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The role of initial cells in maize anther morphogenesis

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Summary

The near absence of cell movement in plants makes clonal analysis a particularly informative method for reconstructing the early events of organ formation. We traced the patterns of cell division during maize anther development by inducing sector boundaries that preceded the earliest events of anther initiation. In doing this, we were able to estimate the smallest number of cells that are fated to form an anther, characteristic cell division patterns that occur during anther morphogenesis, and the relationship between the pre-existing symmetry of the initial cells and the final symmetry of the mature anther. Four general conclusions are made: (1) anthers are initiated from small groups of 12 or fewer cells in each of two floral meristematic layers; (2) the early growth of the anther is more like a shoot than a glume or leaf; (3) cell ancestry does not dictate basic structure and (4) the orientation of initial cells predicts the orientation of the four pollen-containing microsporangia, which define the axes of symmetry on the mature anther. The final point is discussed with other data, and an explanation involving a 'structural template' is invoked. The idea is that the orientation of initial cells within the floral meristem establishes an architectural pattern into which anther cells are recruited without regard to their cellular lineages. The structural template hypothesis may prove to be generally applicable to problems of pattern formation in plants.

Key words: clonal analysis, cell lineage, pattern, polarity, anther, maize.

Introduction

By describing the cellular parameters of stamen morphogenesis, we can understand both the mechanisms that create the stamen and the evolutionary relationship of stamens to other plant structures. Like most angiosperms, the stamen in maize is composed of pollen-bearing anthers supported by thin filaments (see Fig. 1). The anther is bilaterally symmetrical about a central connective, and each side of the anther is further subdivided into two smaller structures called microsporangia (Esau, 1977). How cells in the floral meristem initiate the stamen, and then divide to produce the basic four-chambered structure of the anther is largely unknown. Of particular interest is whether the patterns of cell division in leaf development (Poethig, 1989) also occur in the reproductive structures. Leaves and stamens have been compared in a variety of ways, but there is no consensus on their evolutionary relationship. Many authors have preferred the classical interpretation of the flower, which assumes that stamens evolved from modified leaves (Arber, 1937; Canright, 1952; Haughn and Somerville, 1988), while others have argued that stamens must have evolved from a modified branch system (Hamshaw, 1932-1933; Satina and Blakeslee, 1941; Wilson, 1942).

Clonal analysis provides a unique three-dimensional perspective on the cell division patterns during morphogenesis. While it has been used extensively to study leaves and shoots (Poethig, 1987, 1989 for reviews) it has not been put to similar use in studies of flower development. Because of the brick-and-mortar manner in which plant cells divide, somatic sector boundaries represent a history of cell division that can be traced back to the first division planes of the nascent organ. Thus, using clonal analysis, it is possible to reduce the four-chambered structure of the mature anther to its origin on the floral meristem, and to reconstruct the characteristic sequences of cell division that occurred during morphogenesis.

Our methodology involves somatic sectors of the the purple pigment anthocyanin. In a previous study, unstable mutant alleles of anthocyanin genes were used to determine how the cell layers of the floral meristem contribute to anther morphogenesis (Dawe and Freeling, 1990). A more generally applicable method of inducing somatic sectors is to X-irradiate heterozygous stocks to bring about the loss of the wild-type product. In organs that express anthocyanin, such as the anther and leaf-like glumes of the male flower (Fig. 1), this procedure produces colorless sectors on otherwise purple tissue. While the filament of the maize stamen does not express anthocyanin, all tissues and cell layers of the mature anther are pigmented (Dawe and Freeling, 1990; and unpublished observations). Thus, anthocyanin pigmentation can be used to study early stamen morphogenesis prior to the differentiation of the filament, and all subsequent stages of anther development.

