Cell fate in the shoot apical meristem of *Arabidopsis thaliana*

IAN J. FURNER and JOANNE E. PUMFREY

Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK

Summary

Seeds of *Arabidopsis thaliana,* heterozygous for the *alb1* mutation were treated with X-rays to generate sectors of albino tissue in the mature plants. Sectors were observed in tissues derived from L_2 and L_3 layers of the shoot meristem. Altogether 324 sectors were obtained affecting 512 leaves or the inflorescence. The majority of sectors affected only one or other of the first leaf pair. In later leaves, sectors were less frequent, and often affected more than one leaf. Sectors seen in the flowers almost invariably included some of the cauline leaves. Sectors in any region of the plant were of variable length and width. The axillary meristems of *Arabidopsis* were found to be clonally related to two or more cells near the centre of the subtending leaf. Overall the data are compatible with the idea that there are few, if any, restrictions on cell fate within the cell layers of the dry seed meristem. As in other higher plants, developmental fate could only be predicted in a general and probabilistic way. Such a pattern might be generated if the acquisition of cell fate occurred continuously as the plant grows, in a position-dependent, lineage-independent fashion. A general model of the meristem has been produced to accommodate the observations concerning the great majority of the sectors.

Key words: *Arabidopsis thaliana*, fate map, meristem, sector *alb1*, cell fate.

Introduction

The meristems of higher plants are centres of cell division and organ initiation. The aerial structures of the plant are generated by the shoot apical meristem. Leaves are initiated on the flanks of the apical meristem in a regular pattern (phyllotaxy). Studies of cell fate in higher plants have attempted to relate the cells in the meristem or organ primordia to their final contributions to the mature plant. Typically, genetic events have been used to mark cell lineages at a particular point in development. For example, the fates of cells in the dry seed shoot apical meristem of maize (McDaniel and Poethig, 1988) and sunflower (Jegla and Sussex, 1989) have been mapped by irradiating seeds. Similarly, genetic events occurring early in zygotic development have been used to map the embryo of maize (Poethig et al., 1986) and cotton (Christianson, 1986).

Previous studies of stable periclinal chimeras in annual and perennial plants suggest that dicotyledonous plants typically have a three-layered meristem (Satina et al., 1940 and reviewed in Tilney-Bassett, 1986). The outermost L_1 layer divides anticlinally to form an essentially colourless epidermis over the leaves and stem. The subepidermal L_2 layer divides anticlinally in the meristem, and anticlinally and periclinally in the leaf primordium to give rise to the palisade layer and lower spongy mesophyll layer in the centre of the leaf and all of the mesophyll at the leaf margin. The inner L_3 layer divides anticlinally and periclinally in both the meristem and the leaf primordium, giving rise to the centre of the stem and a rather variable and irregular core in the leaves.

Arabidopsis thaliana, an inconspicuous ephemeral dicotyledonous plant, has become a model organism for studies of the genetics of higher plant development (Mayer et al., 1991). The vegetative shoot apical meristem of *Arabidopsis* is very small, approximately 53 μm in diameter (Vaughn, 1952, 1955). It consists of a flattened dome of cells which becomes more convex when the plant bolts (Miksche and Brown, 1965). The dry seed meristem of *Arabidopsis* is a smooth dome and leaf initiation starts only after germination. The seeds of the Landsberg *erecta* variety grown in long days germinate and the apical meristem produces six leaves in a rosette. The plant then bolts and the meristem produces a stem bearing three cauline leaves. The shoot apical meristem becomes the inflorescence meristem and continues to grow producing an indeterminate number of flowers borne on the stem. The precise number of rosette and cauline leaves is environmentally and genetically controlled. (The numbers given are for this variety in our growth rooms.) The first two leaves appear at opposite positions and subsequent leaves and flowers are initiated in a single phyllotaxy with each at approximately 140° to the preceding organ. The direction of the spiral (clockwise or anti-clockwise) occurs randomly from plant to plant, but is constant within a particular plant (Smyth et al., 1990).

We set out to produce a fate map of the cells within the dry seed shoot apical meristem of *Arabidopsis.* To do this, we modified a procedure that has already been used successfully in maize (Steffenson, 1968; Coe and Neuffer, 1979) and other species. X-irradiation of dry *Arabidopsis* seeds heterozygous for the *alb1* mutation was used to generate albino

sectors in the green tissue derived from the L_2 and L_3 layers in the mature plants. Cell fate in *Arabidopsis* resembled the findings from earlier studies on sunflower (Jegla and Sussex, 1989) and maize (McDaniel and Poethig, 1988). The cell fates observed in this study were variable and we propose that both position and chance play large roles in governing a cell's final contribution to the plant.

Materials and methods

Arabidopsis seeds of the variety Landsberg *erecta* segregating for the *alb1* mutation (Koorneef et al., 1983) were weighed into 1000 seed batches (20 mg). 10-20 such batches were placed into a Torrex 150 X-ray machine (Astrophysics Research Corporation). The seeds were irradiated for varying periods of time at a dose rate estimated to be 300 R/minute (5 mA, 150 KeV, unfiltered). Seeds were mixed with dry sand and sown at 1000 seeds per tray containing a mixture of 4:1 sand : Fison Levington Compost. The trays were placed in propagators approximately 2 ft from two fluorescent (white) 80 watt tubes. The temperature was maintained at 21ºC with an 18 hour day. Each day the trays were examined for sectored plants and these plants were transferred to individual tray sections, grown and scored for the position and extent of sectors and the plant phyllotaxy. The width of sectors was estimated as a proportion of the width of the leaf. The length was estimated as the number of affected structures (leaves or the flowers). In sectors including white and pale green tissue, the sector width was scored as including both. As the first two leaves are morphologically indistinguishable, leaf 1 was arbitrarily defined as the member of this pair closest to leaf 3.

