

## Evolutionary conservation of angiosperm flower development at the molecular and genetic levels

JOHN L BOWMAN\*

Section of Plant Biology, University of California, Davis, Davis California, 95616, USA

MS received 11 June 1997

**Abstract.** Flowers consist primarily of four basic organ types whose relative positions are universally conserved within the angiosperms. A model has been proposed to explain how a small number of regulatory genes, acting alone and in combination, specify floral organ identity. This model, known widely as the ABC model of flower development, is based on molecular genetic experiments in two model organisms, *Arabidopsis thaliana* and *Antirrhinum majus*. Both of these species are considered to be eudicots, a clade within the angiosperms with a relatively conserved floral architecture. In this review, the application of the ABC model derived from studies of these typical eudicot species is considered with respect to angiosperms whose floral structure deviates from that of the eudicots. It is concluded that the model is universally applicable to the angiosperms as a whole, and the enormous diversity seen among angiosperms flowers is due to genetic pathways that are downstream, or independent, of the genetic programme that specifies floral organ identity.

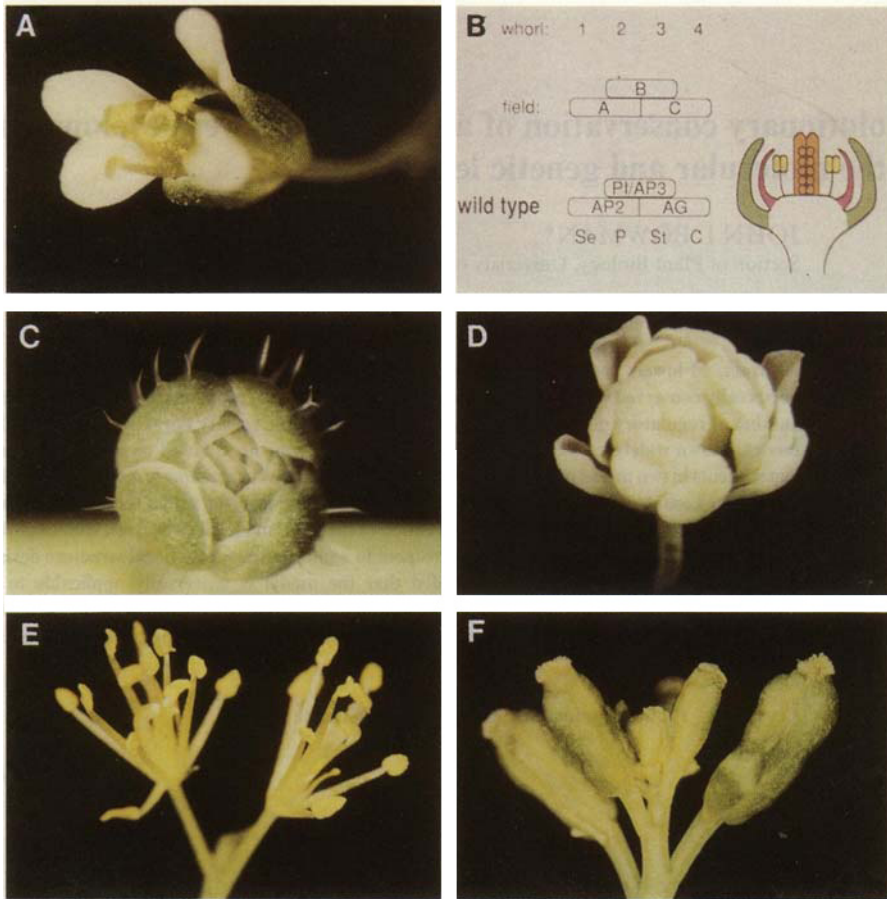
**Keywords.** Flower development; angiosperms; homeotic genes.

### 1. Angiosperm flower

An enormous diversity of size, shape, and complexity exists amongst the flowers of the quarter of a million species of extant angiosperms. Flower size varies over 1000-fold, with *Rafflesia* (Rafflesiaceae) flowers up to a meter in diameter dwarfing the minute flowers of *Wolffia* (Lemnaceae) measuring less than a millimeter across. Floral organ number also varies over several orders of magnitude with the complex flowers of *Tambourissa* species (Monimiaceae) having more than a thousand organs while the simple flowers of the Chloranthaceae may consist of but a few organs. Despite the enormous diversity in numbers, sizes, and shapes of the floral organs within the angiosperms, a universal theme underlies the basic architecture of all angiosperm flowers: flowers are built of four basic organ types whose relative positions are invariant (figure 1A; sepals, petals, stamens, and carpels). Thus, a basic floral ground plan exists that defines the relationship between organ type and position in all angiosperm species. The floral organ types typically develop in concentric rings or whorls, with sepals occupying the outermost positions, and petals, stamens, and carpels occupying successively more interior positions. Because of the constancy in the relative positions of floral organ types, it is hypothesized that a common genetic programme to specify floral organ identity is utilized during the development of all flowers. The striking variation observed among the angiosperm flowers would then be due to additional developmental programmes that operate in parallel with or subsequently to the basic genetic

---

\*Fax, 916 752-5410; Email, jlbowman@ucdavis.edu.



