antirrhinum* flower coloured to emphasize either the radial or dorsoventral axes. The flowers are shown with the stem above and the bract below (black). The radial system shows the four concentric whorls of the flower: sepals (green), petals (red), stamens (yellow) and carpels (brown). The dorsoventral axis illustrates the dorsal (blue), lateral (light brown) and ventral (pale yellow) organ types. Note that the dorsal stamen (circle) is arrested at an early stage in development.

of the promoter were removed. The work on *pal*, together with parallel studies in other systems, showed how the genetic interpretation of prepatterns could depend on the structure of promoter or regulatory regions. The question then became what factors determined the prepattern.

A candidate prepattern gene for flower colour was delila (del). The five petals of an Antirrhinum flower are united for part of their length to form a tube, as distinct from the petal lobes which are more separate. In *del* mutants, the petal lobes are red but the tube of the flower is unpigmented (Figure 1e). Tim Robbins showed that the levels of pal transcript were greatly reduced in the unpigmented regions of *del* mutant flowers, indicating that *del* was involved in regulating *pal* transcription. The normal del gene product could therefore be a component of the prepattern interpreted by pal. To test this further, Jorge Almeida compared the expression of various pal alleles in wild-type and *del* mutant backgrounds. He showed that some alleles interacted with *del* in a different way, confirming that del was indeed one element in the prepattern (Almeida et al., 1989). The simplest explanation was that *del* encoded a transcription factor that bound to particular regions of the *pal* promoter. Several years later, Justin Goodrich isolated del and showed that it did indeed encode a transcription factor, belonging to the helix-loophelix myc family (Goodrich et al., 1992). Many flower colour patterns could therefore be explained as being due either to changes in the regulatory regions of biosynthetic genes or to mutations in components of the prepattern of transcription factors that they interpret.

This approach to analysing colour patterns had a very important limitation. Showing that the pattern of *pal* activity depended on how it interpreted a prepattern of transcription factors simply begged the question of what in turn regulated the prepattern. During the 1980s it became increasingly clear that the answer to this sort of question was not to be found by simply looking at more and more mutants with altered patterns of decoration, be they patterns of bristles or colour. Rather than looking at the decorations, attention switched to looking at mutants affecting the structure being decorated. To understand, for example, why the lobes of a petal may contain a different transcription factor from its tube, it is no good just analysing more flower colour pattern mutants. You have to look for the genes that set up the differences between lobes and tube in the first place. The work on flower colour therefore led back to the question of how floral morphology is determined.

### **Exploring the field**

To get a handle on genes affecting floral morphology, we wished to try and inactivate them with transposon insertions. In 1985, Rosemary Carpenter and I decided to set up a large scale transposon-mutagenesis experiment in Antirrhinum. The idea was to grow and self-pollinate lines carrying active transposons at 15°C, a temperature which favours transposition in Antirrhinum (Harrison and Fincham, 1964; Carpenter et al., 1987). Large numbers of their progeny would then be grown and self-pollinated to reveal homozygous mutant phenotypes in the next generation. Over a 4 year period, we aimed to have about 26 000 plants self-pollinated and screen 80 000 final progeny. One early problem we encountered was getting the funding to grow the plants. It was not that the cost was particularly high-it was less than that needed to support a post-doc. for 2 years. The problem was that it did not involve any new or exciting technology but simply growing lots of plants, more than 99.9% of which would be completely useless. Luckily, we did eventually get funding from the Gatsby Research Foundation, and were able to proceed with the experiment.

The screens turned out to be more productive than we could have dreamed (Carpenter and Coen, 1990). Walking through the fields of *Antirrhinum* flowers, we encountered all sorts of mutants with altered flower shapes and forms. The screens provided, in the most pleasurable way, key material that became the subject of genetic and molecular investigations over the following years. Before describing some of the results, I need to mention briefly the basic layout of an *Antirrhinum* wild-type flower.

Two axes of asymmetry characterize the plan of an *Antirrhinum* flower (Figure 2). Along the *radial axis*, four types of organs are produced in concentric whorls, going from the outside of the flower towards its centre in the order: sepals, petals, stamens and carpels (whorls 1–4, respectively). The *dorsoventral axis* is oriented such that the dorsal (upper) part of the flower is nearer the stem

and the ventral (lower) part is near to the bract, a small leaf-like organ subtending the flower. Dorsoventral asymmetry is most apparent in petals and stamens, each of which can be divided into three types: dorsal, lateral and ventral. I shall treat the genes affecting each axis in turn and then go on to describe some genes that are involved at earlier stages, when the axes are starting to be set up.