Results

The alb1 *mutation and sector phenotypes*
The strategy used in this study was to treat plants heterozygous for the *alb1* mutation with X-rays to induce albino sectors. This mutation is lethal in the homozygous state and the allele is maintained in the heterozygous condition. Seed from heterozygous plants were grown and their seeds bulked. Plants from seeds treated with 16 kR of X-rays showed a three-day delay in leaf emergence. Subsequent growth was normal and no lethality was observed. Less than 1% of plants from treated seeds showed visible sectors. Two observations led us to believe that the albino and pale green sectors were related to the *alb1* mutation. First, no sectors were seen in plants from 4000 wild-type seeds treated with 16 kR of X-rays. Secondly, seeds from X-ray-derived sectored plants showed a higher incidence of plants heterozygous for *alb1* than those of unsectored sibs. Of seeds collected from sectored plants, 34 of 36 (94%) were heterozygous for *alb1*, and of those from unselected sibs, 26 of 62 (42%) were heterozygous.

The sectors induced by X-ray treatment of plants heterozygous for the *alb1* mutation are either albino or pale green, depending on their position in the leaf. Sectors affecting the edges of the leaf were usually albino while those near the midrib were often pale green or albino and pale green (Fig. 1). The near-midrib sectors were sometimes irregular in shape and did not extend to the leaf tip (Fig. 1). These appear to be albino sectors in L_3 overlaid with green L_2 tissue. When large L_2 sectors were found spanning several leaves, a core of pale green L_3 tissue could often be seen (Fig. 1). Since L_3 sectors are usually relatively inconspicuous, it is likely that they were underscored in this analysis.

Large sectors and meristem heterogeneity
Sectors were observed on 324 plants derived from approximately 80,000 seeds treated with X-rays. The width, length and sector frequencies are presented in tabular form (Table 1) and in graphical form (Figs 2, 3 and 4), and representative examples of sectors from leaves 1, 3, 6 and 9 superimposed on the schematics of the developing leaf primordia are shown in Fig. 5. Sectors on the early leaves (1 and 2) are usually

Table 1. *A summary of the positions, width, lengths and frequencies of* alb1 *sectors affecting 324 plants*

		Leaf number										
		1	2	3	4	5	6	7	8	9	Flowers	
1	Number of sectors affecting particular structures	97	96	37	49	49	42	37	27	36	42	512
2	Average length	1.37	1.41	2.35	2.92	2.88	3.97	4.20	5.11	4.77	4.40	
3	Average width	0.33	0.39	0.44	0.44	0.52	0.54	0.71	0.79	0.71		
4	Frequency of sectors including more than a single structure	0.082	0.135	0.486	0.510	0.449	0.952	0.892	0.963	1.000	0.976	
5	Frequency of sectors including the flowers	0.052	0.031	0.189	0.265	0.286	0.619	0.595	0.889	0.806		
6	Number of sectors each divided by their individual lengths	91.04	87.42	25.42	31.38	32.67	15.3	12.52	6.50	8.84	12.18	324
7	Number of sectors divided by the average length	70.8	68.1	15.74	16.78	17.0	10.57	8.81	5.28	7.55	9.55	
8	Size estimate % of total area	28	28	8	10	10	6	5	2	3	4	100

Length is expressed in terms of the number of affected structures (leaves or the flowers) irrespective of the sector starting point. Width is expressed as a fraction of leaf area. The number of sectors divided by their average length is row 1 divided by row 2. The calculation of the number of sectors divided by their individual length (NS/IL) is more complex. Each sector was considered to make fractional contributions to each structure. For example, a sector appearing in leaves 6 and 9 was scored as equivalent to one half of a sector in each. Similarly, a sector spanning leaves 3, 6 and 9 was scored as equivalent to one third of a sector in each and so on up to 10 affected structures. Sectors confined to a single structure were scored as 1. All 324 sectors were analysed in this way and the results summed for each structure. The size estimates are the NS/IL for each structure (row 6) divided by the total number of sectors $324 \times 100\%$ and are presented in row 8.
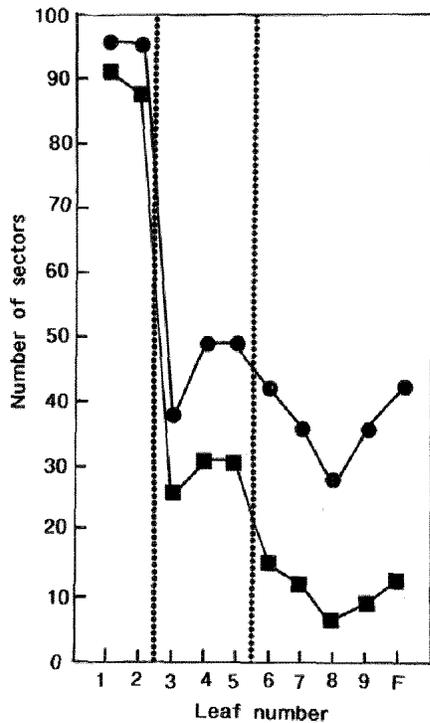
Fig. 2. The number of sectors observed in the leaves or the flowers (circles) and the number of sectors in each structure, each divided by its individual length in affected structures (squares). The data are from Table 1. The dotted vertical lines separate the three ranks of cells proposed in the model (Fig. 8).