**Figure 1.** Wild-type, mutant and transgenic *A. thaliana* flowers. **(A)** Wild-type *Arabidopsis* flower consists of four whorls of floral organs: sepals, petals, stamens, and carpels. **(B)** ABC model of the specification of floral organ identity depicting how three classes of floral homeotic genes could specify the identity of each of the four whorls of floral organs. A section through one-half of a floral promordium is represented as a set of boxes, with the regions representing each whorl shown at the top of each column. Each box represents a single field (A, B, or C) as shown in the top set of boxes. The distribution of floral homeotic gene products present in each of the whorls is shown in the lower set of boxes, with the phenotype of the organs in each whorl shown in the lower set of boxes. Se, Sepal; P, petal; St, stamen; C, carpel. A schematic drawing of a longitudinal cross-section of the *Arabidopsis* flower is shown at the right, with each organ colour coded: sepals = green, petals = pink, stamens = yellow, and carpel = orange. **(C)** An *agamous apetala3* flower is comprised entirely of sepals. **(D)** A 35S-AP3; 35S-PI; *agamous* flower is comprised entirely of petals. Photo compliments of Beth Krizek. **(E)** A 35S-AP3; 35S-PI; *apetala2* flower is comprised entirely of stamens. **(F)** An *apetala2 pistillata* flower is comprised entirely of carpels.

programme that specifies floral organ identity. The first part of this review consists of a brief description of a molecular genetic model of how floral organ identity is specified, while the remainder of the review discusses flower diversity in the framework of this model.

## 2. ABC model of flower development

The recent application of developmental genetics has led to progress in the understanding of how organ type is specified in the flower. The advances are based on both genetic analyses of mutants which specifically disrupt floral development and the molecular cloning of the corresponding genes to reveal the nature of their biochemical function. Due to their ease of genetic manipulation, the focus was initially on two species, *Arabidopsis thaliana* (Brassicaceae) and *Antirrhinum majus* (Scrophulariaceae). A model (now known widely as the ABC model) based on genetic data was proposed to explain how a limited set of genes acting alone and in combination could specify the identity of the floral organs (Bowman *et al* 1989; Carpenter and Coen 1990; Schwarz-Sommer *et al* 1990; Bowman *et al* 1991; reviewed in Coen and Meyerowitz 1991; Weigel and Meyerowitz 1994). Subsequent molecular analyses of the relevant genes and ectopic expression studies in *Arabidopsis* have largely supported the genetic model and led to numerous refinements and further insights (e.g., Drews *et al* 1991; Mandel *et al* 1992; Jack *et al* 1994; Sakai *et al* 1995; Krizek and Meyerowitz 1996; reviewed in Weigel and Meyerowitz 1994).

### 2.1 Floral homeotic mutants of *Arabidopsis*

The *Arabidopsis* flower, as with the flowers of many dicotyledonous species, consists of four concentric whorls of organs. Four sepals occupy cruciform positions in the first whorl, with the second whorl consisting of four petals whose positions are alternate and interior to those of the sepals. Six stamens occupy the third whorl, and a two-carpelled gynoecium forms the fourth whorl (figure 1A). Several studies have focused on a set of floral homeotic genes, mutations in which result in homeotic transformations of one floral organ type into another floral organ type. One interpretation of these mutants is that cells in the developing flower misinterpret their position, and thus, differentiate into inappropriate cell types. The floral homeotic mutants fall into three classes, designated A, B, and C, and mutants in each class result in organ identity defects in two adjacent whorls. A class mutants (*apetala2*[*ap2*] and *apetala1*[*ap1*]) have homeotic conversions in the first two whorls, with, in the case of *ap2*, the first whorl organs developing as carpels rather than sepals, and stamens arising in the place of petals (Komaki *et al* 1988; Bowman *et al* 1989,1991,1993; Kunst *et al* 1989; Irish and Sussex 1990; Gustafson-Brown *et al* 1994; Jofuku *et al* 1994). B class mutants (*pistillata* [*pi*] and *apetala3* [*ap3*]) have alterations in the middle two whorls with sepals developing instead of petals in the second whorl and carpels instead of stamens in the third whorl (Bowman *et al* 1989; Hill and Lord 1990; Bowman *et al* 1991; Jack *et al* 1992; Goto and Meyerowitz 1994). The inner two whorls are affected in C class mutants (*agamous*[*ag*]), with petals developing in place of stamens and another flower replacing the carpels (Bowman *et al* 1989, 1991; Yanofsky *et al* 1990).

### 2.2 ABC Model

The basic tenets of the ABC model (figure 1B; Bowman *et al* 1991) are that: (i) each of the classes of homeotic gene function acts in a field comprised of two adjacent whorls, the particular whorls being those that are altered when the corresponding genes are in

mutant form; (ii) the combination of floral homeotic gene activities present in any particular whorl specifies the type of organ that develops in that whorl, e.g., A class alone specifies sepals, classes A + B specify petals, classes B + C specify stamens, and C class alone specifies carpels; and (iii) the class A and class C activities are mutually antagonistic such that loss of A results in C activity in all four whorls and vice versa. For most of the genes it has been shown that the first and third tenets are satisfied at the transcriptional level, however, for some class A genes (*AP2*), the restriction of their organ identity activity to the outer two whorls must occur post-transcriptionally (Jofuku *et al* 1994; Liu and Meyerowitz 1995). The model successfully predicts the phenotypes of the floral organs for most of the single, double and triple mutant genotypes examined. However, in triple mutants in which all of the A, B and C classes of floral homeotic activities are removed, the model does not predict which type of organ will develop. One might suppose that if all genes required for the specification of floral organ identity were removed, the identity of the resulting organs might represent a ground state, possibly a leaf-like organ. In actuality, organs with features of both carpels (such as stigmatic tissue, fusion of organs along their margins, and ovules) and leaves (stellate trichomes and stipules) develop in the triple mutant flowers (Bowman *et al* 1991). Thus, there must exist genes that specify carpel development in the absence of C class gene function (Bowman *et al* 1991).