## The radial axis

Before beginning the mutagenesis experiment, we were already aware of one mutant that affected organ type along the radial axis. Closer examination of the small green-flowered mutant that I had seen on my first visit to Norwich showed that it had transformed organs in two whorls (Figure 3b). It had sepals growing in whorl 2 instead of petals, and carpels growing in place of stamens in whorl 3. The phenotype could therefore be summarized as sepal, sepal, carpel, carpel, as compared with the wildtype sepal, petal, stamen, carpel (the carpels in whorl 4 of the mutant did not always develop). The mutant had a similar phenotype to that of two previously described Antirrhinum mutants, deficiens (def) and globosa (glo) (Stubbe, 1966), and crosses showed that it was an allele of glo. The phenotype of these mutants suggested that there was a specific genetic function in whorls 2 and 3 that conferred petal and stamen identity. It was unclear, however, how carpel or sepal identity was established.

In the summer of 1988, we found a mutant in which the sepals in whorl 1 had been replaced by carpels, but it was less obvious what had happened to the second whorl, where petals normally form. It seemed that these organs were narrow and strap-like with abnormal structures at the ends. I went home in the evening after having spent some time looking at these flowers and kept thinking about the new phenotype. It occurred to me that if the strange strap-like structures were due to a transformation of petals towards stamens, a simple combinatorial model could account for the mutants. Suppose the wild-type flower contains two genetic functions a and b that are active in whorls 1-4 in the combination a, ab, b, 0, conferring identities sepal, petal, stamen, carpel, respectively. Loss of the b function would give the combination a, a, 0, 0, specifying sepal, sepal, carpel, carpel, the phenotype conferred by def and glo mutants. Plants lacking the a function would have 0, b, b, 0, specifying carpel, stamen, stamen, carpel, the possible phenotype of the new mutant. The next morning, I rushed into the greenhouse to look at the mutant flowers again. To my delight the strap-like organs in whorl 2 did indeed have some telltale features of stamens that I had overlooked the previous day. Later on we obtained some much clearer examples of this type of mutation where there could be little doubt that stamens had replaced petals and carpels replaced sepals (Figure 3a), but the earlier anticipation of the result has remained with me as a striking example of how observations and descriptions are influenced by what you are looking for.

Although this model could account for two phenotypes we also needed to explain a third phenotype, plena, that came from the screens. In flowers of *plena* mutants, the stamens and carpels of whorls 3 and 4 were replaced by



**Fig. 3.** Flowers of wild type and organ identity mutants. (wt) Wild type flower with the phenotype sepal, petal, stamen, carpel (the stamens and carpels are hidden from view inside the flower). (a) Absence of a function leading to the phenotype carpel, stamen, stamen, carpel. (b) Loss of b function leading to sepal, sepal, carpel, carpel (whorl 4 does not always develop). The carpels of whorl 3 are united, so that their styles form a large column that can be seen projecting from the flower. (c) Loss of c function giving sepal, petal, petal, followed by a reiteration of sepal and petal organs (adapted from Coen and Meyerowitz, 1991).

petals or sepals, giving a flower with no reproductive organs (Figure 3c). According to the model, the production of petals and sepals in the outer two whorls required the a function, so their presence in the inner whorls of the mutant suggested that a was ectopically active. This implied that *plena* mutants might lack a third function, c, which was normally expressed in whorls 3 and 4 where it inhibited activity of a (Carpenter and Coen, 1990). In addition to affecting organ identity, normal *plena* function was also needed to limit the number of whorls in the flower because *plena* mutants had a proliferation of extra whorls within whorl 4, giving the flower an indeterminate number of organs.

The following year, I presented our findings at a meeting in the US attended by Marty Yanofsky from Elliot Meyerowitz's lab at Caltech, working on floral mutants in *Arabidopsis*. Marty told me that they were thinking along similar lines in Elliot's lab and had come up with a comparable model. The models were indeed very similar, the main difference being that they had shown that in addition to c inhibiting a, the a function also inhibited c (Bowman *et al.*, 1991; Carpenter and Coen, 1990). The genes needed for the a, b and c functions were christened *organ identity* genes, as their combined action a, ab, bc, c in the four whorls were responsible for the wild-type identities sepal, petal, stamen, carpel (Figure 4; Coen and Meyerowitz, 1991).

