Development 122, 1093-1102 (1996)
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DEV0061

Initiation patterns of flower and floral organ development in *Arabidopsis thaliana*

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SUMMARY

Sector boundary analysis has been used to deduce the number and orientation of cells initiating flower and floral organ development in *Arabidopsis thaliana*. Sectors were produced in transgenic plants carrying the *Ac* transposon from maize inserted between the constitutive 35S promoter and the GUS reporter gene. Excision of the transposon results in a blue-staining sector. Plants were chosen in which an early arising sector passed from vegetative regions into the inflorescence and through a mature flower. The range of sector boundary positions seen in mature flowers indicated that flower primordia usually arise from a group of four cells on the inflorescence flank. The radial axes of the mature flower are apparently set by these cells, supporting the concept that they act as a structural template. Floral organs show two patterns of initiation, a leaf-like pattern with eight cells in a row (sepals and carpels), or a shoot-like pattern with four cells in a block (stamens). The petal initiation pattern involved too few cells to allow assignment. The numbers of initiating cells were close to those seen when organ growth commenced in each case, indicating that earlier specification of floral organ development does not occur. By examining sector boundaries in homeotic mutant flowers in which second whorl organs develop as sepal-like organs rather than petals, we have shown that their pattern of origin is position dependent rather than identity dependent.

Key words: sector boundary analysis, *Arabidopsis*, flower development, floral primordia, organ initiation

INTRODUCTION

The form of the higher plant body is the result of developmental decisions made in meristems. Specific cell divisions in meristematic regions ultimately decide the location and overall shape of mature organs. Factors that control how many cells are involved, where they are located, and when they commence division are largely unknown (Lyndon, 1990; Sussex, 1989).

Cell division patterns in plant meristems have been studied using mosaics, i.e. individuals that carry a cell-autonomous genetic marker in some of their cells (reviewed by Dawe and Freeling, 1991; Poethig, 1987, 1989; Tilney-Bassett, 1986). The first conclusion from such studies has been that plant meristems are stratified, with the epidermis, and in most cases an underlying layer of cells, each maintaining their clonal distinction. Later studies traced cell lineages from specific stages of development, such as those derived from the apical meristem of the mature seed. In general, they showed that cell lineages are variable in extent, and that one lineage eventually displaces the other as meristematic growth continues. Hence it was concluded that defined, lineage-dependent fates are not usually involved in plant morphogenesis (Sussex, 1989).

More recently, cell lineage studies in plants have been used to identify the number of cells set aside as progenitors of specific organs (Dawe and Freeling, 1991). These 'sector boundary' studies make use of mosaic events established well in advance of the origin of the organ. A sample of sectored mature organs is then observed, and the number of different locations where a sector boundary may occur gives an estimate of the number of cells present at the organ's specification. Sector boundary studies were first performed in *Drosophila* (Merriam, 1978) where mosaics arising at the first zygotic division allowed the number of cells generating imaginal discs to be estimated. In plants, the first sector boundary analysis showed that cotton cotyledons each arise from eight cells in a line in the developing embryo (Christianson, 1986). More recently, Dawe and Freeling (1992) used the technique to establish that the maize anther often arises from progenitors occurring as a block of only four cells within the flower primordium.

In the last few years there have been major advances in our understanding of the genetic basis of flower morphogenesis (Weigel and Meyerowitz, 1994). In particular, a category of genes has been discovered that act to specify the identity of flower meristems and of individual floral organ types. These genes define positional information that is interpreted by the newly arising primordia, allowing them to differentiate appropriately. However, our knowledge of factors that control the commencement of flower and floral organ development is still sketchy. To increase our understanding, it would be useful to know when a commitment is made to generate flowers and their component floral organs. Sector boundary analysis can provide this information by allowing us to determine the number and orientation of progenitor cells. Also the pattern of

initial cell division in specific organs, and the geometry of their subsequent growth, can be deduced.

We have therefore used sector boundary analysis in *Arabidopsis thaliana* flowers to test if defined cell lineages are set aside in advance of the commencement of flower and floral organ growth. Also, by observing sectors in homeotic mutant flowers, possible links between organ initiation and organ identity have been examined. Further, variations in cell lineage patterns observed between flowers has thrown light on the role played by the initiating cells in setting the geometric axes of the mature flower. Finally we discuss our findings in relation to evolutionary speculations concerning the leaf-like or shoot-like ancestry of floral organs.

MATERIALS AND METHODS

Plant material

All plants were derived from the Landsberg *erecta* ecotype. To obtain sectored plants, we employed a transformed line (line 5.1) that carried the mobile element *Activator* (*Ac*) from maize. The element was inserted into wild-type *Arabidopsis* using *Agrobacterium tumefaciens* carrying the T DNA plasmid pBI35SAc11 (Finnegan et al., 1993). In this construct the promoter of the *Ac* element has been replaced by a constitutive 35S promoter from the cauliflower mosaic virus (CaMV). The modified *Ac* element is located between another 35S promoter and the bacterial *uid*A reporter gene. The chimeric condition of the plant is caused by excision of the *Ac* transposon leading to cell-autonomous expression of the reporter gene which can now be visualised by β-glucuronidase (GUS) staining of all descendant cells (Finnegan et al., 1989).

To obtain sectored mutant flowers, the transformed line was crossed with various mutant strains, and F_2 progeny raised for analysis. The mutant lines used were *pistillata-1*, *pistillata-1 agamous-1*, *apetala3-1* (Bowman et al., 1989) and *apetala3-3* (Jack et al., 1992).

GUS staining and processing of stained inflorescences

Primary inflorescences were harvested when the oldest flowers were just opening (Fig. 1A; stages 12-13; Smyth et al., 1990). They were vacuum infiltrated with 50 mM sodium phosphate buffer (pH 6.8) containing 1 mM X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) and 0.2% sodium lauryl sulphate and stained overnight at 37°C. The reaction was stopped and chlorophyll removed by incubating in 70% ethanol for several hours, exchanging the liquid from time to time. At this stage the sectored inflorescences were evaluated using a dissecting microscope and relevant stage 12 or 13 flowers were either analysed directly, or fixed for microscope analysis of sections.

To determine the reliability of X-gluc staining, control experiments were carried out using plants that were anticipated to be fully blue. These were derived from a descendant of line 5.1 that had inherited an excised version of the insert from its parent (i.e. a germinal excision). Sepals stained blue regularly and relatively uniformly. Other floral organs stained heaviest in flowers that were just opening (stages 12-13). The gynoecium was stained particularly at the base and in the upper regions (excluding the stigmatic papillae). Stamens were stained in both the filament and the anther. Petals took up the least stain and careful examination was necessary to determine its presence and extent. These staining patterns closely resemble those obtained by Tsukaya et al. (1993) using a similar 35S::GUS construct. Plants lacking the construct showed no blue staining.