narrow and confined to one structure, while sectors from the late leaves (6-9) and flowers are usually wider and contribute to several structures (Fig. 5). However, sectors vary greatly in both width and in the number of affected structures (length). The most extreme example of this is illustrated in
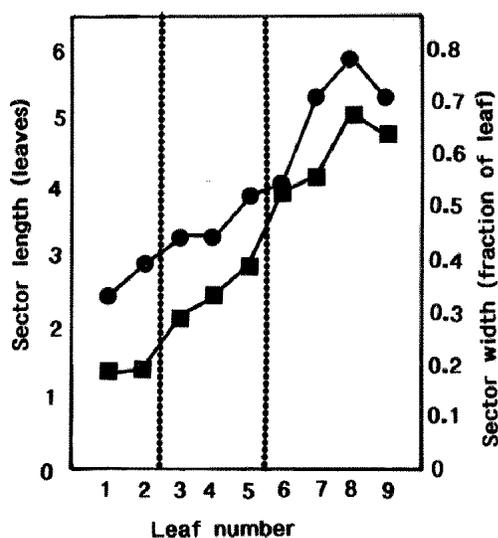

Fig. 3. Average sector length (squares) in affected structures and average sector width (circles) as a fraction of leaf area and as a function of leaf number of the flowers (F). The data are from Table 1. The dotted vertical lines separate the three ranks of cells proposed in the model (Fig. 8).
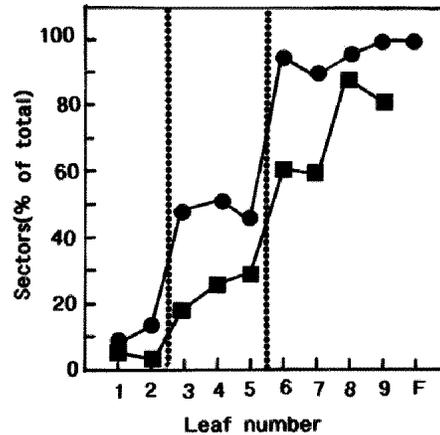

Fig. 4. The percentage of sectors in each leaf or the flowers (F) that also affect another structure (circles) and the percentage of such sectors also observed in the flowers (squares). The data are from Table 1. The dotted vertical lines separate the three ranks of cells proposed in the model (Fig. 8).

Fig. 5, leaf 1 i. In this plant almost all the L_2 layer of the plant is derived from a single albino cell. Similar, but less extreme examples, can be seen in the later leaves, for example in Fig. 5, leaf 3 g, a single albino cell has contributed to all later leaves and the flowers. Similar exceptionally large contributions are shown in some of the sectors in leaves 6 and 9 (Fig. 5). The occurrence of these atypical sectors implies that the means and summary data presented in Table 1 and Figs 6-8 should be interpreted with great caution since relatively few such atypical sectors can greatly affect the mean sector size.

Sector frequency, width and length

Approximately 60% of the total number of sectors (193/324) affected leaves 1 or 2 and, of these, 88% (170/193) were unique to these two leaves (Table 1 and Fig. 2). Other leaves and flowers showed a much more uniform number of sectors (range 27-49, mean 41) and many of these sectors contributed to more than one leaf. Approximately 12% of the sectored plants were affected in any particular structure after the first two leaves.

Sectors in the first two leaves are narrow, averaging only 30% of the width of the leaf, and rarely extend to other leaves (Table 1 and Fig. 3). In addition, we have observed sectors on leaf 1 that appear on only the upper surface or underside of these leaves suggesting that they may be derived from more than one rank of L_2 cells in the dry seed meristem. Sectors of later leaves are longer and wider and some of them are displaced from the meristem, reducing their ultimate length (examples Fig. 5, leaf 3 c, f and i), while others are maintained into the inflorescence apex (examples Fig. 5, leaf 6 i, leaf 9 a, b, e). The remainder become periclinal chimeras (examples Fig. 5, leaf 6 a, d, e).

Estimating relative contributions of areas of the dry seed meristem to the adult structures