Showing that a similar model for floral organ identity applied to distantly related flowering plants, *Antirrhinum* and *Arabidopsis*, had two important consequences. First, it showed that there was a conserved underlying genetic mechanism for the control of identity along the radial axis. This may not seem particularly surprising because the basic organization of the flower into sterile outer



**Fig. 4.** Radial system of *abc* functions determined by the organ identity genes. In whorl 1, *a* specifies sepals (green); in whorl 2, *a* and *b* specify petals (red); in whorl 3, *b* and *c* specify stamens; in whorl 4, *c* specifies carpels. In addition to affecting organ identity, the gene needed for *c* also limits whorl number (i.e. confers determinacy).

organs encircling reproductive organs is highly conserved. However, previous anthologies of floral teratologies had given the impression that almost any organ could be replaced by another without rhyme or reason (Meyer, 1966). It was therefore a considerable surprise to find that the mutants fell into conserved classes that could be accounted for by a simple model.

The second consequence of finding such conservation was at a more practical level. If the mutants were so similar in different species, the genes involved might also be expected to be held in common. Isolation of an organ identity gene from one species could therefore be used to isolate its counterpart in another. For example, the b genes, first isolated from Antirrhinum by the group of Hans Sommer and Zsuzsanna Schwarz-Sommer, were used to isolate the b orthologues from Arabidopsis (Sommer et al., 1990; Jack et al., 1992; Tröbner et al., 1992; Goto and Meyerowitz, 1994). Similarly, we used the c gene, isolated from Arabidopsis in Elliot Meyerowitz's group, to isolate the plena gene from Antirrhinum (Yanofsky et al., 1990; Bradley et al., 1993). All of these genes turned out to code for proteins that belonged to the same family of transcription factors, called the MADS box family. The a function genes of Arabidopsis also code for transcription factors but one of them does not belong to the MADS box family (Mandel et al., 1992b; Jofuku et al., 1994). The *abc* functions therefore reflected the combined action of specific transcription factors in different whorls of the flower.

Further analysis of *plena* gave us some insights into how the *a* and *c* functions interacted in *Antirrhinum*. Rosemary Carpenter had made the curious observation that plants carrying a transposon at *plena*, resulting in loss of *c*, occasionally gave progeny with a loss of *a* phenotype. Given the proposed antagonism between *a* and *c*, I wondered if these exceptional progeny had arisen by a transposon-induced change at the *plena* locus, giving a regulatory mutant that expressed *c* ectopically. In fact, all of the *a* function mutants we had obtained were semidominant, consistent with their being gain-of-function regulatory mutants in the *c* gene. Desmond Bradley tested this by determining the structure and transcriptional pattern of *plena* in the various mutants (Bradley *et al.*, 1993). In wild-type flowers, plena expression was restricted to whorls 3 and 4, the normal domain of c activity. In contrast, all of the mutants classified as lacking a showed ectopic expression of *plena* in whorls 1 and 2. The ectopic mutants had alterations in an intron of *ple*, indicating that the intron carried regulatory elements that were normally involved in preventing *ple* expression in the outer whorls. What had been called the *a* function in Antirrhinum therefore corresponded to factors that were involved in negatively regulating *ple* in whorls 1 and 2. In the ectopic mutants, ple regulation became uncoupled from a, rendering a ineffective and allowing c activity in all whorls. This produced the combination c, bc, bc, c, and the phenotype carpel, stamen, stamen, carpel. The results also showed that expression of *plena* in floral organs was sufficient to confer sex organ identity. This was also demonstrated in Arabidopsis by ectopic expression of the c gene in transgenic plants (Mandel et al., 1992a; Mizukami and Ma, 1992).

#### The dorsoventral axis

A major aim of our screens was to try to find transposoninduced mutants with reduced dorsoventral asymmetry. Lines of *Antirrhinum* in which dorsoventral asymmetry is diminished or lost have been known for many years (Moquin-Tandon, 1841; Darwin, 1868; Masters, 1869; Stubbe, 1966). In the most extreme cases, the lines have flowers that look perfectly radially symmetrical, a phenotype called *peloric*. The petals and stamens of these peloric flowers are not, however, of a completely new type but closely resemble their ventral counterparts in wild-type flowers (Figure 5). The flowers therefore appear to be ventralized, indicating that they lack genetic functions that normally act in dorsal regions to establish asymmetry (Carpenter and Coen, 1990).