It is important for analysis of sectored organs that the GUS staining is cell-autonomous. Evidence for this is that sector boundaries within organs were relatively sharp, with little evidence of a gradient in staining intensity. Further, the majority of boundaries falling within organs bisected them (see Results), unexpected if the blue stain moved into adjacent tissues during the staining procedure.

Fixation, sections and microscopy

Selected flowers that had been GUS-stained were fixed overnight at room temperature in an aqueous solution containing 50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde. After dehydration through an ethanol series (70%, 85% and 95%), flowers were counter-stained overnight with 0.1% eosin in 95% ethanol to allow easy orientation of the specimen during embedding and sectioning. The ethanol was replaced step by step with Histoclear (National Diagnostics) which in turn was replaced by Paraplast Plus (Oxford Labware) in several steps over 2-3 days at 65°C. Individual flowers were then transferred into plastic plug modules and the Paraplast solidified.

Flowers were cross-sectioned using a Reichert and Jung microtome. Relatively thick sections of 14 μm were generated to enhance visualisation of sectors, especially in weaker staining tissues. GUS-stained cells were identified in sections at 100× and 400× magnification by the presence of blue granules using bright-field optics and red granules using dark-field optics.

RESULTS

Wild-type morphology

Flower organs are arranged in concentric whorls as four sepals (two medial and two lateral), four petals, six stamens (four medial and two lateral) and two fused carpels in a lateral orientation (Fig. 1A). Sepals and carpels are sessile organs with three main vascular strands. Petals are attached to the flower axis in a peltate manner and contain one main vascular strand which branches successively. Stamens, in which a tetraloculate anther is present on a long filament, contain only one main vascular strand. The early development of *Arabidopsis* wild-type flowers has been described in detail (Hill and Lord, 1989; Smyth et al., 1990; Vaughan, 1955; Fig. 1E,F).

Sector boundary analysis

In this method, the number of different fractions into which an organ can be divided by a boundary between two types of sector (in this case unstained and GUS-stained) gives an estimate of the number of cells present at the time of organ initiation. It is a requirement that the sectoring event must have occurred well in advance of flower and organ initiation. For this reason an individual transformed line (line 5.1) showing predominantly large sectors (early events) was used. Individual plants were chosen that showed a sector already present in the main stem that continued into the primary inflorescence (Fig. 1B,C). Typically, around one quarter to one half of a sectored stem stained blue. The epidermal (LI) layer was stained relatively heavily, and in most cases the underlying LII layer and deeper cells (LIII) were also stained. Because a sector arises initially as one cell, some movement of cells between layers (cell invasion) presumably occurred following the sector's establishment much earlier.

As boundaries of the stem sector passed into the inflorescence they sometimes dissected individual flowers (Fig. 1D,G). Sectors mostly occupied all cell layers of the flower at approximately corresponding positions, suggesting that the layers grow more or less in concert. Sectored mature flowers were then collected for detailed analysis.

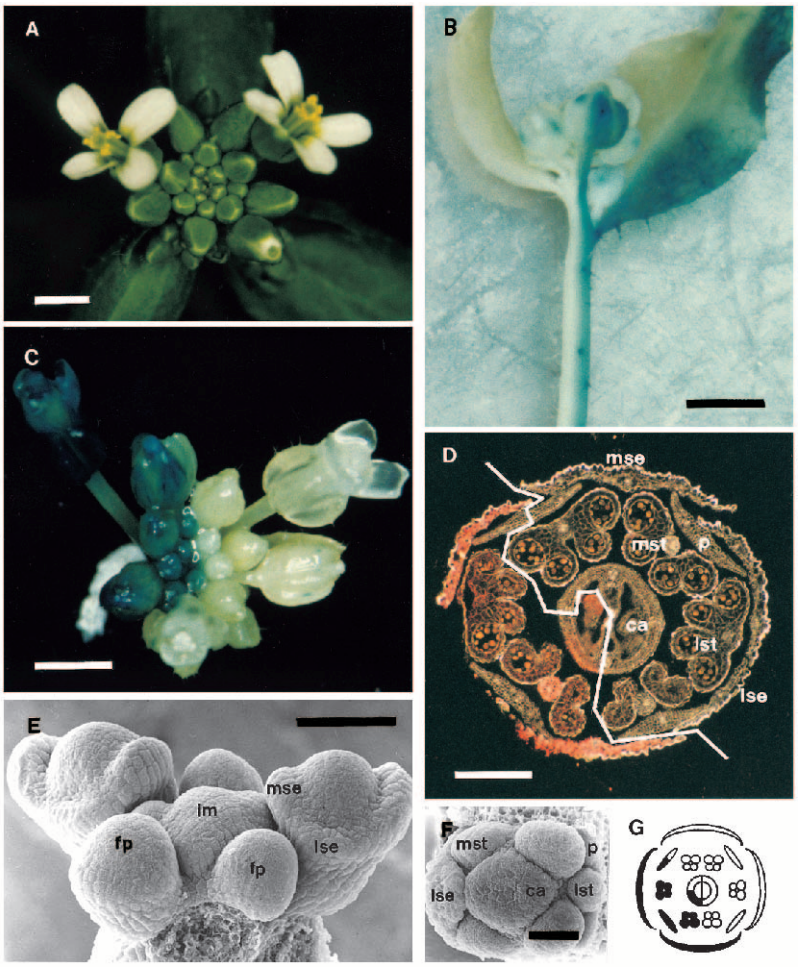

Fig. 1. Inflorescences and flowers of wild-type *Arabidopsis* showing examples of sector boundaries. (A) Vertical view of an inflorescence apex at the stage sampled. (B) Inflorescence with an early, blue-stained GUS sector passing up the stem, into the inflorescence and through a bud (flower number 138-2). (C) Vertical view of a sectored inflorescence. (D) Section of a sectored flower (number 104-3) viewed under dark-field optics. Under these conditions the blue-staining granules appear red. The position of the sector boundary is indicated. mse, medial sepal; lse, lateral sepal; p, petal; mst, medial stamen; lst, lateral stamen; ca, carpel. (E) Side view of a inflorescence apex showing the meristem (im), developing floral primordia (fp), and primordia of medial and lateral sepals (mse and lse). (F) Vertical view of a developing stage 6 flower showing young floral organ primordia. Three sepals have been removed. lse, lateral sepal; p, petal; mst, medial stamen; lse, lateral stamen; ca, carpel. (G) Floral diagram of the sectored flower shown in D, with the staining region shaded. Bars represent 1 mm in A-C, 200 μm in D, 50 μm in E and 20 μm in F.