The frequency of sectors observed in any particular structure should reflect the number of target cells in the dry seed meris-
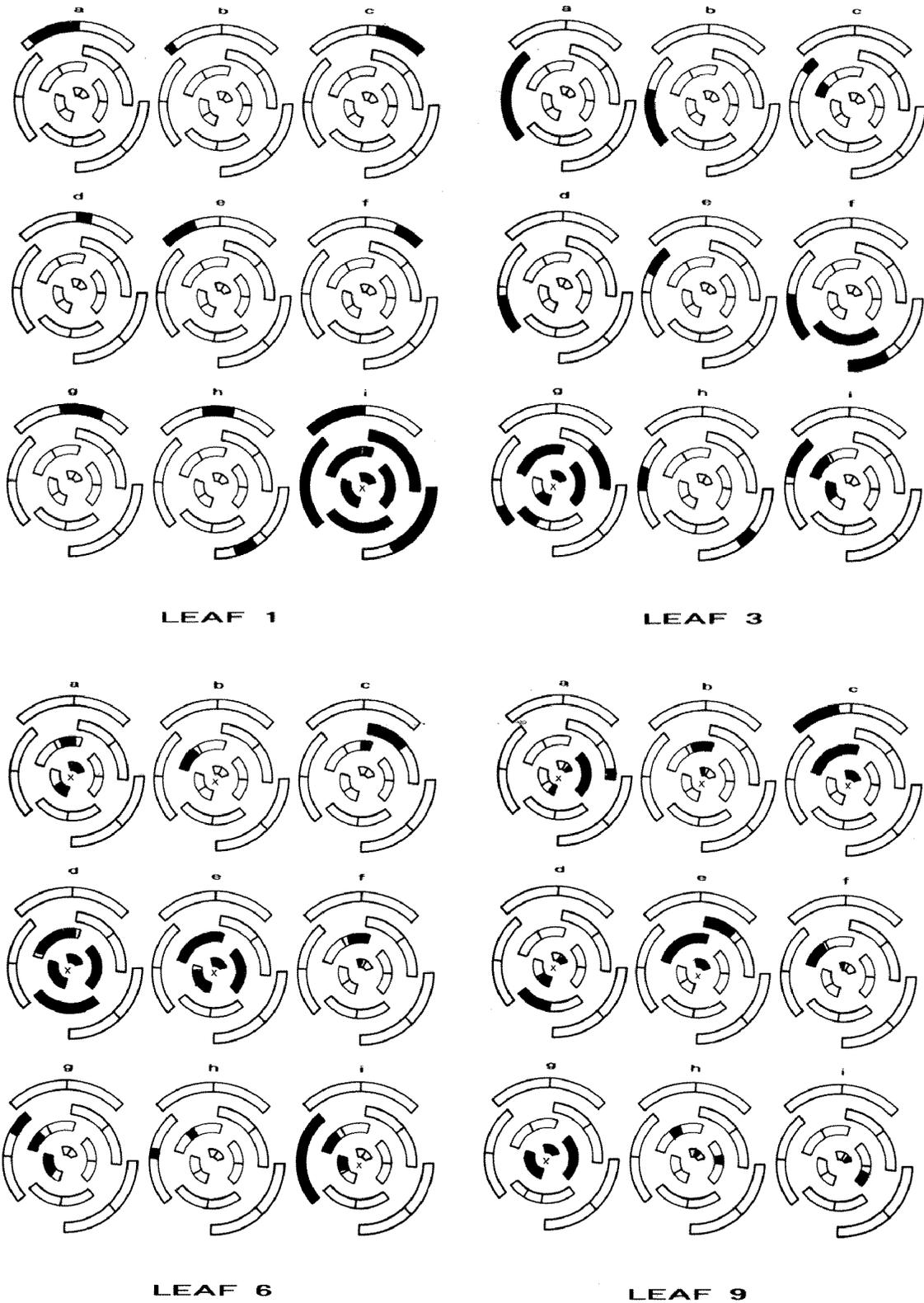
Fig. 5. Typical sectors in leaves 1, 3, 6 and 9. The extent of each sector is indicated by solid black. The leaf pattern is a schematic based on transverse sections of the leaf primordia of *Arabidopsis* . The positions and extents (radially) are approximately correct. An X is indicated in the centre of the diagram if the flowers were wholly or partially affected. In each case, position h is an L3 sector observed in several leaves. Meristem 1 i is atypical and is the largest sector that we have observed in this work.
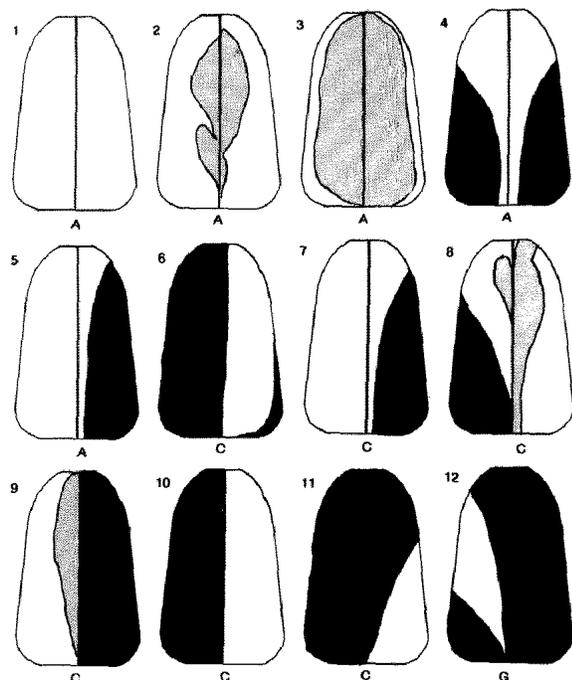
Fig. 7. An example of the relationship of the distances presented in Table 2 to the observed phyllotaxy. The distances from leaf 6R to the other half-leaf primordia and the flowers have been superimposed on the approximate phyllotaxy of the developing leaf primordia. The largest leaf shown is leaf 3 and leaf 9 is the smallest. Leaves 3 to 9 are drawn in descending order of size (leaf 3 is on the left)

Fig. 6. The relationship between sectors in the cauline leaves and the phenotype of shoots from the axillary meristem above it. The axilliary bolting shoot phenotype (A=albino, C=Chimeric and G=Green) is indicated below each leaf. L_3 contributions are stippled, full green tissue is indicated as solid black and albino tissue is white.

tem that give rise to it. For example, over half of the sectors appear only on the first leaf pair and in the dry seed meristem these leaves would represent a target larger than the rest of the plant. Unfortunately, such simple considerations cannot be applied within the rest of the plant since sector frequency is relatively uniform and sectors often affected more than one structure. Leaves 3-9 and the flowers are associated with a progressively increasing probability of sectors including one or more other structures (Table 1 and Fig. 3). The number of other structures affected also increases with later structures.