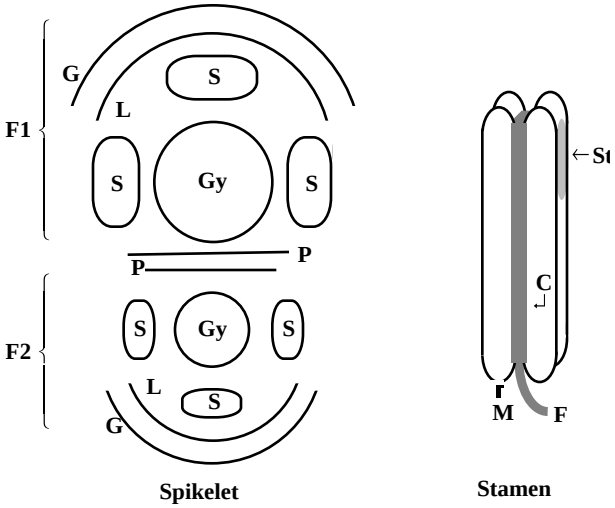

Fig. 1. Diagrammatic representation of the maize spikelet and stamen. The spikelet is shown in cross section, oriented as it would be on the tassel with two flowers lined up along the longitudinal axis of the plant. C, connective; F, filament (thin stalk that bears the anther at maturity); F1, first flower; F2, second flower; G, glume; Gy, gynoecium (which aborts at an early stage); L, lemma; M, microsporangia; P, palea; S, stamen; St, stomium (there are two, one on each side; stomia release pollen at maturity). The microsporangia contain sporogenous cells and four additional cell layers that constitute the anther wall (Esau, 1977; see also Kiesselbach, 1949). F2 is shown as smaller than F1 simply to illustrate that the second flower develops later than the first flower. At maturity, the flowers are the same size.

In previous studies of leaf development, clones of mutant tissue were induced at specific developmental stages to trace cell lineage (reviewed by Poethig, 1987; Dawe and Freeling, 1991). Using this approach, visible mutations induced just prior to leaf initiation indicate how many cells on the meristem produce leaves: the fraction of the total leaf area within the sector is the reciprocal of the number of initial cells. As a result of this type of analysis, leaves are now thought to be derived from over a hundred meristematic cells that divide in a more or less homogeneous fashion from initiation to maturity (Poethig, 1989). The absence of stem cell-like activity among the large number of cells that initiate leaves led Poethig (1984) to term the leaf initials 'founder cells.'

In contrast, we induce somatic color mutations well in advance of flower formation and use the resulting sector boundaries to estimate the smallest number cells that are fated to form the anther. Using this methodology, the boundary is viewed as a line that is drawn randomly about the organism, intercepting fields of cells that are destined to form future body structures (reviewed by Merriam, 1978; Dawe and Freeling, 1991). Given a large number of mosaic structures, the smallest fraction of a given body structure that is of one genotype can be assumed to have been derived from a single cell. The primary advantage of sector boundary analysis is that it requires no prior knowledge of when organ initiation occurs, so long as the sector preexists the body structure under study. It further measures the smallest set of cells with organ-specific fates, which may or may not be smaller than the number of cells at the time when the organ is initiated.

Materials and methods

Generation of sectors

In order to create sector boundaries well before anther initiation, germinating seedlings heterozygous for a number of plant color genes were treated with X-rays to induce chromosome breakage. X-rays frequently cause the loss of dominant markers to reveal the recessive, colorless phenotype in somatic tissues. Seedlings were grown until the coleoptile had just begun to emerge from the seed coat, X-irradiated (1000 rads acute, through a 0.35 mM copper filter with a Phillips model RT250 X-ray machine) and hand planted in the field. Three different genotypes were used. The largest set of kernels was heterozygous for eight genes necessary for plant anthocyanin pigmentation: *R-ch, B, Pl, A1, A2, C2, Bz1* and *Bz2* (this stock is described in Dawe and Freeling, 1990). Plants of this constitution have purple leaves, glumes and anther walls. There are no alleles in maize that color the filament of the stamen. Each of the recessive alleles (except *b* and *r-g*, an allele recessive to *R-ch*) are genetically epistatic, such that loss of any one of the six dominant alleles by chromosome breakage results in colorless sectors in leaves and flower parts. Seven sectors were found on a set of 855 plants of this genotype (355 grown in 1989 and 500 grown in 1990). Two sectors were found on 80 plants that were heterozygous for *R-ch* or *B, Pl* and *A2* and one sector was found on 100 plants that were heterozygous for *R-ch* or *B, Pl* and *Bz2*. These stocks were generated from seed supplied by the Maize Genetics Cooperative Stock Center, University of Illinois, Urbana. The stock of Dr Barbara McClintock's *a2-m1* was obtained from Dr Ben Burr, Brookhaven National Laboratory.