Although we did not obtain any peloric mutants from our screens, we did get several mutants with a 'semipeloric' phenotype, intermediate between peloric and wild type (Figure 5). The flowers from these mutants had lateral and ventral organs that resembled those of peloric flowers, but the remaining organs had a combination of dorsal and lateral characteristics (see petal diagrams in Figure 5). The semipeloric mutants fell into two complementation groups: *cycloidea* (*cyc*) and *radialis* (*rad*) (Carpenter and Coen, 1990; Luo *et al.*, 1996). The *cyc* mutations were particularly interesting because they were allelic with mutations carried in lines with peloric flowers, indicating that *cyc* might be a key component in setting up dorsoventral asymmetry.

Da Luo identified the transposon responsible for one of the cyc mutations and was then able to isolate and analyse cyc (Luo et al., 1996). The predicted CYC protein showed no homology with other proteins of known function, although it did contain a consensus nuclear localization signal, consistent with its playing a role in transcriptional regulation. The most exciting results came from RNA *in situ* hybridizations with cyc. They revealed that cyc was expressed specifically in dorsal regions at a very early stage of floral development, before any morphological asymmetry along the dorsoventral axis was manifest (Figure 6). Expression continued through to later stages in the dorsal petal and stamen primordia. This indicated



Fig. 5. Wild type, semipeloric and peloric flowers. On the left, flowers are photographed in face view with the dorsal (d), lateral (l) and ventral (v) petal lobes indicated for wild type. The characteristics of the individual petal lobes are diagrammed to the right of each flower, colour coded as in Figure 2 to indicate dorsal (blue), lateral (light brown) and ventral (pale yellow). For the semipeloric flower, petals with characteristics of both dorsal and lateral petals are indicated as part blue, part light brown. In all cases only five petal lobes are shown for simplicity (mutant flowers often have an extra petal). Modified from Luo *et al.* (1996).



Fig. 6. Expression patterns of cyc, flo and cen as determined by RNA in situ hybridizations. A longitudinal section through the growing tip of the inflorescence is shown with inflorescence meristem (I), floral meristems (F) and bract primordia (B) indicated. The cyc gene is only expressed in a dorsal region of the floral meristem. Expression of flo is in bract primordia and floral meristems but not in the inflorescence meristem. Expression of cen is just below the inflorescence apex.

that floral asymmetry depended on establishing spatially specific expression of *cyc*.

One surprise was that no cyc expression was detected in lateral organs, even though they are ventralized in cycmutants. The most likely explanation is that cyc expression in the dorsal domain acts through signalling between cells, to influence the behaviour of lateral organ primordia. One possibility is that the flower is first divided into two regions: a dorsal domain expressing cyc and the remaining domain with no cyc expression. This early partition could then be further elaborated by cell-cell interactions to generate three distinct domains: dorsal, lateral and ventral. Candidate genes involved in this elaboration are rad, which gives a similar mutant phenotype to cyc, and *divaricata* which confers a lateralized mutant phenotype (J.Almeida, M.Rocheta and L.Galego, personal communication).

It is essential that the domain of cyc expression is precisely aligned with respect to the organ primordia in the flower, otherwise inappropriate organ types would develop. A clue to how this might be achieved came from the observation that cyc mutants often had six organs per outer whorl rather than the normal five organs per whorl. By comparing the development of mutant and wild type, we showed that this was because  $Cyc^+$  acts very early to repress growth and primordium initiation in dorsal regions of the flower. Perhaps this provides a mechanism for coupling cyc expression with organ position. For example,



Fig. 7. The flower and the fly.

by retarding growth, early  $Cyc^+$  activity could ensure that primordium development in dorsal regions is centred within the cyc expression domain.