Estimates of target size based on sector frequency do not work for the later leaves because the average sector results from a dry seed meristem cell which gives rise to portions of several structures. For example, 37 leaf 3 sectors and 36 leaf 9 sectors were found suggesting that each was derived from approximately the same number of target cells (Table 1). However, the average length of the leaf 3 sectors was half that of those seen in leaf 9 (2.35 and 4.77 structures, respectively). The target size estimate was compensated for the contribution of the late sectors to several structures by dividing the number of sectors affecting each structure by their average length (Table 1 row 7). This converts the raw data from the biological situation in which marked cells give rise to sectors affecting parts one *or more structures* to a mathematically simpler, but unrealistic set of model data in which marked cells only give rise to parts of a single structure.

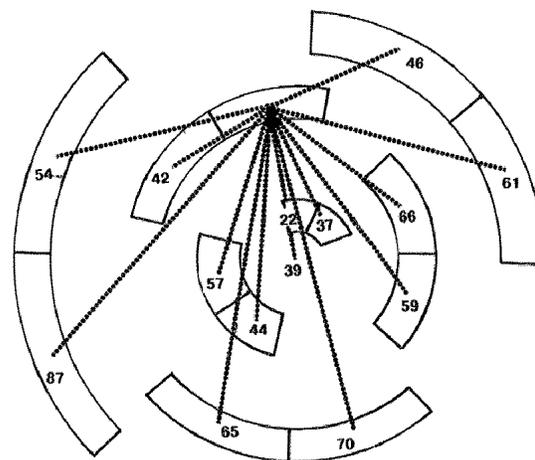Dividing the number of sectors affecting each structure by the average number of structures affected by each sector could compensate for the sector length increase and provide an index of target size. In practice this proved very sensitive to effects from the exceptional sectors; therefore each individual sector was divided by its individual length and summed to provide an index of size. (The details of the calculation are given in Table 1 legend). This size estimate attributes fractions of each of the total 324 sectors to each leaf and can be converted to area merely by dividing by this figure. (The details of this calculation are given in Table 1 legend). Leaves 1 and 2 each account for 28% of the area, the next three leaves account for 9% each and the last four leaves account for an average of only 3% each. Approximately 4% of the area can be accounted for by the flowers.

Contributions to the flowers

98% of floral sectors were also present on the leaves. The proximity of the flower and leaf primordia in the meristem appears to be an important factor in determining the pattern of sectors which are present in both the flowers and leaves. For example, 73% of sectors in leaves 6-9 extended into the inflorescence (Table 1 and Fig. 4), while sectors affecting leaves 1 and 2 rarely made floral contribution (4% of cases). Leaves 3, 4 and 5 were intermediate (24% of cases).

The origin of axillary meristems

The axillary meristems associated with the cauline leaves (7-9) of *Arabidopsis* usually produce a stem bearing further cauline leaves and an inflorescence. The origin of these meristems was studied in a number of the chimeras affecting the cauline leaves. When L_2 sectors affected the centre of the leaf, the associated side-branch was also affected. Green L_3 sectors of the cauline leaves with albino L_2 did not extend into the axilliary branches associated with this leaf suggesting the L_3 of the main stem made no contribution to the axilliary bud. L_2 sectors affecting only the edge of the leaf beneath an axillary bud frequently did not affect the bud (data

Table 2. *Relative distances between half-leaf primordia deduced from sectors affecting more than 1 structure; L$_2$ sectors affecting two or more leaves 3-9, and the flowers were categorized as to which half-leaves were affected*

| | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | | | |
R	L	R	L	R	L	R	L	R	L	R	L	R	F			
75	86	92	92	75	86	87	94	94	85	92	82	98	78	L		3
	100	100	100	100	55	94	100	100	93	72	100	100	92	R		
		38	68	68	61	46	46	60	71	76	65	62	66	L		4
			60	60	65	61	33	41	53	62	64	52	61	R		
				30	69	70	46	36	57	56	63	56	54	L		5
					59	65	55	49	46	56	58	56	57	R		
						42	55	58	48	42	50	52	48	L		6
							66	59	44	57	23	37	39	R		
								17	45	52	51	38	36	L		7
									44	51	58	45	42	R		
										23	35	29	39	L		8
											47	45	45	R		
												22	29	L		9
													28	R		

Arabidopsis plants show a random direction of phyllotaxy. All sectors on anti-clockwise plants were converted to an artificial clockwise phyllotaxy by inversion and R (right) is the direction of phyllotaxy. L (left) is opposite to the direction of phyllotaxy. The distances were deduced using the formula

$$1 - \frac{2X_{a-b}}{X_a + X_b} \times 100\%$$

where X_{a-b} = Number sectors observed in structure a and b
X_a = Total of structure a sectors
X_b = Total of structure b sectors

not shown). Fig. 6 shows the sector patterns on 12 leaves and phenotypes of the associated side-branches. The phenotype of the centre of the leaf determines whether a side-branch will be albino, chimaeric or green. Large contributions of albino tissue at the centre of the leaf are associated with albino side-branches (Fig. 6, 1-5). Chimeric leaves with borders near the centre are often associated with chimeric side-branches (Fig. 6, 6-11). Occasionally, sectors fail to be seen in the side-branch despite occupying a nearly central position (Fig. 6, 12). Such sectors might represent L$_3$ intrusion into normally L$_2$ positions in the leaf primordium or the end of an L$_2$ sector between the leaf primordium and its associated axillary meristem. These results suggest that the axillary meristem is derived from two or more L$_2$ cells clonally related cells at the centre of the leaf beneath it.

Phyllotaxy and sector pattern

To examine the relationship between L2 sectors affecting two or more structures and the phyllotaxy, an analysis of the number of sectors affecting any pair of half-leaves was performed. The results are presented in Table 2 and graphically in Fig. 7.