The pigmentation and basic cell lineages of maize anthers have been described previously (Dawe and Freeling, 1990). Briefly, somatic sectors induced at the seedling stage are most often restricted to one of the two major apical cell layers of the maize plant, the LI or the LII. In maize, the outer cell layer of the bilayered anther wall (the epidermis) is derived from the LI, while the inner layer (the endothecium) is derived from the LII. Color must be absent from both the LI and the LII for the anther to appear colorless. Therefore colorless anthers in X-ray-induced sectors must be the result of either two independently induced sectors (one in each apical cell lineage) or the 'invasion' of cells from a colorless lineage into the adjacent pigmented lineage. Cell layer invasion was sufficiently high following X-irradiation to provide nine LI+LII tassel sectors with chimeric anthers. Approximately half of the sectors analyzed here are described in more general terms in Dawe and Freeling (1990).

Scanning electron microscopy

Tassels from the inbred B73 were removed at a stage when two internodes had fully elongated, and processed for scanning electron microscopy by the replica plating technique (Williams et al., 1987) as modified by Bertrand-Garcia and Freeling (1991). Flowers were staged according to Bonnett (1953) and Cheng et al. (1983).

Results

Glume sectors

Seedlings heterozygous for a number of plant anthocyanin genes were irradiated to induce the somatic loss of domi-

nant alleles and subsequent expression of the recessive, colorless phenotype. Nine sectors extended from the base of the plant well into the tassel. On the edges of tassel sectors, the boundaries between pigmented and non-pigmented tissue produced chimeric spikelets (Fig. 2A, Fig. 1 for a description of the male flower). We analyzed how the boundaries between mutant and wild-type tissue intercepted mature glumes and anthers. This differs from the more familiar approach of analyzing the areas within entire mutant sectors, which, in the present study, were designed to be much larger than the organs under study.

Glumes are the outermost structure of the maize spikelet, and by histological analysis are thought to be homologous to leaves (Bonnett, 1953). Our preliminary clonal analysis further suggests a strong similarity between glumes and leaves. On 58 spikelets, the sector boundaries intercepted the base of the outer glume and followed the pattern of venation to the tip. This was true whether the boundary fell towards the center or towards the margins of the glume. Thus, like leaves (Poethig, 1984), the polarity of cell division is relatively uniform in a base-to-tip direction. In six of the chimeric glumes, the sector boundary intercepted the initial cell group twice, leaving a colorless region in the center of a purple glume (Figs 2B,C). One such sector contained the smallest clonal region of a single color, which contained 1/19 of the total width of the glume (Fig. 2C). The small size of this sector demonstrates that the group of glume initials can be subdivided into many parts, i.e. about 19 cells in the dimension that produces the width of the glume. Thus, glume sectors illustrate the basic features of leaf formation, where initiation occurs from many founder cells and no single cell functions as an initial cell (Poethig, 1984). Since we will refer to the differences between anther and leaf initiation, the sector boundaries on the leaf-like glumes are used a source of comparison.