### 2.3 Manipulation of floral organ identity in *arabidopsis*

All of the known floral homeotic genes encoding the A, B, and C functions have been cloned in *Arabidopsis*, and likely encode transcription factors. Intriguingly, one of the A class genes (*API*) and all of the B and C class genes (*AP3*, *PI*, *AG*) encode transcription factors belonging to the MADS box gene family, suggesting that a diversification of this gene family may have been instrumental in the evolution of flowers (Purugganan *et al* 1995; Theissen *et al* 1996). Ectopic expression studies of the B and C class genes have shown that they are necessary and sufficient to specify organ identity within the flower (Mandel *et al* 1992; Mizukami and Ma 1992; Jack *et al* 1994; Krizek and Meyerowitz 1996). Utilizing combinations of mutant and transgenic lines in which the A, B, or C genes have been ectopically expressed it is possible to manipulate the floral organ identity in each whorl of the flower. There are few constraints on the identities and positions of the different floral organs. For example, a flower consisting entirely of sepals can be formed if both the B and C functions are removed, as in an *ag ap3* double mutant flower (figure 1C; Bowman *et al* 1991). Likewise, a flower consisting entirely of carpels develops when both the A and B functions are removed, as in an *ap2 pi* double mutant flower (figure 1F; Bowman *et al* 1991). Flowers consisting of all petals or all stamens can also be generated. Expression of the B class genes (*AP3* and *PI*) in all four whorls of a C class mutant (*ag*) result in a flower consisting of petals in all whorls (figure 1D; Krizek and Meyerowitz 1996). Conversely, expression of the B class genes in all four whorls of A class mutant (*ap2*) result in a flower consisting of stamens in every whorl (figure 1E; Krizek and Meyerowitz 1996). Thus, all of the different types of floral organs can potentially develop in any of the four floral whorls. One of the few floral architectures that may not be easily obtainable is a flower with a reverse organ order (i.e., carpels, stamens, petals, sepals). This may be due to the antagonistic nature of the A and C activities which in some cases has been shown to be at the transcriptional level

and may be direct. A further complication is that both A class genes are also involved in earlier events in floral development, namely the specification of the flower meristem.

### 3. Universality of the ABC model

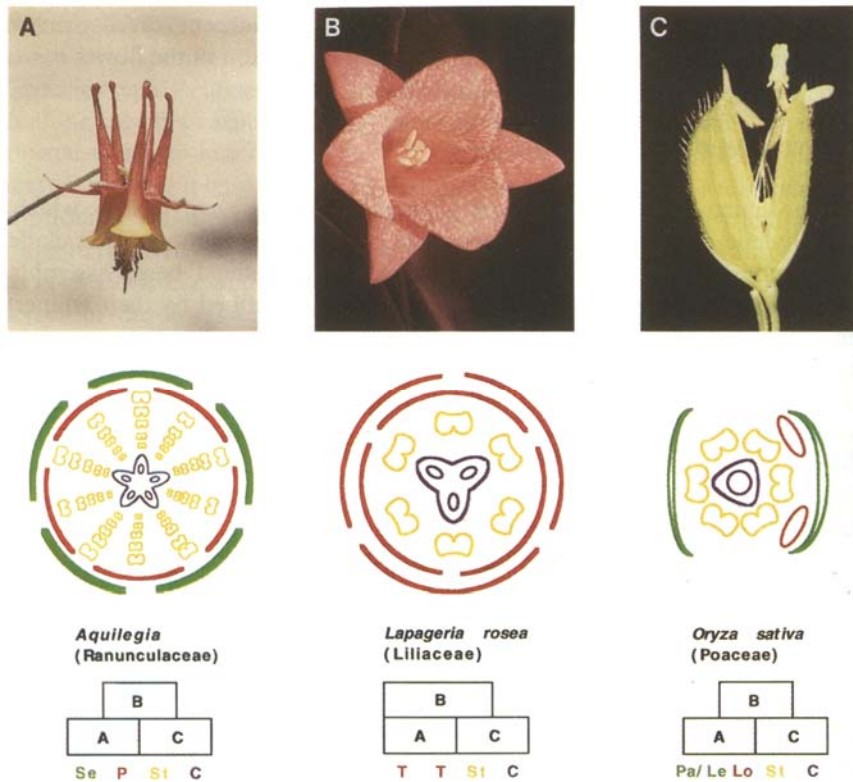
Recent molecular and morphological phylogenies of the angiosperms, which form a monophyletic group, suggest the presence of two large monophyletic clades, the eudicots and monocots, embedded within a poorly defined basal assemblage of Magnoliid dicots (Crane *et al* 1995). The eudicots are defined by their triaperturate pollen, while the monocots are defined by their single cotyledon amongst other features. The basal assemblage of Magnoliid dicots appears polyphyletic and the relationships between them are not well established. Identifying the ancestral characters of the flower will rely on elucidating the phylogenetic patterns among the Magnoliid dicots. Although their relationships are an enigma at present, the application of large scale molecular phylogenetic analysis is beginning to resolve the early diversification of this group of angiosperms (Chase *et al* 1993; Soltis *et al* 1997). How universal is the ABC model with respect to floral organ specification in the angiosperms as a whole?

#### 3.1 ABC genes from other eudicot species

Initially, the molecular genetic experiments focused on two evolutionarily divergent dicotyledonous species, *A. thaliana* and *A. majus* (Schwarz-Sommer *et al* 1990; Coen and Meyerowitz 1991; Weigel and Meyerowitz 1994). Both of these species, like most other species of eudicots, have flowers that consist of the four floral organ types mentioned above organized in four concentric whorls. Thus, although more than 100 million years separates these two species, their floral architecture is very similar. Thus, it was not surprising to find that they share a common genetic network for specifying floral organ identity. Subsequently, investigations have shown that the model originally proposed for *Arabidopsis* and *Antirrhinum*, is likely to be directly applicable to all eudicots (figure 2A). This is based on the observation that similar sets of orthologous floral homeotic genes appear to be active in the Solanaceae (*Petunia hybrida*, van der Krol and Chua 1993; *Nicotiana tabacum*, [tobacco], Hansen *et al* 1993; Kempin *et al* 1993; *Lycopersicon esculentum* [tomato], Pnueli *et al* 1991), the Myrtaceae (*Eucalyptus globulus*, Kyozyuka *et al* 1997a), the Polygonaceae (*Rumex acetosa*, Ainsworth *et al* 1995), the Caryophyllaceae (*Silene latifolia*, Hardenack *et al* 1994), the Salicaceae (*Populus trichocarpa*, Brunner *et al* 1997), and the Asteraceae (*Gerbera* sp., Yu *et al* 1997). The general applicability of the model is further strengthened by that fact that heterologous floral homeotic genes can function in widely divergent eudicots (Mandel *et al* 1992). Thus, the genetic programme that specifies floral organ identity in eudicots appears to be conserved in all species examined to date.