Although cvc clearly played an important role in establishing dorsoventral asymmetry, we still had to explain why cyc mutant flowers gave a semipeloric rather than peloric phenotype. It seemed that either all our cvc mutants were leaky or genetic factors other than cyc were responsible for the residual asymmetry of semipeloric flowers. This was further investigated by screening cyc mutants for derivatives that had completely peloric flowers. We derived two peloric mutants in this way. Neither of these had detectable changes at the cyc locus, indicating that the peloric derivatives were due to mutations in a second gene. While we were carrying out this work, Jorge Almeida's group in Lisbon had shown that a peloric line of Antirrhinum carried two mutations: cyc and a mutation that gave flowers in which the dorsal petals were deeply separated. I mentioned to Jorge that this second mutation gave a similar phenotype to that of *dichotoma* (*dich*), a mutation first described in the 1930s (Kuckuck and Schick. 1930). He confirmed by allelism tests that the peloric line was indeed doubly mutant for cyc and dich (J.Almeida, M.Rocheta and L.Galego, personal communication). Da Luo then showed that the peloric plants we had derived from cyc had also arisen through mutations in the dich gene. Thus, the cyc and dich genes are both needed to set up full dorsoventrality and can partially substitute for each other, allowing for residual asymmetry in each single mutant from Luo et al. (1996). However, the role of cvc appears to be more critical than that of *dich* because its mutant phenotype is more extreme.

In addition to establishing differences between dorsal, lateral and ventral organs, the *cyc* and *dich* genes are also required to set up dorsoventral asymmetry within individual organs. Unlike the ventral petal, which is bilaterally symmetrical, dorsal and lateral petals are asymmetrical along the dorsoventral axis of wild-type flowers (Figure 5). This internal asymmetry is lost in *cyc:dich* double mutants and reduced in *cyc* or *dich* single mutants, suggesting that in addition to setting up differences between organs, *cyc* and *dich* are needed to establish subdomains within organs along the dorsoventral axis.

How do the radial and dorsoventral axes of the flower interact with each other? This can be answered by comparing dorsoventral asymmetry in different whorls. In whorl 2

of wild type, the dorsal petal lobes grow to be larger than the other petal lobes of the flower whereas in whorl 3, the dorsal stamen is retarded in growth relative to the other stamens and is eventually arrested in development. Thus, petal growth is enhanced in dorsal regions but stamen growth is repressed. This difference in behaviour of petals and stamens could reflect either their location in different whorls or their distinct organ identities. We were able to test this by looking at dorsoventral asymmetry in organ identity mutants. If repression of growth in dorsal regions was a consequence of stamen identity, it should occur whether the stamens were in whorl 3 or 2. In mutants having stamens in place of petals, the dorsal stamens of whorl 2 were indeed retarded in growth, showing that the fate of primordia in dorsal regions depended on their radial organ identity. There is therefore a combinatorial interaction between the dorsoventral and radial systems. It follows that the only primordia in the flower with the same combination of gene activities are those located at mirror image positions on either side of the dorsoventral axis, accounting for the bilateral symmetry of the flower (Carpenter and Coen, 1990).

#### The flower and the fly

There are many similarities between the ways in which the basic plan of a flower and a fly are genetically determined. To see this more clearly, I have illustrated both the *Antirrhinum* flower and *Drosophila* body from a botanical viewpoint (Figure 7). Both systems depend on the combined action of genes defining regional identities along two axes. Floral whorls along the radial axis can be compared with fly segments along the anterior–posterior axis. Regional identities along both of these axes involve spatially specific expression of transcription factors, belonging mostly to the MADS box family in flowers and the homeobox family in flies. These axes interact combinatorially with dorsoventral axes of each system to provide a bilaterally symmetrical plan.

These similarities are the more striking because the two systems are thought to have evolved independently from unicellular ancestors. They reflect a common way that multicellular organisms have recruited mechanisms of transcriptional regulation to generate regional identities. Although many of the mechanisms appear to be similar, they can nevertheless be superimposed on quite different



Fig. 8. Part of the inflorescence of a flo mutant (left) compared with wild type (right). In the mutant, a shoot instead of a flower grows in the axil of a bract (all but one of the axillary shoots on the flo mutant or of the flowers of wild type have been removed for clarity) (from Coen *et al.*, 1990).

growth patterns. The segments of *Drosophila*, for example, arise by synchronous subdivision of an egg whereas the whorls of a flower arise by sequential growth on the periphery of a growing tip, the meristem. This is reflected in the mutants: *Drosophila* mutants with altered segment number generally have fewer segments than wild type whereas mutants affecting whorl number in flowers can give a proliferation of whorls that continue to be added indeterminately.