The sector boundaries on glumes also show that the polarity of cell division preceding glume initiation is highly variable. In the six cases where a sector boundary intercepted the glume initials twice, the spikelet was flanked above and beneath by additional chimeric spikelets. Since maize flowers are oriented with the glumes perpendicular to the long axis of the plant (Fig. 1), the sector boundary must have traveled back and forth through the glume initial cells. Thus, it is clear that sector boundaries do not run straight along the longitudinal axis of the tassel, but are pushed and molded by the cell divisions that occur during spikelet morphogenesis. Other visual evidence of unorganized cell division can be seen in scanning electron micrographs, where the cells on the floral meristem occur in an apparently random pattern (Fig. 3).

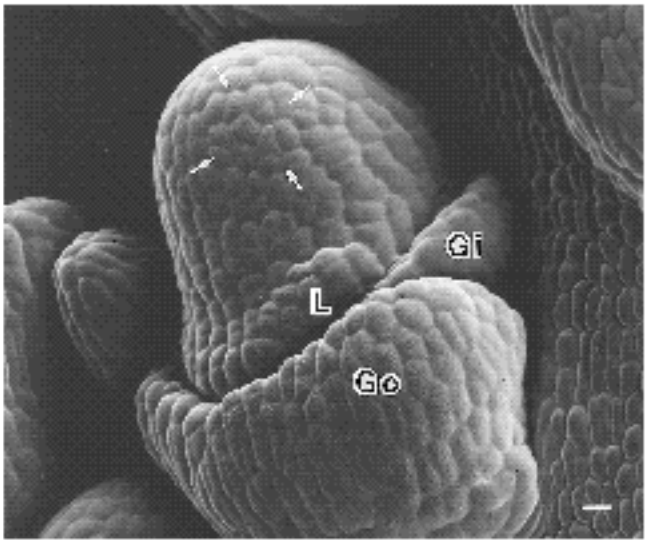

Fig. 3. Scanning electron micrograph of a male flower primordium. This shows the apical dome of the first flower in a stage just prior to stamen initiation. Outer (Go) and inner (Gi) glumes and the lemma (L) of the second flower are visible. Note the unorganized pattern of cells on the floral meristem. The region enclosed by arrows shows the approximate location of where the stamen will be initiated. The number of cells in the enclosed area provides an upper estimate of the number of stamen initial cells. Scale bar, 10 μm.

Anther sectors

Among the nine chimeric tassels, 101 chimeric anthers were observed. In an additional 11 cases, sector boundaries fell among the three stamens without producing chimeric anthers. A prominent feature of sector boundaries within anthers was that the proportion of colored tissue at the base of the anther often differed from the proportion at the tip of the anther (Figs 2D,F-H). For instance, 37 sector boundaries fell between microsporangia such that the anther was divided into halves at its base. However, on 14 of these anthers, the boundaries did not follow the midline from the base to the tip of the anther, but were displaced to the side so that the apex was entirely one color (Figs 2G, 4). Fig. 4 shows that the tips of anthers with such displaced boundaries were pigmented purple as frequently as they were colorless, indicating that this phenomenon is not a result of reduced cell division in the genetically deficient (colorless) tissue. We will subsequently use the term 'boundary displacement' to describe a boundary that shifts from an otherwise straight course along the longitudinal axis of an anther.

In leaves, cell division is directed towards the margins at particular stages in development (Poethig and Sussex, 1985). If this were true in anthers, it might explain the displacement of boundaries from the tip. The natural polarity of cell division was investigated using the transposon-induced mutation *a2-m1*, which is unstable in the presence of the regulating element *Suppressor-mutator* (McClintock, 1957). In *a2-m1* stocks, reversion events from colorless to purple occur spontaneously throughout development (Fig. 5). Because of their random nature, spontaneous sectors provide information about how cell division was oriented following the reversion event, but not when or where on the primordium the event occurred. If growth were oriented preferentially towards the margins, oblong or rectangular sectors oriented away from the connective would be observed; however, sectors occurring at various stages reveal that the growth direction is invariably with the long axis (Fig. 5).