#### 3.2 Tepals versus sepals and petals

In contrast to the eudicots, the perianth (sterile organs of the flower, e.g., sepals and petals of eudicots) of many species of monocots and basal dicots consists of only a single



**Figure 2.** The top panel in each column is a colour photo of a representative species of a higher eudicot (A), a monocot (B), and a grass flower (C), with the respective floral diagram depicted in the middle panel. The application of the model of the specification of floral organ identity is shown in the lower panel, in the same convention as in figure 1. (A) *Aquilegia* sp. (Ranunculaceae). Flowers of *Aquilegia* (columbine) are typical of the higher eudicots, being composed of the four basic floral organs: sepals, petals, stamens, and carpels. There is one whorl each of sepals, petals and carpels, and approximately ten whorls of stamens. Each of the whorls is composed of five members, again typical of many, but not all, higher eudicots. The flowers are pendant with the spurs of the petals containing the nectaries. In the case of *Aquilegia*, the model for the specification of organ identity applies as was postulated for *Arabidopsis* and *Antirrhinum*. (B) *Lapageria rosea* (Liliaceae). Flowers of many lily family species have a rather undifferentiated perianth, as in this *L. rosea*. In these cases the perianth members are often referred to as tepals. Thus, the *Lapageria* flower consists of two outer whorls of tepals, a third whorl of six stamens, and a fourth whorl of three united carpels. The basic number of floral organ parts per whorl in this flower is three, a characteristic of many monocot flowers. In the case of *Lapageria*, the model must be modified such that the B class genes are active in the first whorl as well as the second and third whorls, thus giving rise to a situation in which the outer two whorls are essentially identical. (C) *O. sativa* (Poaceae). Flowers of the grass family are highly derived and reduced. The typical grass flower has an outer whorl composed of a palea (left outer organs in figure) and a lemma (right outer organ in figure). The second whorl contains organs referred to as lodicules, and the third and fourth whorls contain stamens and carpels respectively. In the case of *O. sativa* (rice), the model of organ identity need not be modified if one makes the assumptions that the palea and/or lemma are equivalent to the sepals of most other flowers and the lodicules are equivalent to petals. That the lodicules are homologous to petals is strongly suggested by rice B class mutants described in the text. The case of the palea and/or lemma being homologous to sepals of other angiosperms is at present equivocal; examination of the expression patterns of A class genes in these organs may help clarify their homology to floral organs of other angiosperms. Se, Sepals; P, petals; St, stamens; C, carpels; T, tepals; Pa, palea; Le, lemma; Lo, lodicules.

type of organ, often referred to as tepals. In many cases, the tepals are in two distinct whorls and phenotypically resemble petals. For example, flowers of the Liliaceae consist of two outer whorls of pigmented tepals, a third whorls of stamens, and a fourth whorl of carpels (figure 2B). One interpretation of the architecture of these flowers is that they develop a second whorl of petals in the positions usually occupied by sepals in eudicots. This suggests that in these flowers the outer boundary of the B class genes has been shifted to the perimeter of the flower such that the B class genes are now active in the first whorl as well as the second and third whorls (figure 2B). Support for this hypothesis comes from genetic analyses of *Tulipa*. A spontaneous mutant, *Viridiflora*, was identified in which the two whorls of tepals were homeotically transformed into sepal-like organs while the stamens were transformed into carpels, a phenotype reminiscent of the B class mutants of *Arabidopsis* (van Tunen *et al* 1993). The *Viridiflora* mutant phenotype suggests that B class genes are active in the outer three whorls of wild-type *Tulipa* flowers.

It is likely that a perianth composed of a single type of organ, rather than one which was differentiated into distinct sepal and petal organ types, represents the ancestral condition in angiosperms. Evolution of a differentiated perianth could occur by differentiation of the existing perianth into two distinct organ types, or alternatively, by conversion of stamens to petaloid organs. In the former case, a shift in the outer boundary of B class gene function could account for the differentiation into two distinct organ types, the direction of the shift being dependent on the original condition of the undifferentiated perianth (e.g., sepaloid versus petaloid). In the latter case, a centripetal shift in the boundary demarking the A and C class functions could account for the recruitment of stamens to become petaloid organs. It seems probable that both of these scenarios, as well as others, occurred in various angiosperm lineages (Cronquist 1988; Endress 1994). For example, within the Magnoliaceae, all transitional stages from an undifferentiated perianth consisting of petaloid organs (tepals) to well differentiated perianths consisting of sepals and petals occur. In this case it appears that the sepals might have been derived from tepals. In contrast, within the Ranunculaceae, the petals appear to be staminodal in origin. Regardless of the evolutionary mechanism by which a bifurcated perianth arose, simple shifts in the boundaries of the homeotic gene functions can account for the observed variation.