One important difference between the systems is that dorsoventrality evolved much more recently in flowers than in flies. Dorsoventral asymmetry of flowers is thought to have arisen many times independently from a radially symmetrical ancestral condition, as a specialized adaptation to animal pollinators. In matching the shape and behaviour of pollinators more precisely, dorsoventrality in flowers is therefore a relatively recent evolutionary response to the much more ancient dorsoventral asymmetry of animals. This raises the question of how genes like cyc and dich became recruited to confer a new axis of asymmetry and what role, if any, these genes play in species, such as Arabidopsis, that have radially symmetrical flowers. It will also be interesting to determine whether the same genes have been recruited in species that are thought to have evolved dorsoventrality independently (Coen and Nugent, 1994).

## Early activators of floral development

The radial and dorsoventral systems themselves depend on earlier acting genes that are needed to initiate floral development. The first of these to be isolated and analysed was the *floricaula* (*flo*) gene (Coen *et al.*, 1990). Wildtype *Antirrhinum* plants produce flowers in the axils of bracts along the main inflorescence. The flowers first arise as groups of dividing cells, floral meristems, on the periphery of the main growing tip, the inflorescence meristem. Instead of flowers, the *flo* mutant produced side shoots that resembled the main inflorescence shoot (Figure 8). These side shoots could themselves produce further shoots so that the whole inflorescence grew indeterminately. The *flo* gene was therefore needed for promoting floral meristem identity and in its absence, inflorescence identity was simply reiterated.

We were first struck by the *flo* mutant in our screens of 1987, as an individual lacking flowers amidst a sea of flowering plants. The problem was that because it produced no flowers, there was little we could do with the mutant other than propagate it vegetatively. Our only hope was that by placing it at 15°C, a temperature which increases transposition, the mutant might revert back to flower production as a consequence of transposon excision. Fortunately, a few revertant flowers did eventually appear on the *flo* mutant treated this way, making further analysis possible. The flo mutant turned out to be caused by a Tam3 insertion, allowing the gene to be isolated and its RNA expression pattern determined (Coen et al., 1990). Expression of *flo* was first detected at a very early stage in bract primordia and their axillary floral meristems, consistent with its role in defining meristem identity (Figure 6). At later stages, flo was transiently expressed in sepal, petal and carpel primordia. José Romero and Bob Elliott cloned and sequenced the flo cDNA and showed that it had no extensive homology to any other known sequences, although it did have some features consistent with its coding for a transcription factor.

The major effect of *flo* on *Antirrhinum* flower development raised the question of whether it played a similar role in other flowering plants. Detlef Weigel in Elliot Meyerowitz's lab used *flo* to isolate its *Arabidopsis* counterpart and showed that it corresponded to *leafy*, a gene with a comparable mutant phenotype to *flo* (Weigel *et al.*, 1992). Thus *flo* and *leafy* were members of a general class of genes needed to promote flower development, termed *meristem identity* genes (Coen and Meyerowitz, 1991). Other members of this class were subsequently isolated from *Antirrhinum* and *Arabidopsis* and shown to interact with *flo/leafy* to confer floral identity (Huala and Sussex, 1992; Huijser *et al.*, 1992; Mandel *et al.*, 1993; Schultz and Haughn, 1993; Carpenter *et al.*, 1995; Kempin *et al.*, 1995).

The phenotype of meristem identity gene mutants suggested that one of the roles of these genes might be to activate the radial system of organ identity genes (Coen et al., 1990; Wiegel and Meyerowitz, 1993). We showed that this activation was mediated, at least in part, by another gene, fimbriata (fim). The phenotype of fim mutants had features of both meristem and organ identity mutants, displaying transformations of floral organ identity and reduced determinacy of the floral meristem (Carpenter and Coen, 1990; Simon et al., 1994). Rüdiger Simon and Sandra Doyle identified the transposon inserted at fim and were able to isolate the locus. Rüdiger showed that fim occupies an intermediate position in a sequence of gene activation that starts with the meristem identity genes and leads to the expression of organ identity genes in specific whorls of the floral meristem (Simon et al., 1994). Analysis of cell lineages in the flower suggests that organ identity gene expression may be allocated to cells at a very early stage in floral development (Vincent et al., 1995). One role of fim may be to ensure that this allocation is precisely aligned with the morphological boundaries between whorls. A comparable function may be carried out by the



**Fig. 9.** Origin of periclinal chimeras in meristems with three layers. An L1 cell carrying a mutation, shown black, divides to produce a patch of cells that overlies the developing side branch, thus giving rise to a periclinal chimera (from Carpenter and Coen, 1995).

unusual floral organs (ufo) gene of Arabidopsis (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995) which was isolated by homology with *fim* (Ingram *et al.*, 1995).