Selection of sectored flowers

Wild-type families segregating for a single 35SAc11 insert were grown until the first flowers on the main inflorescence had just opened (Fig. 1A). 1,083 such inflorescences were excised and stained for the presence of GUS activity. Of these, 747 were either unstained or stained in only a few small, isolated patches. The plants without stain were those that lacked the insert through segregation, and plants in which no excision events had occurred. Those stained in small patches demonstrated late excision events, and for the purposes of this analysis are combined with the unstained class. The remaining 336 inflorescences were either variegated with large sectors (Fig. 1B,C), or stained completely blue. The fully blue plants had either inherited an insert from which the *Ac* element had already excised in one or other parent, or the inflorescence had been fully taken over by a blue sector from an early excision event. In either case they are not relevant to the present analysis. 52 of the remaining variegated inflorescences had a sector in the main stem that passed into the inflorescence and through at least one mature flower where it could be clearly seen in the pedicel. 56 sectored flowers that were just opened were scored in detail, either by direct observation or from sections (Fig. 1D,G). In addition, four sectored wild-type flowers were obtained as segregants from the mutant experiments (see below).

Floral diagrams of the 60 sectored flowers are depicted in Fig. 2. In scoring these, the medial and lateral positions were recorded but not the abaxial (outer) versus adaxial (inner) orientation with respect to the inflorescence apex. Before analysing the sectors in detail, the flowers were examined for the relative amounts of fully blue-stained tissues versus unstained tissues. These should occur in equal proportions in the absence of bias caused by such factors as differential growth of the different sector types, and non-autonomous or incomplete staining. The latter is particularly relevant for weaker staining organs such as petals and stamens. Table 1 shows that the two patterns occur in close to equal frequencies for all four floral organ types in both medial and lateral positions.

Table 1. Staining patterns of floral organs in wild-type flowers

	Number of organs				
Floral organ	Fully stained	Unstained	Sectored	Unknown	Total
Sepals, medial	31	17	71	1	120
Sepals, lateral	58	58	3	1	120
Petals	107	113	7	13	240
Stamens, medial	94	101	32	13	240
Stamens, lateral	57	53	1	9	120
Carpels	50	48	10	12	120

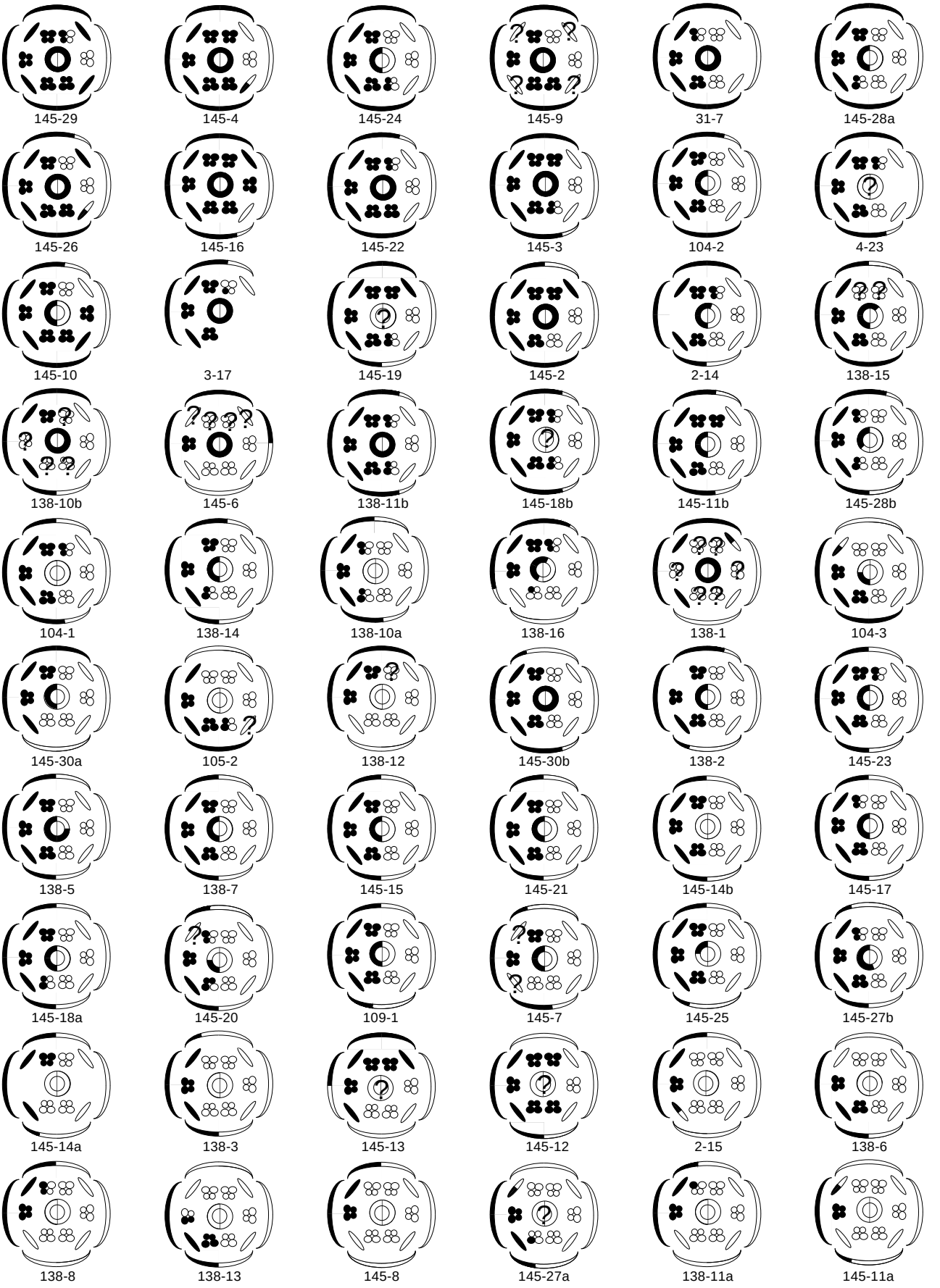
145-29
145-4
145-24
145-9
31-7
145-28a
145-26
145-16
145-22
145-3
104-2
4-23
145-10
3-17
145-19
145-2
2-14
138-15
138-10b
145-6
138-11b
145-18b
145-11b
145-28b
104-1
138-14
138-10a
138-16
138-1
104-3
145-30a
105-2
138-12
145-30b
138-2
145-23
138-5
138-7
145-15
145-21
145-14b
145-17
145-18a
145-20
109-1
145-7
145-25
145-27b
145-14a
138-3
145-13
145-12
2-15
138-6
138-8
138-13
145-8
145-27a
138-11a
145-11a