Due to the prevalence of boundary displacement, sector boundaries were classified into six classes according to

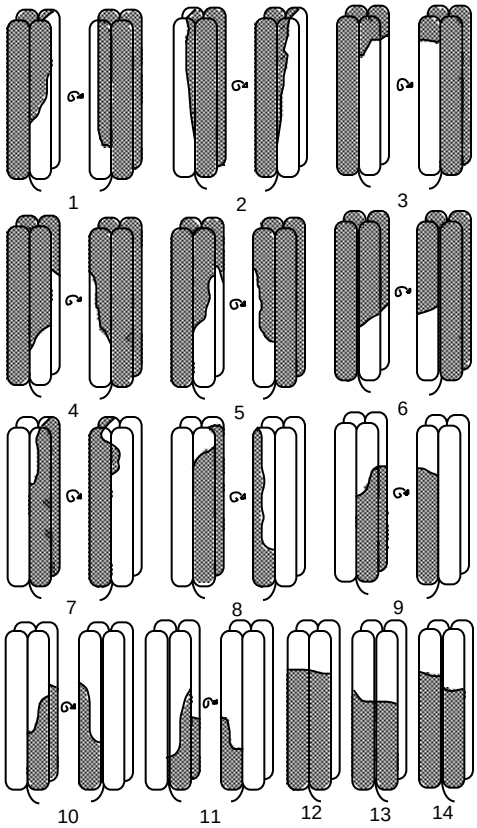

Fig. 4. Boundaries displaced from the midline of the anther. All of the anthers were half yellow and half purple at the base, but at the tip were entirely one color. Anthers are presented with the two stomia (which release pollen) oriented with the plane of the page. Drawings joined by a curled arrow represent two sides of a single anther. Unless specifically illustrated, all boundaries fell along microsporangium borders. Note that sectors 1-6 are purple at the tip, whereas 7-14 are yellow at the tip.

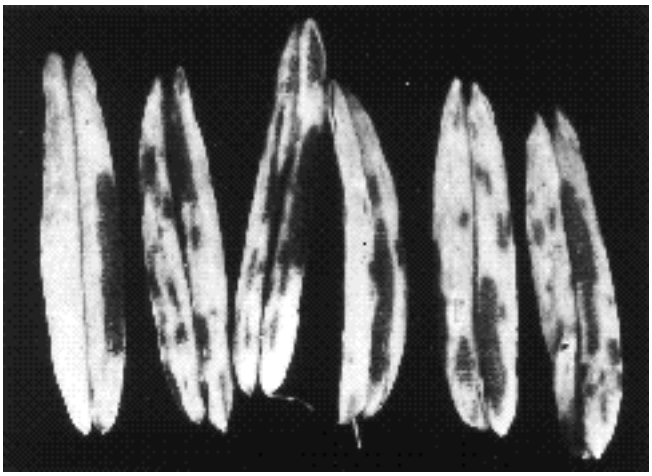

Fig. 5. Spontaneous sectors generated by *a2-m1*. The unstable mutation *a2-m1* was discovered by McClintock (1957) as a mutation controlled by the *Spm* family of transposable elements. It reverts spontaneously from colorless to purple. This allele was chosen simply because the stock tends to revert infrequently at different stages during anther development (the sectors from other unstable alleles often converge and obscure the shape of individual events). These are representative of many similar anthers. Note that sectors of different sizes are strictly oriented with respect to the long axis of the anthers.

where they fell relative to the microsporangia at the base of the anther (Fig. 6). If the sector boundary fell between microsporangia at the base of the anther and visibly extended along the border between microsporangia for any length, it was considered as lying between microsporangia. If the sector boundary did not intercept an anther between microsporangia at the base, it was classified as lying within a microsporangium. Fig. 6 shows the percentage of total for each sector class, a schematic top view of sector boundaries and three to six representative anthers for each class. The six classes were distributed evenly among the nine tassel sectors (Table 1), indicating that the sector boundaries on individual tassels did not consistently intercept consecutive anther primordia in the same or similar ways.