### Chimeras

In addition to the radial and dorsoventral systems, flowers have an axis that distinguishes internal from external tissues. In many animals, the inside-outside axis is elaborated early in development through cell movements that establish three embryonic layers: endoderm, mesoderm and ectoderm. In contrast to the situation in animals, cells do not move relative to each other during plant development so the three-dimensional structure of plants depends entirely on patterns of cell growth and division. These patterns result in three layers of dividing cells, L1-L3, in the shoot meristems of many flowering plants (Figure 9; Tilney-Bassett, 1986). Each layer corresponds to a largely distinct cell lineage. The cells descended from the L1 mainly give rise to the epidermis, those from the L2 produce the subepidermal tissues and the germ cells of reproductive organs, and those from the L3 give rise to core tissues. If a somatic mutation occurs early in meristem development, it may give a branch in which only one layer of the meristem is mutant (Figure 9); this is called a 'periclinal chimera'. Vegetative propagation of the branch allows the chimera to be maintained.

The genes involved in establishing the radial and dorsoventral axes are expressed in all layers, raising the question of how their expression can be coordinated across different cell lineages. We were able to address this problem by analysing flo chimeras. Plants carrying a transposon in the flo gene occasionally produced flowering branches on otherwise mutant inflorescences (Carpenter and Coen, 1990). Seed collected from these branches gave either mutant progeny alone or they segregated for wild type and mutant in a 3:1 ratio. The simplest explanation was that the transposon had excised from flo early in development, giving a chimeric branch in which gene function was restored in one layer (Figure 9; Coen, 1991). Transmission of the revertant allele to progeny would only be observed in those cases where excision had occurred in L2, the source of germ cells. Previous studies on plant chimeras had used indirect methods to determine the gene activity in the various layers. However, we were able to use a more direct approach with flo chimeras because we had isolated flo and knew it was expressed in all three layers of wild type. The prediction was that the revertant branches should express flo only in single layers of their meristematic tips. To test this, we needed to propagate



Fig. 10. Top view of a *cen* mutant inflorescence showing the radially symmetrical (peloric) flower at the top with normal flowers below it.

the flowering branches so that their gene expression patterns could be determined. Normally if you want to propagate *Antirrhinum*, you take cuttings from young vegetative shoots. In our case, this would not have been very useful because we needed to propagate branches only after we had seen that they had flowers on them. I suggested we try and take cuttings from the growing tips of the flowering branches. The gardener, Peter Walker, was not optimistic about this working but said he would give it a go anyway. Luckily he got it to work and this allowed us to produce and maintain entire plants derived from the revertant branches.

Sabine Hantke analysed *flo* expression in the propagated plants and showed that it was only in one layer, either L1, L2 or L3, confirming that the plants were periclinal chimeras (Carpenter and Coen, 1995; Hantke et al., 1995). She went on to determine the expression pattern of the downstream organ identity genes in the chimeras, showing that they were expressed in all three layers. Thus, expression of *flo* in any one layer could activate organ identity genes in all three layers, showing that  $Flo^+$  activity could be transmitted from one cell to another to promote gene transcription. This may reflect movement of the FLO protein between layers through cytoplasmic connections between cells, plasmodesmata, or it might be mediated by a distinct cell-cell signalling mechanism (Carpenter and Coen, 1995; Lucas et al., 1995). In either case, these experiments revealed a process that coordinated gene activity between layers at a very early stage of development.