### 3.3 Grass flowers

While flowers of most eudicots and some monocots resemble those of *Arabidopsis* and *Antirrhinum* (composed of sepals, petals, stamens, and carpels) flowers of some other monocots are often quite distinct, making homologies between floral organs difficult. For example, flowers of the Poaceae consist of an outer set of organs called the palea and lemma; internal to these outer organs are lodicules, and interior to the lodicules are the sexual organs, the stamens and carpels (figure 2C). Grass flowers are subtended by leafy organs termed glumes which are likely homologous to the bracts subtending flowers of many other angiosperms. Studies of two Poaceae species, *Oryza sativa* (rice) and *Zea mays* (maize), suggest that the genetic programme specifying floral organ identity in grass flowers shares remarkable conservation with that of eudicots. Rice mutants have been isolated that result in homeotic transformations of the lodicules into palea-, lemma-, or glume-like structures and the stamens into carpels (Nagato 1994). These mutants resemble the B class mutants of *Arabidopsis* and *Antirrhinum* and their

phenotype strongly suggests that the lodicules of the grass flower are homologous to petals (figure 2C). Orthologs of the A and C class genes have also been isolated from rice (Kyoizuka *et al* 1997b), suggesting that the molecular control of floral organ identity in rice is conserved as well. Similar studies in maize support these conclusions (Veit *et al* 1993), but in the case of maize, the interpretations are complicated by the evolutionarily recent tetraploidization of maize. In general, two copies of each of the A, B, and C genes appear to be present in the genome of maize (Veit *et al* 1993; Mena *et al* 1996). This results in either complete or partial redundancy such that when one of the copies is rendered non-functional by mutation, the functional copy mitigates the mutant phenotype (Mena *et al* 1996). In the case of the C function, there is only partial redundancy suggesting that in the time since maize acquired its tetraploid nature, the two C class genes have evolved to have both distinct and overlapping functions (Mena *et al* 1996). A similar situation has been observed in the eudicot *Petunia hybrida*, a tetraploid species derived from two different diploid species (Angenent *et al* 1993; van der Krol and Chua 1993; Halfter *et al* 1994). Since the evolutionary divergence between the Poaceae and the eudicots likely occurred early in the radiation of the angiosperms, it is likely that all angiosperms utilize the same basic genetic programme for specifying floral organ identity.

### 3.4 *Monoecious and dioecious species*

While hermaphroditic flowers are likely to be the ancestral condition, monoecism and dioecism have evolved in many separate lineages within the angiosperms (e.g., Irish and Nelson 1989; Dellaporta and Calderon-Urrea 1993; Grant *et al* 1994). Perhaps due to the polyphyletic nature of their origin, the mechanisms by which unisexual flowers develop are diverse as well. Plant species usually achieve unisexuality by selective abortion of the male or female reproductive organs, however, this abortion can occur at any stage in the development of the sexual organs. For example, in *Asparagus* (Liliaceae), development is arrested at micro- or megagametogenesis in the appropriate flowers, while in maize (Poaceae) the stamen or carpel primordia are aborted at an early stage of development, and in *Mecurialis* (Euphorbiaceae) no vestiges of the inappropriate sexual organ primordia emerge from the floral meristem. Since most flowers of monoecious and dioecious plants begin their development as bisexual flowers, it seems unlikely that the ABC genes are involved with the selective abortion of the sex organs. In support of this, when ABC gene expression has been examined in flowers of monoecious or dioecious plants (*Rumex acetosa*, Ainsworth *et al* 1995; *S. Latifolia*, Hardenack *et al* 1994; *P. trichocarpa*, Brunner *et al* 1997), their expression patterns resemble those of perfect (hermaphroditic) flowers. This suggests that the genetic programmes specifying sex determination act downstream or independently of the floral organ identity specification programme. Indeed, it may be a general phenomenon that floral homeotic gene expression is not causally involved in most cases where organs of any particular type, sexual or perianth, fail to develop due to either selective suppression or abortion.

### 3.5 *One in a quarter of a million*

There are always exceptions to the rule. In the case of the quarter of a million or so documented angiosperm species, there is one species, *Lacandonia schismatica*

(Lacandoniaceae) in which the stamens are interior to the carpels (Martinez and Ramos 1989). This small, saprophytic plant has flowers that consist of two outer whorls of three tepals each, 60-80 free carpels interior to the tepals, and then three central stamens. The flowers of *L. schismatica* resemble those of the most closely related family, the Triuridaceae, a family of small tropical herbs that live on dead or decaying matter. Flowers of the Triuridaceae consist of two outer whorls of tepals, a small number of stamens, and numerous free carpels in the center of the flower, thus, they are identical to those of *Lacandonia* except for the placement of the stamens (Heywood 1993). However, within the Triuridaceae both monoecious and dioecious species exist, with the female flowers of dioecious species consisting of two outer whorls of tepals and numerous carpels interior to the tepals. One possible explanation of the unusual flower architecture exhibited by *L. schismatica* is that this species evolved from a dioecious ancestor within the Triuridaceae in which the B class genes were reactivated in the centre of the flower resulting in the development of central stamens (Vergara and Alvarez-Buyela 1997).

### 3.6 Variations on a theme

Flower mutants have fascinated man for centuries, and some of these variants have become a large fraction of the commodities of the cut flower industry (e.g., Meyerowitz *et al* 1989). The literature is replete with reports of variation occurring between flowers of a single species as well as variations of flower architecture between closely related species (see Meyerowitz *et al* 1989 and references therein). Many of these flower variants can be explained by simple shifts in the boundaries of homeotic gene activity with respect to the positions of floral organs. For example, 'double flowers', in which additional whorls of petals develop, have been described in a variety of species. Obvious examples are roses and carnations, which in wild-type form have only five petals. In cases where the development of these 'double flowers' has been examined, the extra petals arise in positions normally occupied by stamens. For example, *Potentilla fruticosa* (Rosaceae) flowers usually consist of five sepals in an outer whorl, five petals in a second whorl, 20-25 stamens in 4-5 whorls, and numerous carpels interior to the stamens. In 'double flowers' of this species, an extra whorl, or sometimes two, of petals develops at the expense of the outermost whorl (s) of stamens (Innes *et al* 1989). This phenotype can be explained by a shift in the A/C boundary towards the centre of the flower. This would result in the outermost whorl of organs that would normally develop as stamens expressing A and B class activities rather than B and C, and thus they would differentiate into petals. Similar floral variants have been described in a variety of eudicot species [(e.g., *Sanguinaria canadensis* (Papaveraceae; Lehmann and Sattler 1993), *Actaea rubra* (Ranunculaceae; Lehmann and Sattler 1994), *Hibiscus rosa-sinensis* (Malvaceae; MacIntyre and Lacroix 1996)] suggesting that shifts in the A/C boundary may be quite common.