Sector boundaries most frequently split anthers into halves (class A), and second most frequently separated one microsporangium from the other three (class B). Combined, class A and B sectors constitute 63% of all sector boundaries observed (Fig. 6). Class C and D sectors differ in that one of the points of interception lies between microsporangia while the other lies within a microsporangium. Thus, instead of the smallest fraction of a given constitution being $\frac{1}{2}$ or $\frac{1}{4}$ of the anther, the smallest fractions were approximately $\frac{3}{8}$ (class C) or $\frac{1}{8}$ (class D). In a minority of cases, both points of intersection fell within microsporangia, creating $\frac{1}{2}$ sectors (class E) or $\frac{1}{4}$ sectors (class F) that were entirely removed from the borders between microsporangia. In other very small sectors, it was not possible to discern whether the boundaries fell within microsporangia or were displaced very early in development (class G). Since the eight class G sectors could not be placed into one of the six major classes, the percentage data in Fig. 6 are based on a total of 93 anthers.

Displacement events of small proportions were found along the entire length of anthers (e.g. Fig. 6, class A no. 2-6); and in three cases displacement occurred at the very base (two are shown: Fig. 4 no.7 and Fig. 6., class B no. 2). However, boundaries were most frequently displaced at the tip. Table 1 shows the frequency of boundary displacement from the tip of sectors in each size class. When sector boundaries delineated smaller fractions of the anther, the boundaries were more likely to be displaced from the tip (Table 1). For instance, 62% of the Class B sectors (smallest fraction, $\frac{1}{4}$) were displaced from the tip, while displacement occurred in only 38% of the Class A sectors (smallest fraction, $\frac{1}{2}$).

In 91% of the sector boundaries, at least one point of interception fell between the microsporangia at the base of the anther (Fig. 6). On the microsporangium borders that did not contact the connective (Fig. 1, the border along the plane of the page), the boundaries fell precisely along the visual interface between microsporangia. On the microsporangium borders that contacted the connective, the boundaries did not lie within the connective, but at the border between microsporangia and connective. Of 27 cases where