Discussion

X-irradiation of *Arabidopsis* seeds heterozygous for the *alb1* mutation resulted in albino sectors on the leaves, stem and inflorescence. The dose of X-rays (16 kR) used resulted in a slight developmental delay (3 days), but the survival, plastochron and phyllotaxy of the treated plants were unaltered. While the nature of the genetic lesions resulting in the albinism is uncertain, the data suggest that the sectors occur almost exclusively in *alb1* heterozygotes. Chlorophyll-deficient sectors were observed in tissues derived from the L$_2$ and L$_3$ layers of the meristem. Of a total of 324 sectors found, 26 (8%) appeared to be of L$_3$ origin. This is likely to be an

underestimate, since they are relatively inconspicuous and may have been missed. We suspect some may have been misclassified as affecting L_2. L_2 sectors were similar in shape to large sectors observed in irradiated tobacco (Poethig and Sussex, 1985).

The extent of sectors showed very great variation, ranging from plants in which a single albino cell produced most of the aerial tissue, to more typical sectors which affected one of the early leaves or several of the late leaves. It is possible that the size of the dry seed meristem was not constant and a low proportion of relatively "underdeveloped" seeds could account for these exceptional plants. The X-ray treatment produces only a slight developmental delay with no plant lethality or morphological abnormality to the *average* seed. However, the possibility of a small X-ray-sensitive subpopulation cannot be ruled out. In either case, subsequent proliferation to generate a normal meristem size could expand the sector contribution to account for these large sectors. If the first explanation is true, sieving seeds might be a possible method to generate a more homogeneous seed and meristem size and hence sector size. The second explanation is more difficult to test and sector expansion owing to extensive damage could be occurring in a small subpopulation of meristems. Slight damage might also be occurring in the average meristem and this could generate a small increase in sector size.

The frequency of sectors over the plant is non-uniform and the majority of sectors appear only on one or other of the first two leaves. Presuming the X-ray sensitivity of the cells in the shoot meristem is similar, a larger populaton of cells in the dry seed meristem gave rise to these structures. Sectors affecting later leaves showed an increasing probability of contributing to more than one structure, suggesting that the seed meristem cells that generate them also often contribute to other leaves and/or the flowers. This property makes it difficult to produce size estimates for structures in the dry seed, since each is formed from fractional contributions from cells which also contribute to other organs. Calculations based on sector frequency divided by length were used to give an estimate of primordium size as a percentage of visible scored structures. This should not be interpreted as a percentage of the meristem area, since stems were not scored and some meristem cells might contribute to inconspicuous structures such as stipules and rosette leaf axillary meristems, or cease dividing and give no obvious structure at all. Given this reservation, the first two leaves each account for 28% of the area, the next three leaves for 9% each and the last four leaves an average of 3% each. The flowers accounted for 4%.

Sector width as a fraction of leaf width increased with time (later leaves). Similarly, sectors in later leaves affected a greater number of structures (length). The increase in sector length is a reflection of an increased proportion of persistent sectorial chimeras contributing to several leaves and the occurrence of a few periclinal chimeras in which all L_2 tissue was displaced by albino tissue. Nearly all sectorial chimeras affecting the inflorescence are eventually lost from the meristem or become periclinal chimeras (I.J. Furner and J.E. Pumfrey, unpublished work). In *Arabidopsis* the apical meristem becomes the inflorescence meristem after leaf 9. The sectorial and periclinal chimeras continue into the flowers in 41 of 324 sectors suggesting that there is no distinction between germinal and somatic tissue and that no group of cells are

specifically set aside to form the inflorescence. All but one of the 42 sectors affecting the flowers also appeared on the cauline leaves.

Few L_3 sectors were observed (approximately 8% of total sectors). In general the L_3 sectors appear similar to L_2, i.e. small narrow sectors on the early leaves and large sectors affecting several leaves later. L_3 does not contribute to the flowers and the leaf contribution varies from no contribution to a large patch of tissue in the centre of the leaf. Persistent L_3 sectorial chimeras were also seen and these are illustrated in Fig. 5, leaf 1 - leaf 9, position h. Since L_3 contributes only the core of the leaf, these sectors appear at the centre of two or more leaves without appearing in the L_2 - derived tissue between them. In addition L_3 does not contribute to the axillary meristems of the cauline leaves. Such meristems are derived from two or more L_2 cells, clonally related to the leaf beneath them.

There is no relationship between particular lineages and particular structures supporting the importance of position in deciding cell fate (Fig. 5). The L_2 sectors shown in Fig. 5 appear continuous and could provide a means to prepare a map of the meristem based on the probability that any two structures contain tissue from the same sector. Such a map might be expected to reflect the physical distance within and between organ primordia in terms of probability. To attempt this, all sectors affecting more than one leaf were categorized for which leaf halves they appeared on. "Distances" between half-leaves were deduced from the formula, 1-2 × (numbers of sectors appearing on both structures/sum of sectors appearing on either). This empirical formula equals 1 if no sectors include both structures and equals 0 if all sectors include both structures (results are presented in Table 2). Maps of the this type were developed in *Drosophila* (Sturtevant, 1929) and the frequency units in the text are similar to sturts (Hotta and Benzer, 1973), i.e. the frequency with which two structures are not affected by the same sector ×100 (%). Sturt maps relate the relative positions of cells that will eventually give rise to different adult organs on the surface of the blastoderm. This method relates the relative positions of cells that will eventually give rise to the leaves and flowers in the growing meristem. The marked cells were generated in the seed meristem before leaf initiation and earlier or later irradiation would be expected to decrease or increase these distances, respectively.