As discussed in a previous section, shifts in the relative position of the outer boundary of B class homeotic gene function could result in a homogeneous perianth. Ford and Gottlieb (1992) reported a naturally occurring variant of *Clarkia concinna* (Onagraceae), *bicalyx*, in which the outer two whorls consist of sepal-like organs, rather than being differentiated into sepals and petals as they are in normal *Clarkia* flowers. This phenotype could be explained by a shift in the outer boundary of B class function

towards the centre of the flower resulting in two whorls of organs having only A class gene function. A shift in B class activity in the opposite direction, towards the periphery of the flower, appears to have occurred in the genus *Clermontia* (Lobeliaceae; Di Laurenzio *et al* 1997). In this case, several species have a perianth consisting of two whorls of petals, as opposed to the ancestral condition which was a perianth consisting of both sepals and petals.

In the examples above, it appears that the floral homeotic gene activity boundaries may have shifted in closely related species or in genetic variants within a single species. Similar shifts may account for variation across broader ranges of taxa. For example, the families within the Zingiberales each have their own characteristic floral structure (Kirchoff 1991). Some families, such as the Strelitziaceae (birds-of-paradise) and Musaceae (bananas), have flowers typical of monocots: three sepals, three petals, six stamens (in two whorls), and three carpels. In other families (e.g. Zingiberaceae [ginger], Cannaceae, and Marantaceae), sterile petaloid structures, often referred to as staminodes, develop in the positions normally occupied by stamens (Kirchoff 1991). In the most extreme cases, as in the Cannaceae and Marantaceae, four of the six positions normally occupied by stamens are occupied by petaloid organs, and a fifth position contains an aborted organ. The final stamen position is occupied by an organ that is one-half petaloid and one-half stamenoid, such that only one functional theca is formed (Kirchoff 1991). The phenotypes of each of the families could be explained by a shift in the A/C boundary towards the centre of the flower, the extent of the shift varying between the families. In this scenario, the shift in the A/C boundary would not be symmetric, but rather it would occur in an irregular manner such that organs within a single whorl, and in some cases individual organs, would have different combinations of floral homeotic gene activity (i.e., A + B versus B + C). This hypothesis could be tested by examining A and C class activity in various Zingiberales families.

#### 4. Conclusions

If the genetic programme that specifies floral organ identity is conserved throughout the angiosperms, what then is responsible for creating the enormous diversity between flowers of different species? The simplest explanation is that other genetic programmes that act independent of or subsequent to the floral organ identity programme are responsible for generating diversity in floral form. For example, the number of whorls of floral organs and the number of floral organs within a whorl are probably controlled by genetic programmes that dictate species specific cell division patterns in the floral meristem, and these programmes likely function independently of that specifying floral organ identity. Other genetic programmes that likely operate in a species specific manner include ones influencing floral organ size, shape, and colour. Such programmes could either act independently of the specification of floral organ identity, or be ultimately activated (i.e., downstream) by the genes controlling floral organ identity. For instance, the genetic programme controlling the zygomorphy (asymmetry) of the *Antirrhinum* flower operates independently of that Controlling floral organ identity, while the programmes controlling organ size and shape appear to be activated by the floral organ identity programme (Luo *et al* 1996).

Presently several major issues remain unresolved. Firstly, how are the ABC genes which specify floral organ identity initially activated? Genetic analyses have identified

several potential positive regulators, but the relationship between these earlier acting genes and the ABC genes is currently an enigma. Secondly, how is the positional information encoded in the distribution of the ABC homeotic gene products translated into the differentiation of particular organ types? The identification of the genetic programmes 'downstream' of the homeotic genes remains one of the biggest challenges in developmental biology. Finally, from where did the genetic programme specifying floral organ identity evolve? Similar, perhaps orthologous, genes are found in non-flowering seed plants (e.g., Tandre *et al* 1995) suggesting that the ABC genes may have been appropriated from a pre-existing genetic programme in a progenitor of flowering plants to assume its present role in angiosperms. Knowledge of the function of these orthologous genes in non-flowering plants such as the Gnetales and gymnosperms could illuminate both the evolutionary origin of the flower and potential homologies between the floral organs of angiosperms and the reproductive organs of non-flowering seed plants.

### Acknowledgements

I thank members of my laboratory for stimulating discussions and comments on this manuscript, Beth Krizek for supplying photographs and seed stocks, and Ernesto Sandoval for the *Lapageria rosea* flower. My laboratory's research on flower development in *Arabidopsis* is supported by the National Science Foundation (IBN-96-31458).