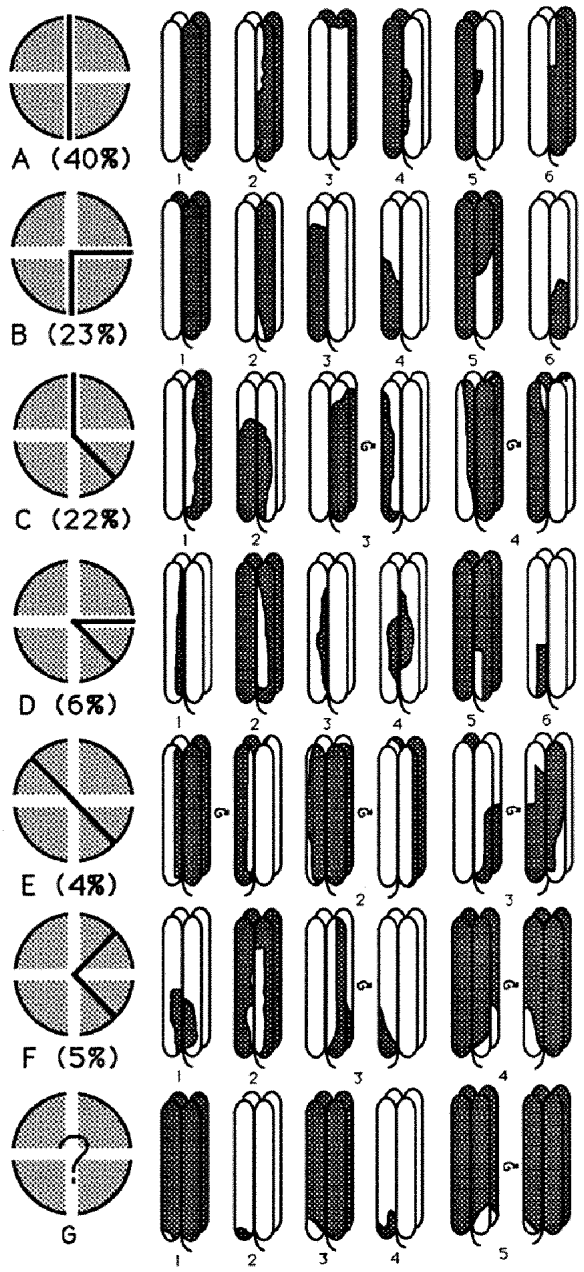

Fig. 6. Classes of anther sectors and drawings of representative anthers. At the left, each class of anther sectors is provided with a letter designation, a percentage figure, and a pie-shaped top view of how the boundaries of each class intercepted the anthers at the base. At the right, 3 to 6 drawings of representative anthers are provided. The percentage figures are based on a total of 93 sectors because the Class G sectors were excluded. In the top views provided for each class, wide, unshaded areas represent microsporangia borders and the bold lines represent sector boundaries. Representative anthers are presented with the two stomia (which release pollen) oriented with the plane of the page. Anther drawings joined by a curled arrow represent two sides of a single anther. Unless specifically illustrated, all boundaries fell along microsporangium borders. The Class A1 type of sector was found ten times and the class B1 type of sector was found three times (in one the colors were reversed).

sector boundaries were clearly visible at microsporangium/connective borders, the connective was either entirely purple or entirely yellow (12 were purple and 15 were yellow; for examples, see Fig. 2D,E). There were also no sectors confined to the connective, i.e., there were no yellow anthers with sectors of purple on the connective, or purple anthers with sectors of yellow on the connective.

Those sectors where the boundaries did not fall at microsporangium borders were often particularly irregular (e.g. Fig. 6, class D no.4, class E no.3 and class F no.2). In one class C sector, the boundary extended in a linear diagonal direction from one corner of a microsporangium to the opposite corner (Fig. 2F). Although the cause of this sector is not known, one of the tassel sectors observed in this study underwent a similar (180°) twist on the main tassel branch without changing the placement of spikelets on the branch (not shown).

Discussion

Initial cell number and sector boundary displacement

In this report, we use glumes to illustrate the previously described properties of maize leaves (Poethig, 1984). Glumes provide an appropriate comparison because of their similarity to anthers with respect to mature size (Kiesselbach, 1949) and the size and character of the meristem from which they are initiated (Cheng et al., 1983). From the size of the smallest glume sector, we can conclude that there are about 19 initial cells along the direction that generates the width of the glume. Regardless of where a sector boundary intercepted these cells, it followed a direct path towards the tip of the mature glume (Fig. 2B,C). This is the expected result when growth is distributed evenly among the initial cells of an organ, and coincides well with our current understanding of leaf development (Poethig, 1989; Sylvester et al., 1990). The effect of growth on a boundary across leaf initial cells can be likened to marking a line around a balloon and blowing it up: cell division is roughly equal over the surface and does not alter the shape or location of the sector boundary.