Fig. 2. Floral diagrams of 60 sectored wild-type flowers. Each flower carries a sector that was already present on the inflorescence flank before the flower arose. Occasional late excision events, occurring as small, isolated patches of blue-stained cells, are omitted. The side of the flower containing the larger proportion of blue-staining (shaded) is placed consistently on the left, and flowers are arranged from left to right and down the page in decreasing order of the cumulative amount of blue staining in the sepals. The orientation of individual flowers with respect to the inflorescence apex was not recorded. Organs in which staining could not be resolved are shown with a question mark, and missing organs are shown as blank positions.

Table 2. Staining patterns* of pairs of lateral organs in wild-type flowers

	Number of flowers							
Floral organs	BB		BU		UU	Sectored	Unknown	Total
Lateral sepals	0		56		0	3	1	60
Lateral stamens	2		50		0	1	7	60
Carpels	15		16		14	9	6	60
	BB BB	BU BB	BU BU	BU UU	UU UU			
Petals	1	5	32	7	0	7	8	60

*BB - both organs fully blue; BU - one fully blue, one unstained; UU - both organs unstained; Sectored - one or more organs sectored.

General properties of flower sector boundaries

Three generalisations can be made about the sector patterns recorded in the 60 flowers (Fig. 2). Firstly, only one sector boundary passed through each flower. Secondly, flowers were divided approximately into halves. Thirdly, the boundary was almost always closer to the medial (vertical) plane than to the lateral (horizontal) plane. These three properties can be illustrated by pooling flowers that demonstrate the same staining patterns for pairs of lateral organs (Table 2). For example, the two lateral sepals almost always occur as one stained fully blue and the other unstained. The four petals also frequently display a medial sector boundary (32 flowers had two blue petals on one side, two unstained on the other). Lateral stamens are similarly divided. For the carpels, some flowers had one blue and one unstained carpel although the proportion was lower (16 out of 60 flowers).

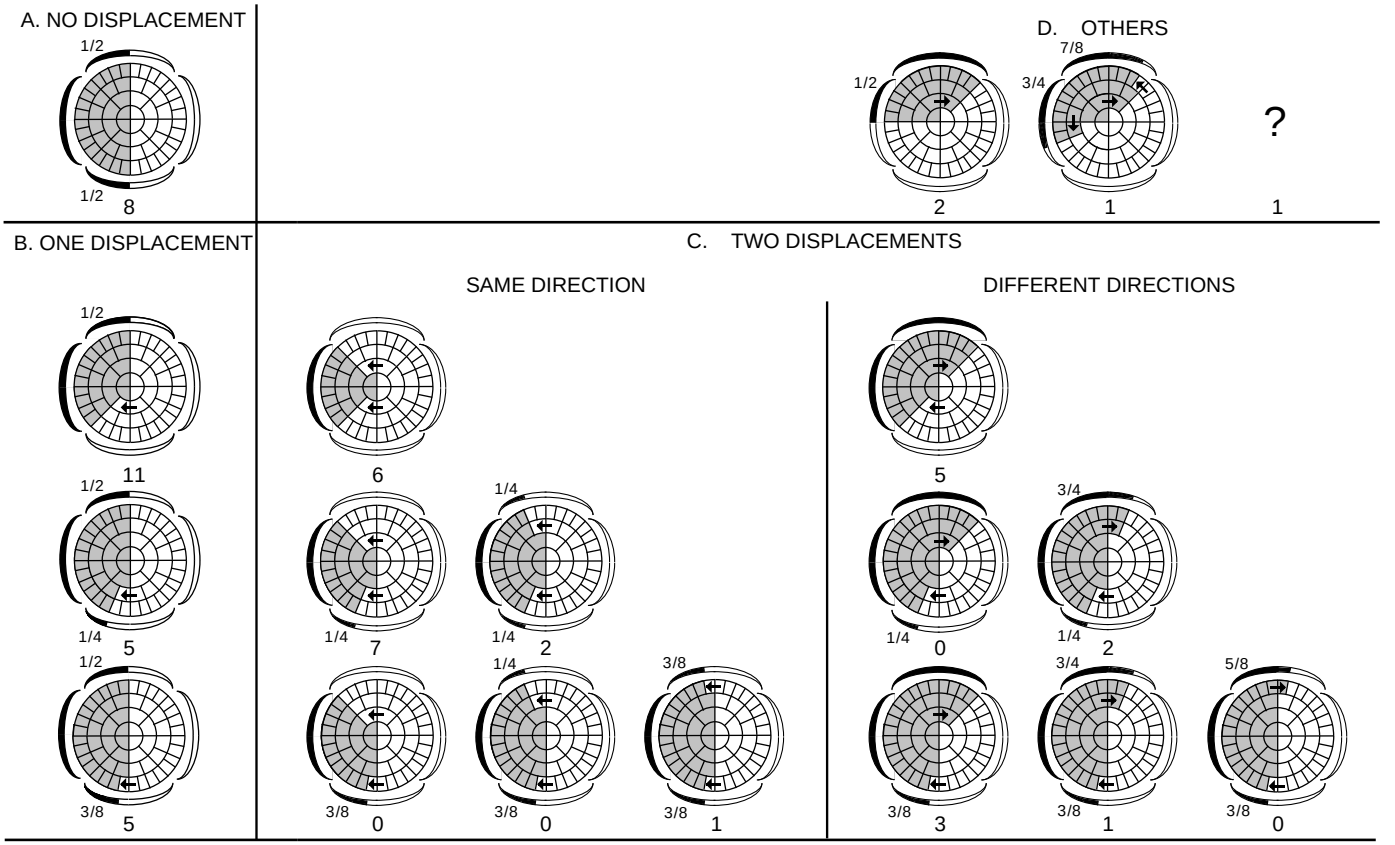

Fig. 3. Interpretation of early cell division patterns generating the 60 sectored wild-type flowers in Fig. 2. Flowers with equivalent patterns of sepal sectoring have been combined without regard to which part of the sector is blue. It is proposed that flowers develop from four cells in a block (central positions in each diagram) that are usually bissected vertically by a sector boundary. These cells then divide to generate concentric rings of descendant cells. Sometimes displacement events occur (arrows) that distort the regular radial expansion of cells. (A) Eight flowers showing an exact medial sector boundary. (B) Twenty one flowers in which early displacement events have resulted in cells from one side contributing descendants to the other. (C) Flowers in which early displacement events have occurred in both the upper and lower halves of the flower, either to the same side (16 flowers) or to opposite sides (11 flowers). (D) Unusual flowers in which the initial boundary divided the four initiating cells in a three to one pattern, following which various displacement events occurred.