### References

- Ainsworth C, Crossley S, Buchanan-Wollaston V, Thangavelu M and Parker J 1995 Male and female flowers of the dioecious plant sorrel show different patterns of MADS box gene expression; *Plant Cell* **7** 1583-1598
- Angenent G C, Franken J, Busscher M, Colombo L and van Tunen A J 1993 Petal and stamen formation in petunia is regulated by the homeotic gene *fbp 1*; *Plant J.* **4** 101—112
- Bowman J L, Alvarez J, Weigel D, Meyerowitz E M and Smyth D R 1993 Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes; *Development* **119** 721-743
- Bowman J L, Smyth D K and Meyerowitz E M 1989 Genes directing flower development in *Arabidopsis*; *Plant Cell* **1** 37-52
- Bowman J L, Smyth D R and Meyerowitz E M 1991 Genetic interactions among floral homeotic genes of *Arabidopsis*; *Development* **112** 1-20
- Brunner A, Rottmann W, Sheppard L, Ktutovskii K and Strauss S 1997 Characterization of *PTAG1* and *PTAG2*, two genes from *Populus trichocarpa* homologous to the *Arabidopsis* floral homeotic gene *AGAMOUS*; *Keystone Symposium: Evolution of Plant Development*, Taos, NM, p 22
- Carpenter R and Coen E S 1990 Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*; *Genes Dev.* **4** 1483-1493
- Chase M W, Soltis D E, Olmstead R G, Morgan D, Les D H, Mishler B D, Duvall M R, Price R A, Hills H G, Qiu Y-L, Kron K A, Rettig J H, Conti E, Palmer J D, Manhart J R, Systsma K J, Michaels H J, Kress W J, Karol K G, Clark W D, Hedrén M, Gaut B S, Jansen R K, Kim K-J, Wimpee C F, Smith J F, Furnier G R, Strauss S H, Xiang Q Y, Plunkett G M, Soltis P S, Swensen S M, Williams S E, Gadek P A, Quinn C J, Eguiarte L E, Golenberg E, Learn Jr G H, Graham S W, Barrett S C H, Dayanandan S and Albert V 1993 Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcl*; *Ann. Mo. Bot. Gard.* **80** 528-580
- Coen E S and Meyerowitz E M 1991 The war of the whorls: genetic interactions controlling flower development; *Nature (London)* **353** 31-37
- Crane P R, Friis E M and Pedersen K R 1995 The origin and early diversification of angiosperms; *Nature (London)* **374** 27-33

- Cronquist A 1988 *The Evolution and Classification of Flowering Plants* (New York: New York Bot. Garden)
- Dellaporta S L and Calderon-Urrea A 1993 Sex determination in flowering plants; *Plant Cell* **5** 1241-1251
- Di Laurenzio L, Struwe L, Pepper A, Kizirian D and Albert V 1997 Gene expression analysis of sepal identity in Clermontia (Lobelioideae: Campanulaceae): homeosis and floral diversification in the Hawaiian archipelago; *Keystone Symposium: Evolution of Plant Development*, Taos, NM, p 24
- Drews G N, Bowman J L and Meyerowitz E M 1991 Negative Regulation of the *Arabidopsis* Homeotic Gene *AGAMOUS* by the *APETALA2* Product; *Cell* **65** 991-1002
- Endress P K 1994 *Diversity and Evolutionary biology of Tropical Flowers* (Cambridge: Cambridge University Press)
- Ford V S and Gottlieb L D 1992 *Bicalyx* is a natural homeotic floral variant; *Nature (London)* **358** 671-673
- Goto K and Meyerowitz E M 1994 Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*; *Genes Dev.* **8** 1548-1560
- Grant S, Houben A, Vyskot B, Siroky J, Pan W-H, Macas J and Saedler H 1994 Genetics of sex determination in flowering plants; *Dev. Genet.* **15** 214-230
- Gustafson-Brown C, Savidge B and Yanofsky M F 1994 Regulation of the *Arabidopsis* floral homeotic gene *APETALA1*; *Cell* **76** 131-143
- Halfter U, Ali N, Stockhaus J, Ren L and Chua N H 1994 Ectopic expression of a single homeotic gene, the *Petunia* gene green petal, is sufficient to convert sepals to petaloid organs; *EMBO J.* **13** 1443-1449
- Hansen G, Estruch J J, Sommer H and Spena A 1993 NTGLO: a tobacco homologue of the *GLOBOSA* floral homeotic gene of *Antirrhinum majus*: cDNA sequence and expression pattern; *Mol. Gen. Genet.* **239** 310-312
- Hardenack S, Ye D, Saedler H and Grant S 1994 Comparison of MADS box gene expression in developing male and female flowers of the dioecious plant white campion; *Plant. Cell* **6** 1775-1787
- Heywood V H 1993 *Flowering Plants of the World* (New York: Oxford University Press)
- Hill J P and Lord E M 1989 Floral development in *Arabidopsis thaliana*: a comparison of the wild type and the homeotic *pistillata* mutant; *Can. J. Bot.* **67** 2922-2936
- Innes R L, Remphey W R and Lenz L M 1989 An analysis of the development of single and double flowers in *Potentilla frutocosa*; *Can. J. Bot.* **67** 1071-1079
- Irish E E and Nelson T 1989 Sex determination in monoecious and dioecious plants; *Plant Cell* **1** 737-744
- Irish V F and Sussex I M 1990 Function of the *apetala1-1* gene during *Arabidopsis* floral development; *Plant Cell* **2** 741-751
- Jack T, Brockman L L and Meyerowitz E M 1992 The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS-box and is expressed in petals and stamens; *Cell* **68** 683-697
- Jack T, Fox G L, and Meyerowitz E M 1994 *Arabidopsis* homeotic gene *APETALA3* ectopic expression: transcriptional and post-transcriptional regulation determine floral organ identity; *Cell.* **76** 703-716
- Jofuku K D, den Boer B G W, Van Montagu M and Okamoto J K 1994 Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*; *Plant Cell* **6** 1211-1225
- Kempin S A, Mandel M A and Yanofsky M F 1993 Conversion of perianth into reproductive organs by ectopic expression of the tobacco floral homeotic gene *NAG 1*; *Plant Physiol.* **103** 10041-1046
- Kirchoff B K 1991 Homeosis in the flowers of the Zingiberales; *Am. J. Bot.* **78** 833-837
- Komaki M K, Okada K, Nishino E and Shimura Y 1988 Isolation and characterization of novel mutants of *Arabidopsis thaliana* defective in flower development; *Development* **104** 195-203
- Krizek B A and Meyerowitz E M 1996 The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function; *Development* **122** 11-22
- Kunst L, Klenz J E, Martinez-Zapater J and Haughn G W 1989 *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana*; *Plant Cell* **1** 1195-1208
- Kyozuka J, Southerton S and Dennis E S 1997a *Eucalyptus* has functional equivalents of the *Arabidopsis API1* gene; *Plant. Mol. Biol.* (in press)
- Kyozuka J, Konishi S, Morita M, Izawa T, Kobayashi T, Nakagawa M, Amano E and Shimamoto K 1997b Molecular mechanisms determining rice panicle and spikelet formation; *Keystone Symposium: Evolution of Plant Development*, Taos, NM, p 26
- Lehmann N L and Sattler R 1993 Homeosis in floral development of *Sanguinaria canadensis* and *S. canadensis* 'Multiplex' (Papaveraceae); *Am. J. Bot.* **80** 1323-1335
- Lehmann N L and Sattler R 1994 Floral development and homeosis in *Actaea rubra* (Ranunculaceae); *Int. J. Plant Sci.* **155** 658-671
- Liu Z and Meyerowitz E M 1995 *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers; *Development* **121** 975-991