From such an analysis it is possible to draw a number of conclusions. The apparent width of leaf primordia decreases with time (in plastochrons). For example, leaf 3 is 75% wide while leaf 9 is only 22% wide. Distances between leaf primordia decrease with time. For example, the average distance between primordium 3 and 4 is 94% and between primordium 8 and 9 is 39%. The closest other half-leaf primordium to each half-leaf is usually five half-leaves later. For example, 5L is close to 7R while 5R is close to 8L and so on. This result suggests the approximate phyllotaxy used in Fig. 5 is a fair representation of the biological reality as it suggests five half-leaves later should be directly inward of the starting half, irrespective of the starting point. The relationship of the distance estimates to a schematic diagram of the developing primordium is shown in Fig. 7. The distances of all other half-leaves to 6R are indicated. The distances seem a good fit to the diagram. However, a similarly good fit

could be made to a figure with all of the space filled by the leaf primordia.

The decreasing sizes of and distances between later leaf primordia are both the result of the decreasing number of dry seed meristem cell lineages persisting in the meristem at later times. Early leaves receive contributions from a relatively large number of basal seed meristem cells, while late leaves receive contributions from relatively few more apical seed meristem cells. The large sectors generated by the latter group of cells usually contribute to several structures and this is associated with an increased frequency of affecting any particular pair of structures and a decreased distance between them. The data were insufficiently detailed and precise to allow the construction of a three-dimensional model of the apex. We have recently generated such a model of the inflorescence apex based on sepal phenotypes of large *alb1* sectors (I.J. Furner and J.E. Pumfrey, unpublished work). Since the leaf phyllotaxy continues into the inflorescence, it is reasonable to propose the same fundamental mechanism governs both, i.e. recruitment of cells in a lineage-independent and position-dependent way. The two data sets are similar in that leaf width and flower size decrease with successive initiations as do interleaf and interflower distances.

No single model with relatively fixed cell fates could be produced to account for all of this data. However, a rather general map could be generated which would account for sector frequencies and the patterns seen with the majority of sectors. This is presented in Fig. 8. The model represents the surface of the L_2 of the meristem. Area was made proportional to (the sums of the number of sectors divided by their individual length) divided by the total number of sectors (as outlined above). It consists of three concentric ranks of cells. The outermost rank gives rise to the first two leaves only and constitutes over half the cells in the meristem. This rank only contributes to unstable sectorial chimeras which are rapidly lost from the meristem. The second rank usually gives rise to leaves 3,4 and 5 and constitutes 28% of the area of the meristem. By plastochron 3 the outer rank cells have been displaced and middle rank cells have divided and their contributions are on average longer and slightly wider than the first rank. By plastochron 6, these cells have been displaced and the central rank of cells dominates the meristem. We propose that these cells compete with one another for position in the meristem and can be displaced, generating unstable sectorial chimeras; or can remain, resulting in persistent sectorial chimeras, or can displace all other lineages, resulting in periclinal chimeras. Cells might also occasionally displace one another between ranks and this could explain some of the larger sectors and variability in sector shape. Like most plants the shoot apex of *Arabidopsis* does not appear to have stable initial cells, and sectoral chimeras are unstable (Fig. 5 and I.J. Furner and J.E. Pumfrey unpublished work).

The typical fates of cells in each of the three ranks is illustrated in Fig. 9. Such fates would account for the majority of the data. The greatest variability in cell fate was associated with the innermost cells. Since there are few of these cells (perhaps four), chance events affecting the planes of division might be important in determining a cell's ultimate contribution. For example, the inner-rank cell illustrated in Fig. 9 resulted in a persistent sectorial chimera. If it had divided to displace the cell to its left the result would have been a peri-
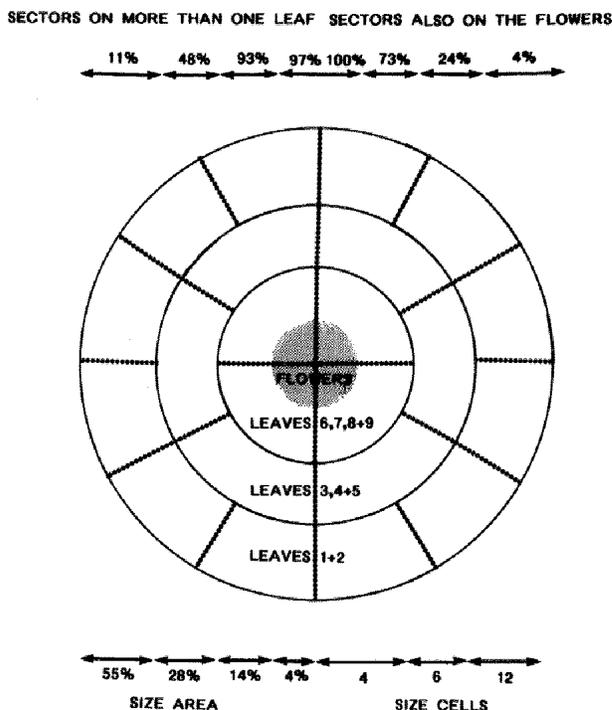

Fig. 8. A model of the *Arabidopsis* apex. The model consists of three ranks of cells. The cells are bounded by continuous and dotted lines. The areas attributed to each rank are based on (the number of observed sectors divided by their individual lengths), divided by the total number of sectors. Also shown above and below the model are the characteristics of each group of cells.

clinal chimera. Similarly, a particular middle-rank cell might divide in a longitudinal plane generating a long thin sector or in a radial plane generating a shorter but wider contribution. Finally, a particular outer-rank cell might or might not divide before its recruitment into leaves 1 and 2, and a single division in a radial plane could double sector width.