Sector analysis of the flower primordium

To deduce the number of cells present when specification of flower primordium development occurs, the overall range of sector boundary patterns defined by the outer perimeter of the flower was analysed (Fig. 3). The four sepals provide appropriate reference points for the perimeter because neighbouring sepals abut and sepals are the first-formed organs.

From the examination of the sector patterns, we propose that flowers ultimately develop from a block of four cells occurring on the flank of the inflorescence apex. Sector boundaries mostly fall medially between these four cells, dividing them into two pairs. This presumably reflects the fact that cells on the inflorescence flank are laid down predominantly in the longitudinal direction (Vaughan, 1955). Because the sector boundary exists within the inflorescence apex from well before floral initiation, it normally passes vertically down its flank. The original four cells then divide and expand the size of the flower primordium outwards. In some cases the descendant cells of each of the four ancestors maintain their radial orientation (e.g. 8 flowers in Fig. 3A). However, displacements of the sector boundary often occur and indicate that asymmetric or asynchronous cell divisions may modify this radial pattern. In this scheme, the earliest displacements result in unsectored medial sepals, slightly later events generate one quarter:three quarters sectors, and an even later events three eighths:five eighths sectors (Fig. 3B). It is proposed that such displacement events occur in both the upper and lower parts of the flower primordium, and in the same or opposing directions (Fig. 3C). A few flowers do not fit this scheme (Fig. 3D), and these may have arisen if the original sector boundary fell between one of the initial cells and the other three.

Overall, the relative frequencies of the sector patterns indicate that the top and bottom halves of the flower behave independently (the numbers of flowers showing displacements in the same and opposite directions were approximately equal; Fig. 3C). They also suggest that the absence of displacement and early displacement events occurred at similar frequencies (37 versus 43 occasions respectively), while later displacements become rapidly and progressively less frequent, with 21 events generating $\frac{1}{4}$:$\frac{3}{4}$ medial sepal sectors, and 11 leading to $\frac{3}{8}$:$\frac{5}{8}$ sectors.

Thus the sector results are consistent with the flower primordium arising from a group of four cells lying square on the flank of the inflorescence apex at the time of floral initiation. Most flowers show variation in the resulting sector patterns, but these can be accounted for simply in terms of variation in the occurrence and direction of early asymmetric cell divisions. Other explanations may be possible, but our scheme accounts for the data in a simple and straightforward way.

Sector analysis of floral organ primordia

Moving now to an analysis of sector boundaries for each of the four types of floral organ, the number of times each was divided into various fractions was summed across all 60 sectored wild-type flowers (Table 3).

Medial sepals were dissected by a boundary 71 times, with the boundary in more than half the cases falling along the main midrib dissecting the sepal into halves (Fig. 4A). About one

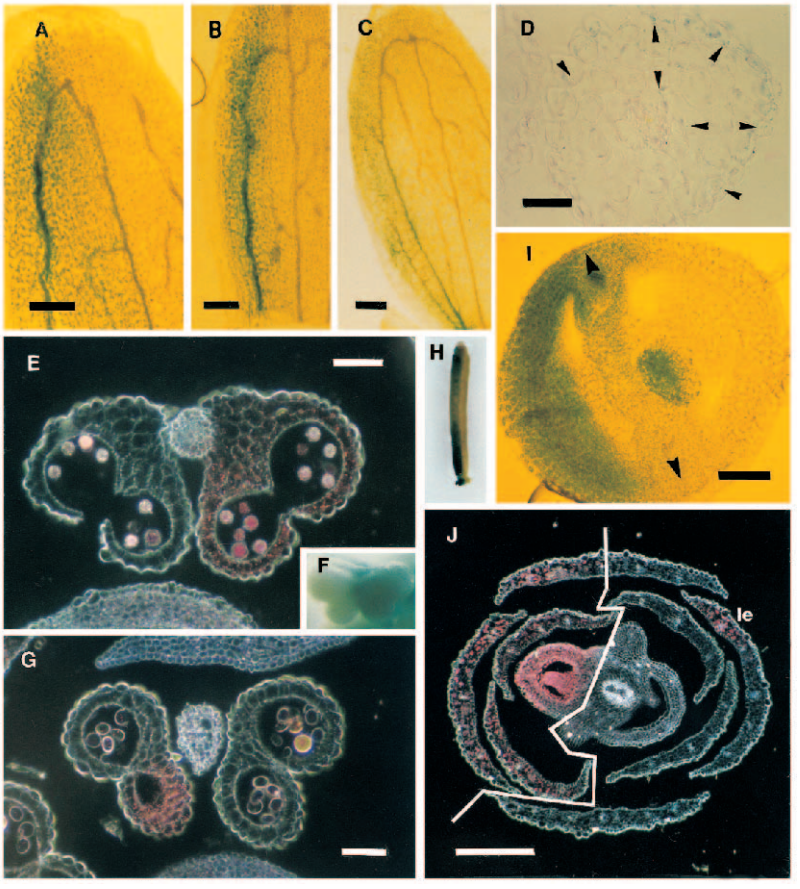

Fig. 4. Sector boundary patterns in individual wild-type floral organs, and in a *pistillata* mutant flower. (A-C) Wild type sepals showing blue sectors occupying one half (A; flower number 145-20 in Fig. 2), three-eighths (B; flower 145-20) and one quarter (C; flower 145-14a) of the sepal's width. (D) Bright field photo of a sectioned wild-type petal close to the base showing its division into halves by a sector boundary (flower 2-15). Arrowheads indicate some of the cells with blue granules. (E, F) Sectioned anthers showing a medial sector boundary under dark field (E; flower 105-2), and an anther's appearance before embedding and sectioning (F; flower 145-18a). (G) Sectioned anther showing staining of a single small locule (flower 3-17). (H) Maturing silique showing a sector boundary falling exactly between the two carpels throughout its length. (I) Hand section of a sectored gynoecium showing the left carpel with a 7/8 blue sector, and the right carpel with a 1/8 blue sector (flower 138-16). Arrowheads indicate boundaries between the two carpels. (J) Dark field photo of the sectioned *pistillata* mutant flower number 3-19 (see Fig. 6) in which second whorl organs develop as sepals. A sector boundary passes between the medial and lateral sepal on the bottom left but does not dissect the second whorl sepal lying between them. le, late excision event generating a small patch of stained tissue. Bars represent 100 μm in A-C, 25 μm in D, 50 μm in E-I and 200 μm in J.