#### Inflorescence architecture

The regulation of floral meristem identity genes can determine the architecture of inflorescences. Inflorescences

can be classified into two basic types, 'determinate' and 'indeterminate'. In plants with determinate inflorescences, the main axis of growth terminates in a flower and further flowers are produced by branching events that occur below this. In species with indeterminate inflorescences, such as Antirrhinum, the main growing tip is prevented from forming a flower and can therefore continue indefinitely, allowing more flowers to form on an elongated stem. Evolution is thought to have proceeded from determinate to indeterminate growth, presumably by recruiting genes to repress the formation of terminal flowers (Stebbins, 1974). A key gene involved in the distinction between these growth patterns is centroradialis (cen) in Antirrhinum (Kuckuck and Schick, 1930). In cen mutants the inflorescence terminates in a flower, switching the pattern from indeterminate to determinate. The simplest explanation is that cen normally represses the activity of meristem identity genes like flo in the inflorescence apex.

We wished to analyse the interaction between *cen* and *flo* but unfortunately did not get any *cen* mutants from our initial screens. We therefore used a more targeted approach involving F1 screens to obtain transposon-induced *cen* mutants. This proved successful and allowed Desmond Bradley to isolate and characterize *cen* (Bradley *et al.*, 1996a). Expression of *cen* was observed just below the main inflorescence apex, consistent with its normal role in repressing the formation of terminal flowers (Figure 6). The CEN protein may be involved in cell-cell signalling, as it shows similarities to animal proteins that associate with lipids and GTP-binding proteins.

In preventing the main inflorescence meristem from adopting floral identity, the *cen* gene acts in the opposite fashion to meristem identity genes, such as flo, which promote floral identity. To study this interaction further, we exploited the ability of plants to respond to day length (Bradley et al., 1996a,b). Antirrhinum plants flower much earlier when grown in long days rather than short days. By shifting plants from short to long day conditions, it was therefore possible to induce them to initiate flower development at specific times in wild-type and mutant backgrounds, and monitor the subsequent expression of flo and cen. In this way, Desmond Bradley showed that flo expression was activated 1-2 days after floral induction in very young meristems that were destined to form the first flowers. Following this, there was a series of interactions between *flo* and *cen* that appeared to involve signalling between cells. Activation of *flo* led to *cen* being expressed, which in turn prevented flo from being switched on in the main apex. This tight coupling between cen and flo may ensure that cen is not activated ectopically and thus avoid a more complete repression of flower development.

A further feature of *cen* mutants is that the terminal flower is radially symmetrical, whereas the axillary flowers retain dorsoventral asymmetry (Figure 10). The terminal flower of *cen* therefore only expresses the radial system whereas the axillary flowers below express both dorsoventral and radial systems. This suggests that in addition to the floral meristem identity genes, the dorsoventral system depends on a specific feature of axillary meristems. Axillary meristems are in an asymmetric environment, with the inflorescence apex above them and the bract primordium below. This polarized environment could provided the necessary cues for activating genes like *cyc* in dorsal regions (dorsal is nearer to the apex than ventral, Figure 6). Unlike axillary flowers, a terminal flower meristem is in a symmetrical environment and may lack the cues required to activate *cyc*. Thus, the production of floral asymmetry appears to be intimately connected with inflorescence architecture.

## **Future challenges**

A major achievement of the last 15 years has been to reveal how organisms contain a whole series of regional differences that were previously hidden from view. In many cases, these hidden identities reflect the expression of specific transcription factors, in patterns that can be elaborated during growth and development through signalling between cells. This shifting prepattern can be interpreted by the regulatory regions of genes, ensuring that they are expressed at appropriate times and places. We are still very far from a complete description of all the molecular territories involved and how they become refined. Even if we knew this, however, there is still the fundamental problem of how this prepattern eventually becomes manifest in the growth and behaviour of cells. In the case of flowers, we encounter this problem from the very earliest stages when meristems and primordia are being formed through to later steps that determine the shape and form of a petal or sepal. One of the major future challenges is to try to understand how these morphological changes are brought about.

Another major problem is how various developmental systems have evolved. Development is often treated as a separate problem from evolution but it seems to me that we will only have a satisfactory picture of development when we can understand how it is that an organism develops in one way rather than another. So far, evolutionary studies have tended to concentrate on looking for conservation as a guide to what is important. However, the more interesting challenge is to try to understand the basis of differences. What role, for example, do the *cyc* and *cen* genes play in species with different symmetries or inflorescence architectures from *Antirrhinum*? Organisms come in many different styles and we can only fully appreciate these once we understand how they have evolved.

## Note

This review describes the field from a personal standpoint. For more balanced reviews see Weigel and Meyerowitz (1994) and Coen and Carpenter (1993).

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