- Luo D, Carpenter R, Vincent C, Copsey L and Coen E 1996 Origin of floral asymmetry in *Antirrhinum*; *Nature (London)* **383** 794-799
- MacIntyre J P and Lacroix C R 1996 Comparative development of perianth and androecial primordia of the single flower and the homeotic double-flowered mutant in *Hibiscus rosa-sinensis* (Malvaceae); *Can. J. Bot.* **74** 1871-1882
- Mandel M A, Bowman J L, Kempin S A, Ma H, Meyerowitz E M and Yanofsky M F 1992 Manipulation of Flower Structure in Transgenic Tobacco; *Cell* **71** 133-143
- Martinez E and Ramos C H 1989 Lacandoniaceae (Triuridales): Una nueva familia de Mexico; *Ann. M. Bot. Gard.* **76** 128-135
- Mena M, Ambrose B A, Meeley R B, Briggs S P, Yanofsky M F and Schmidt R J 1996 Diversification of C-function activity in maize flower development; *Science* **274** 1537-1540
- Meyerowitz E M, Smyth D R and Bowman J L 1989 Abnormal flowers and pattern formation in floral development; *Development* **106** 209-217
- Mizukami Y and Ma H 1992 Ectopic expression of the floral homeotic gene *AGAMOUS* in transgenic *Arabidopsis* plants alters floral organ identity; *Cell* **71** 119-131
- Nagato Y 1994 Embryo and flower development in rice and apomixis in Chinese chive (*Allium tuberosum*); in *Apomixis: exploiting hybrid vigor in rice* (ed.) G S Khush (Los Banos, Phillipines: IRRRI)
- Pnueli L, Abu-Abeid M, Zamir D, Nacken W, Schwarz-Sommer Z and Lifschitz E 1991 The MADS box gene family in tomato: temporal expression during floral development, conserved secondary structures and homology with homeotic genes from *Antirrhinum* and *Arabidopsis*; *Plant J.* **1** 255-266
- Purugganan M.D, Rounsley S D, Schmidt R J and Yanofsky M F 1995 Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family; *Genetics* **140** 345-356
- Sakai H, Medrano L J and Meyerowitz E M 1995 *Arabidopsis* floral boundary maintenance by *SUPERMAN*; *Nature (London)* **378** 199-203
- Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H and Sommer H 1990 Genetic control of flower development: homeotic genes of *Antirrhinum majus*; *Science* **250** 931-936
- Soltis D E, Soltis P S, Nickrent D L, Johnson L A, Hahn W J, Hoot S B, Sweere J A, Kuzoff R K, Kron K A, Chase M W, Swensen S M, Zimmer E A, Chaw. S-M, Gillespie L J, Kress W J and Sytsma K J 1977 Angiosperm phylogeny inferred from 18S ribosomal DNA sequences; *Ann. Mo. Bot. Gard.* **84** 1-49
- Tandre K, Albert V A, Sundas A and Engstrom P 1995 Conifer homologues to genes that control floral development in angiosperms; *Plant Mol. Biol.* **27** 69-78
- Theissen G, Kim J T and Saedler H 1996 Classification and phylogeny of the MADS-box multigene family suggest defined roles of MADS-box gene subfamilies in the morphological evolution of eukaryotes; *J. Mol. Evol.* **43** 484-516
- van der Krol A R and Chua N -H 1993 Flower development in petunia; *Plant Cell* **5** 1195-1203
- van Tunen A J, Eikelboom W and Angenent G C 1993 Floral organogenesis in *Tulipa*; *Flowering Newsl.* **16** 33-37
- Veit B, Schmidt R J, Hake S and Yanofsky M F 1993 Maize floral development: new genes and old mutants; *Plant Cell* **5** 1205-1215 .
- Vergara F S and Alvarez-Buyela E 1977 Developmental genetic basis of the origin and evolution of the homeotic phenotype of *Lacandonia schismatica*: an endemic angiosperm from Chiapas (Mexico) with inverted reproductive whorls; *Keystone Symposium: Evolution of Plant Development*, Taos, NM, p 28
- Weigel D and Meyerowitz E M 1994 The ABCs of floral homeotic genes; *Cell* **78** 203-209
- Yanofsky M F, Ma H, Bowman J L, Drews G N, Feldmann K A and Meyerowitz E M 1990 The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors; *Nature (London)* **346** 35-40
- Yu D, Kotilained P, Elomaa P, Mehto M, Helariutta Albert V and Teeri T H 1977 Inflorescence development in Asteraceae: B-function MADS-box genes are required for congenital fusion in corolla and stamen and the DEFICIENS ortholog has differential radial effects within capitulum; *Keystone Symposium: Evolution of Plant Development*, Taos, NM p 28 .