Table 3. Sectoring patterns of floral organs in wild-type flowers

	Number of organs		Area occupied by blue sector						
Floral organ	Scored	Sectored	1/8	1/4	3/8	1/2	5/8	3/4	7/8
Sepals, medial	119	71	0	8	4	37	8	13	1
Sepals, lateral	119	3	0	0	0	2	0	1	0
Petals	227	7				7			
Stamens, medial	227	32		4		27	1		
Stamens, lateral	111	1		0		1	0		
Carpels	108	10	3	1	0	4	0	1	1

third of the boundaries dissected the sepal into either one-quarter (Fig. 4C) or three-quarters stained sections, with the boundary again falling along major veins. Sectors falling between veins were less frequent, usually revealing a three-eighths (Fig. 4B) or a five-eighths staining pattern. The fact that they can be divided into most of the possible one-eighth incremental divisions indicates that medial sepals are initiated from a ridge extending for eight cells on the flower meristem (Fig. 5A). Lateral sepals were dissected very rarely (Table 3), presumably as a consequence of the way in which sectored flowers were obtained rather than through any major differences from medial sepals in their mode of initiation.

Second whorl petals were sectored on only 7 occasions (Table 3), and in each case the sector divided the organ into halves (Fig. 4D). This suggests that petals arise from two cells in the flower primordium. Additional indirect evidence supports this limited ancestral number. Petal primordia arise in a position lying between medial and lateral sepals, and in 45 cases a sector boundary occurred between two neighboring sepals in this position (Fig. 2), potentially falling between ancestral petal cells. However, it did so on only 7 occasions.

Medial stamens were dissected into halves on 27 occasions (Table 3), always in the medial plane (Fig. 4E,F). Five stamens were stained in either one-quarter or three-quarter fractions, with the stained sectors coinciding with locules. One of the larger locules had a differential staining pattern in three flowers (flower numbers 138-11a, 145-20 and 145-27a in Fig. 2), whereas one of the smaller locules was differentially stained in two flowers (138-16 and 3-17; Fig. 4G). These staining patterns indicate that stamens are derived from four cells in the flower primordium, with the position of the locules being defined by the initial cells. Because we found that either a large or a small locule can be stained individually, the four ancestral cells are much more likely to be clustered than arranged in a line (Fig. 5B).

Finally, the two carpels were analysed. These occur in lateral positions in the *Arabidopsis* flower, and, as with other lateral organs, they were divided relatively infrequently by a longitudinal sector boundary (Fig. 4H). Even so, of the 10 carpels that were dissected, five of the possible one eighth fractions were seen (Table 3; Fig. 4I). This indicates that carpels, like sepals, are initiated from eight cells in line in the flower primordium.

Sector analysis of homeotic second whorl sepal primordia

To test if the mode of organ initiation is independent of the organ's position within the developing flower, we made use of homeotic mutants. In these the identity of a floral organ may be separated from its wild-type position. Specifically, we performed sector boundary analysis on flowers in *apetala3* and *pistillata* mutants where sepal-like organs develop in place of petals in the second whorl.

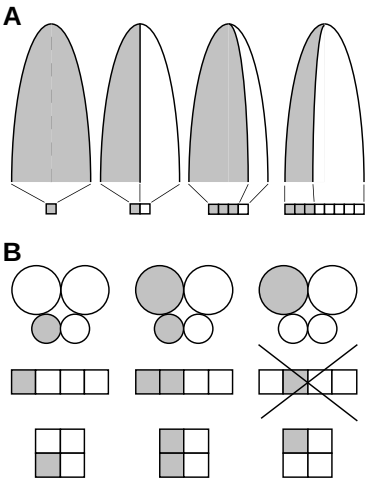

Fig. 5. Diagrams showing alternative patterns of initiating cells, and examples of resulting sector patterns in mature organs. (A) Sectors present in sepals if they were initiated from one, two, four or eight cells in a line. Our observations are consistent with the eight cell pattern. (B) Examples of sectors present in stamens if they were initiated from four cells in a line, or four cells in a block. The former is unlikely because it does not predict the observation that large locules can be differentially stained on occasion (right) as well as small locules (left). The flowers seen contained only one sector boundary, and the 'large only' pattern requires two.

The main observation from 20 sectored mutant flowers (Fig. 6A) was that only one of the 80 second whorl sepals was divided by a sector boundary (flower 1-6), even though sectors passed in the vicinity (i.e. between lateral and medial first whorl sepals) on 13 occasions. If second whorl sepals arose from eight cells in a line (as proposed for first whorl sepals), rather than two adjacent cells (as for second whorl petals), it is likely that relatively more of the mutant organs would have been sectored (Fig. 6B). The observation of 1 sectored mutant organ out of 80 is not significantly different from the 7 sectored out of 227 observed for wild-type petals (Table 3). Importantly, it was divided into halves in the same way as were wild-type petals.

We conclude that when sepal-like organs develop in petal positions in the second whorl, their ancestral cells are arranged like petal initials rather than first whorl sepal initials.

DISCUSSION

The flower primordium occurs as a block of four cells at initiation

Our first major conclusion is that the *Arabidopsis* flower primordium is initiated from very few cells that divide in a regular pattern. The data are consistent with initiation from four cells in a block, which usually derive from longitudinal division of two, side by side cells. Such longitudinal cell division is the usual pattern on the inflorescence flank meristem where flower primordia arise (Vaughan, 1955). The four cells apparently then divide and expand radially in a relatively regular way to generate a concentric group of cells from which all floral tissues are derived. To account for sector boundary displacements from an exact medial division of the flower, we propose that descendants of one or two of these four cells usually