The cell numbers indicated on the model are little more than an informed guess based on typical sectors presented in Fig. 5 and are not deduced from accurate apparent cell
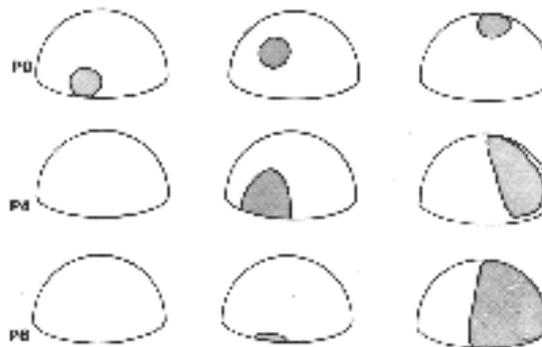

Fig. 9. A diagram illustrating the most probable fates of cells in differing regions of the meristematic dome. P0, P4, P6 represent time in plastochrons. (Left) an outer-rank cell; (centre) a middle-rank cell, and (right) an inner- rank cell. The original albino cell and its clonal derivatives are indicated as stippled areas.

number calculations (Poethig, 1987). The space was arbitrarily divided into cells and is not meant to imply their precise physical positions. Given the complexity of sector patterns seen in the first leaf pair, the average sector width (0.3 leaf) and the very high sector incidence, it seems unlikely the cells are organized in a single rank (as shown in Fig. 8). Contributions from two ranks of cells seems more likely. The flowers represent only 4% of the surface of the model. This is approximately equivalent to one cell, though they usually appear to receive contributions from 1 to 4 lineages which also contribute to the cauline leaves. This is in good agreement with estimates of the genetically effective cell number of 2 in this species based on seed mutagenesis and segregation of M1 traits (Li and Redei, 1969 and I.J. Furner unpublished work).

The data and model presented here show some similarities and some differences to comparable work on sunflowers. The sunflower is a larger plant than *Arabidopsis* and produces approximately 30 leaves before flowering. It has no rosette phase and the inflorescence is terminal. Studies of sectors induced by gamma-rays suggest approximately two layers of 40 cells each of the dry seed contribute to L_1 and L_2 of the mature plant (Jegla and Sussex, 1989). Our data suggest approximately 20 cells in the L_2 layer of the dry seed meristem of *Arabidopsis*. The pattern of sectors in sunflowers was broadly similar to our results, i.e. short narrow sectors in the early leaves and long (affecting several leaves) wide sectors in the late ones and the inflorescence. Sectors affecting a particular region of the plant showed great variation within this general pattern, as do our sectors in *Arabidopsis*. All the plants studied by these workers appear to have remained sectorial chimeras. Several of the *Arabidopsis* plants in this study became periclinal chimeras within the first 10 nodes and more did in the next 10 nodes (I.J. Furner and J.E. Pumfrey, unpublished work). This result suggests that the *Arabidopsis* apex is less stable than that of sunflower, in that lineages rapidly come to dominate the meristem or are eliminated from it. This might be a consequence of the relatively small size of the *Arabidopsis* meristem. This might be expected to allow a greater opportunity for chance events to result in elimination or fixation of the marked cells.

The shoot apical meristem of maize is far larger than that of sunflower and is highly developed in the dry seed (McDaniel and Poethig, 1988). It has over 300 cells organized into two layers (L_1 and L_2). In general, the same pattern of X-ray-induced sectors was observed, small sectors observed in the early leaves and long wide sectors in the late ones. These authors found the terminal tassel is frequently included in sectors affecting the late leaves. These authors concluded that "(1) at the time of germination the fate of any cell or group of cells was not fixed and (2) at the time of germination cells at the same location in the meristem could produce greatly different amounts of tissue in the adult plant". These conclusions are largely true in this work on *Arabidopsis* and the variability in sector size seems greater in this species than in maize.

In all three studies, a similar overall pattern was found despite the obvious differences in size of the meristems and the subsequent development of the plant. In none of the studies is there compelling evidence for strict developmental restrictions on the fate of cells. A general pattern of small sectors on the early leaves from peripheral meristem cells and larger and more variable contributions from more central cells to the late leaves is evident. In all three plants the central structure is clonally related to the adjacent leaves. The inflorescence meristem of *Arabidopsis*, the terminal flower of sunflower and the tassel of maize all show this property. The great variability in sector size in the same region of the plant evident in all three species suggests that the pattern of division in the meristem is not highly ordered. It seems likely that chance events affecting the planes of periclinal division in the meristem greatly influence the retention of a lineage in the meristem and the size of its ultimate contribution.

In these plants, the mechanisms that generates phyllotaxy, the initiation of axillary meristem (in *Arabidopsis*) and the production of terminal structures all do so in a position-dependent, lineage-independent way. Further insight into how these mechanisms operate might be obtained by looking at cell fate in, and cell autonomy of, mutants disturbed in these developmental processes.

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Fig. 1. The pattern of *alb1* sectors on the leaves of *Arabidopsis*. (A) An albino L_2 sector at the margin of a rosette leaf. (B) An albino and pale green L_2 sector near the midrib of the rosette leaf (the pale green is from wild-type L_3 tissue). (C) An inconspicuous pale green L_3 sector in a rosette leaf. (D) Green L_3 tissue within a large *alb1* L_2 sector in the cauline leaves.

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