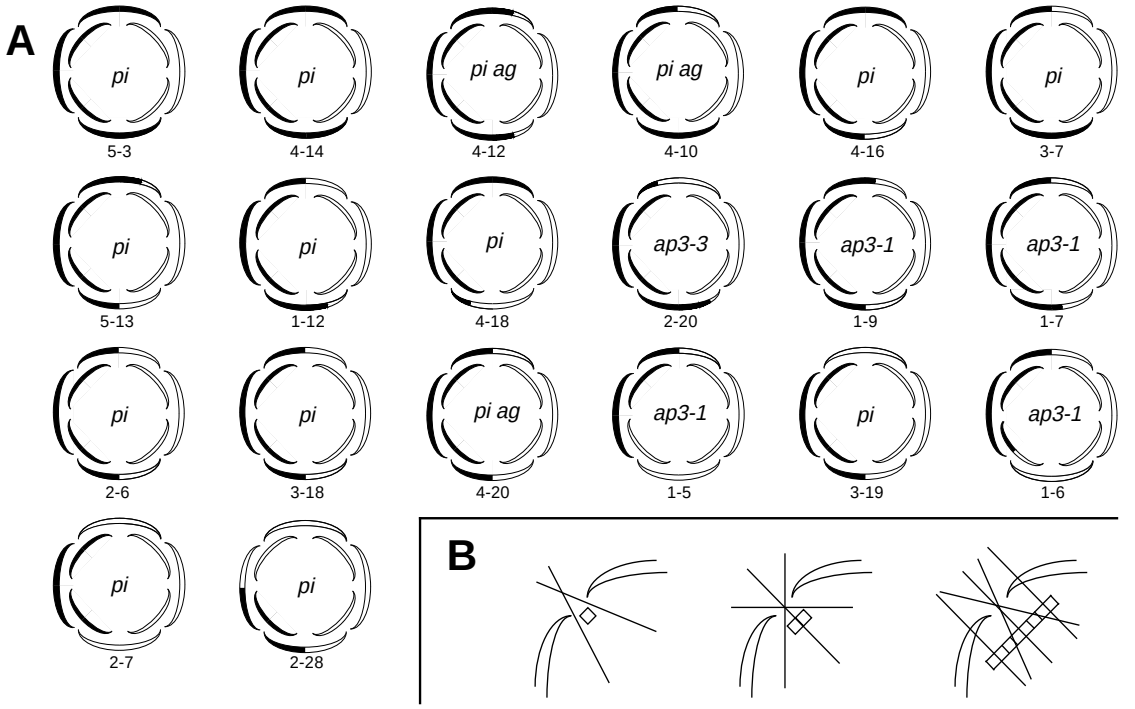

Fig. 6. (A) Floral diagrams of 20 sectored *apetala3* and *pistillata* mutant flowers in which second whorl organs develop as sepals rather than petals. For simplicity, organs in third and further internal whorls, which are variable between the four genotypes analysed, are not shown. (B) Diagram showing the possible sectoring patterns of second whorl organs arising from a row of cells in the region between a medial and a lateral sepal. The outcomes from one-, two-, and eight-cell alternatives are shown.

occupy a wider fraction of the circle than average as the result of early asymmetric cell divisions (Fig. 3).

Other flower initiation patterns are possible although we argue they are less likely. For example, if flowers were initiated from three cells in a cluster rather than four, sectoring of the lateral sepals would presumably occur often but it was rarely seen. Also, if the initiating cell cluster were three or more cells across rather than two, medial sepals would presumably be divided relatively frequently into fractions other than the usual halves or quarters. Finally, some flowers may have been initiated from one cell, but because such flowers cannot be sectored they would not have entered our analysis.

Our results are similar to those of Furner and Pumfrey (1993) in-so-far as they overlap. These authors generated *albino1* sectors in the *Arabidopsis* inflorescence by irradiating dry seeds of heterozygous plants. They then scored sepal patterns in 92 sectored flowers. Like us, they found that medial sepals were sectored much more often than laterals. However, they did not record the individual sector fractions. This precludes the type of analysis we have carried out, but their data do show that the two lateral sepals are usually derived from different ancestral cells (79 flowers out of 92).

The first known indication that a flower primordium is about to develop on the *Arabidopsis* inflorescence flank is expression of the *LEAFY* gene (Weigel et al., 1992). This expression occurs in a small number of cells in the anlagen of floral primordia before any growth is seen, cells that may correspond to the four initial cells indicated by our data.

Floral organs arise from defined numbers of cells

Our second main conclusion is that the number of cells committed to generating specific floral organs is close to the number present when organ growth commences. An indication of the latter can be obtained from surface views of the flower primordium (Fig. 1E,F; Smyth et al., 1990). For each organ type the number is close to that estimated from sector boundary analysis, viz. eight for sepals and carpels, four for stamens and two for petals. If organ-specific cell lineages were set aside earlier, the number derived from the sector analysis would have been fewer. Thus floral organs show no significant lag period between the determination that an organ will arise and the initiation of its growth. This is in line with other findings that developmental decisions in plants are generally made relatively late (Lyndon, 1990; Sussex, 1989).

Although surface cells are easily observed, floral organ growth in *Arabidopsis* is actually initiated from internal cells (Vaughan, 1955). Studies of sectioned buds reveal that the first cell divisions generating sepals, petals and stamens usually occur in the LII layer, whereas carpels may commence growth from either LII or LIII cells (Crone and Lord, 1994; Hill and Lord, 1989). The numbers of cells involved in the first divisions are difficult to deduce from sections but are unlikely to differ significantly from the number of overlying surface cells.

Initiation and establishment of floral organ identity are separable processes

The third major conclusion we can draw is that the initiation and identity of floral organs are controlled independently. When the identity of second whorl organs was changed from petals to sepal-like organs by mutation, the number of cells involved in their initiation was not affected. This confirms conclusions based on cytological studies of cell division patterns that initiate homeotic organ growth. These also reported that patterns are whorl-specific rather than organ-specific (Crone and Lord, 1994; Hill and Lord, 1989). Furthermore, the time-course of development of homeotically altered organs is characteristic of their position rather than their identity (Bowman et al., 1989, 1991; Crone and Lord, 1994; Hill and Lord, 1989), and the effect of the temperature-sensitive mutant *ap3-1* is not

apparent until after its modified second whorl organs have begun to differentiate (Bowman et al., 1989).

Thus the outcome of an organ's differentiation can be readily uncoupled from its pattern of initiation. A search for genes controlling organ initiation is now important, although we already know that some of the organ identity genes also have roles in this earlier process (Bowman et al., 1991).

It is interesting that the maintenance of identity in developing floral organs, once established, is likely to be dependent on cell lineage (Bowman et al., 1989). Such lineage dependent processes are infrequent in plant development. Evidence for this exception comes from the sharp boundaries that separate sepal, petal, stamen and carpel tissue types within mosaic organs that arise in certain mutants of *Arabidopsis* including *superman* and *unusual floral organs* (Weigel and Meyerowitz, 1994). The prediction in this case is that sector boundaries within such structurally mosaic organs will not cross boundaries between the different tissue types.

Compartments in plant development?

In animal development, a compartment has been defined as 'an area of the developing or mature [organism] that is constructed by all the descendants of a founding set of cells' (Lawrence, 1992). In mosaic organisms, sector boundaries do not cross from one compartment to another. In *Drosophila*, for example, sectors were shown not to cross between anterior and posterior halves of adult segments (García-Bellido et al., 1976). This ultimately lead to the discovery of parasegments as a key part of the segmentation process that occurs much earlier in development.

In plants the concept of compartments has not been commonly applied. Christianson (1986) first proposed their existence for cotton cotyledons. The evidence is that sector boundaries arising in embryos at fertilisation result in cotyledons being divided into a range of 1/8 sectors but never into smaller segments. By similar arguments, we could propose that *Arabidopsis* sepals and carpels, for example, are each made up of eight compartments. However, the concept is not particularly useful here because it defines an entity we can already see as a structure in the flower primordium rather than identifying an otherwise unrecognised group of cells set aside much earlier.

Even so, sector analysis has been useful in identifying constrained cell lineage patterns in other plant organs. In maize leaves, for example, one compartment contains half of each vein and its surrounding bundle sheath cells along with an adjacent mesophyll cell, all derived from two adjacent middle layer cells (Langdale et al., 1989). Another, larger compartment apparently occupies a longitudinal segment of epidermal cells lying between two lateral ribs (Cerioli et al., 1994). The latter compartments (called 'modules' by Cerioli et al.) regularly arise from a strip of tissue four cells wide. In *Arabidopsis* root development, too, mosaic lineage studies have revealed that the columellar root cap cells have a different ancestral origin from surrounding cells (Dolan et al., 1994), and that they are ultimately descended from one hypophoseal cell present much earlier in the triangular-stage embryo (Scheres et al., 1994). Lineage restrictions may yet be present in the later stages of development of *Arabidopsis* floral organs, especially the relatively complex reproductive organs, and it may be worthwhile searching for them using mosaics generated during their growth.

The concept of the 'structural template'

Recently, Dawe and Freeling (1992) proposed that for developing plant organs "initial cells serve as templates for the axes of symmetry by initiating a pattern of polarised cell division that is self-propagated". They had found that the majority of sector boundaries that divided maize anthers fell between the four locules rather than within them. They suggested that anthers often arise from four cells in a block, or eight cells in a ring surrounding four cells, and that the limitation to possible sector boundaries occurred because the axes of symmetry of the mature anthers were defined by the orientation of the initial cells. Later modifications to sector boundary positions were possible through displacement events as anther growth continued.

Our results from the *Arabidopsis* flower are consistent with such an interpretation. The regularity of sectors in the *Arabidopsis* flowers is striking given the apparent variation in cell patterns on the inflorescence flank where they arise (e.g. Fig. 1E). The structural template idea provides an explanation for this regularity. Even though the proposed four initiating cells may not be set exactly at right angles on the inflorescence flank, or meet exactly in a square pattern, subsequent growth means that the four quarters of the flower are established from these four cells irrespective of such variations in their initial orientation.

There is an important corollary to the structural template hypothesis (Dawe and Freeling, 1992). When the early displacement of a sector boundary occurs as the consequence of an irregular cell division, the developmental pathway followed by the affected cell lineage does *not* follow the boundary exactly. This means that the morphogenesis of structural units within the flower is independent of cell lineage. If they were lineage-dependent, boundaries would always fall between structural components and never within them.

The structural template concept can also be applied to embryos (Dawe and Freeling, 1991). In 46 semi-gametic cotton seedlings scored by Christianson (1986), sectors frequently occupied one quarter or one half of the combined cotyledon area (22 seedlings). Also they often fell between cotyledons (18 occasions), or divided them into halves (28 times). If four initial template cells are present at the earliest stage of embryo development, they could lead to these relatively stable medial and lateral axes in the developing cotyledons. The other patterns could arise through early displacement events. The maize embryo, and the shoot apical meristem within it, also seem to have initial cells present in a defined orientation with respect to the resulting plant axis (Bossinger et al., 1992; Poethig et al., 1986; Steffenson, 1968).

Thus our study has added flowers to the growing list of plant structures whose axes of radial symmetry are apparently controlled by the orientation of relatively few initial cells.

Evolutionary origin of floral organs

Shoots and leaves differ in their initiation patterns (Lyndon, 1990; Sussex, 1989). The former arise as blocks of cells whereas the latter are usually ridge-like. If these patterns are conserved through evolution, the patterns currently shown by floral organ types may reflect their shoot-like or leaf-like ancestry.

Stamen primordia in *Arabidopsis*, like those of maize (Dawe and Freeling, 1992), are shoot-like. This is in line with stamens being viewed as being reduced, microsporophyll-bearing

shoots rather than sporophylls per se, thus supporting a pseudanthial rather than a euanthial origin of the flower (Doyle, 1994). In contrast, carpels develop from a leaf-like ridge. Carpels evolved at the time of divergence of angiosperms (Doyle, 1994), and our data are in line with the carpel wall being derived from a leaf- or bract-like structure. Sepals are also generally viewed as modified leaves, and the sepal initiation patterns we observed are closely similar to those of *Arabidopsis* leaves (Furner and Pumfrey, 1992; Irish and Sussex, 1992). Finally, because very few cells are involved in the origin of *Arabidopsis* petals, the question of their shoot-like or leaf-like origin remains open.

It has recently become possible to test if shoot- and leaf-like initiation patterns are fundamentally distinct. Genes have been identified that have a role in the development of shoot apical meristems but not leaf primordia. *Knotted-1* and related homeobox genes of maize are expressed in the meristems of vegetative shoots, inflorescences and flowers, but not in regions on the meristem flanks that will soon develop as leaves or glumes (Jackson et al., 1994). It will be interesting to test if these genes, and related ones from *Arabidopsis* (see Lincoln et al., 1994), act as markers of shoot-like versus leaf-like organ initiation within flowers, and further if they have a role in establishing basic differences between these two growth patterns.

We thank Jean Finnegan for providing the *Ac* construct, Peter Bundock for help in setting up the *Agrobacterium* transformation system, John Alvarez for providing the SEMs in Fig. 1, John Alvarez, John Bowman, Bob Elliott, Megan Griffith and Marcus Heisler for critical reading of the manuscript, and colleagues from our laboratory for their advice and comments. This study was funded in part by a DFG Forschungsstipendium Bo 1086/2-1 to G. B., and supported by the Australian Research Council.

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(Accepted 21 December 1995)