Similar reasoning can be used to analyze the initiation of the anther. From previous work it is known that the group anther initial cells has a depth of at least two cell layers (Bonnett, 1953; Dawe and Freeling, 1990). In some previous cases, it was possible to determine which layers of the floral meristem produced anther sectors (Dawe and Freeling, 1990), but it was not possible in the large sectors reported here. We will assume in our discussion that the two layers the floral meristem participated equally in anther initiation, or that the initial cells are composed of two equally sized tiers of cells stacked one over the other. The length and width of the tiers can be determined by the size of the smallest fraction of one color, which was one eighth at the base (Fig. 6, class D). However, analysis is complicated by the fact that on anthers, sector boundaries did not follow direct paths from base to tip. Regardless of where a sector boundary fell along the base of an anther, there was a marked tendency for the boundary to be displaced from the apex (Table 1). The common displacement of boundaries from the tip is not a natural outcome of the cylindri-

Table 1. *Classes of sector boundaries*

Class*	Boundaries in class	Boundaries extended to tip	Boundaries displaced from tip	Anther boundaries on each of nine tassels								
				1	2	3	4	5	6	7	8	9
A	37	23	14	5	1	4	1	2	6	11	3	4
B	21	8	13	4	3	3	1	2	0	4	4	0
C	20	13	7	4	1	1	0	1	3	6	4	0
D	6	1	5	0	1	2	0	0	0	1	2	0
E	4	3	1	0	1	1	0	0	1	1	0	0
F	5	0	5	1	2	0	0	0	1	0	1	0
Total	93	48	45									

*Classes of sector boundaries are labelled A through F as in Fig. 6.

cal growth pattern of the anther. Spontaneous sectors generated by *a2-m1* are oriented with the long axis of the anther, showing that the natural orientation of cell division is lengthwise, not towards the margins (Fig. 5).

It is possible to explain both the prevalent displacement of boundaries from the tip and the six classes of sectors (Fig. 6) by assuming that anthers are initiated from cells organized in concentric rings (Fig. 7). This type of model is based on the suggested arrangement of cells in the shoot apical meristem of maize and sunflower seeds (Poethig et al., 1986; Jegla and Sussex, 1989). Fig. 7 shows twelve initial cells organized with an inner ring of four cells contained within an outer ring of eight cells. If the sector boundary bisected the outer but not the inner ring of cells, subsequent cell division would generate the type of boundary displacement shown in Fig. 4. It is clear from the data (Figs 4, 6), that there is a great deal of variation in the degree of boundary displacement from the tip in each class. This is also typical of shoot development, where the actual fate of any shoot progenitor cell can only be assigned in a general way (McDaniel and Poethig, 1988).

The quantitative aspects of the data are not entirely consistent with this model. If there were always eight cells in the outer ring, sector boundaries should fall within one of the four microsporangia at least as frequently as they fell between microsporangia. However, 77% of all boundaries fell between microsporangia. Similarly, sector boundaries that divided the anther in half (40%) were much more frequent than those that produced a fraction of one eighth (6%), yet based on an eight cell ring, they would be expected in similar proportions. The fact that so many sectors (63%) divided the anther into fractions of four suggests that anthers are often initiated from tiers of four cells. In order to accommodate all aspects of the data, we suggest that an inner ring of four cells is sufficient to initiate the anther, but that in many cases an additional ring of eight cells is also recruited directly from the meristem.

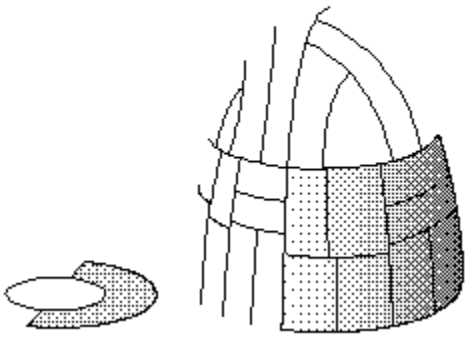

Fig. 7. The effect of growth on a sector boundary through 12 anther initial cells. A depiction of 12 cells arranged as a four cell ring inside an eight cell ring is shown on the left. A sector boundary, separating pigmented and non-pigmented cells, bisects the outer but not the inner ring of cells (shown as a bold line). Subsequent cell division produces an anther primordium with the boundary displaced from the tip (mature anthers with this type of boundary are shown in Figs 2G, 4).

Another potentially important mechanism for the extensive displacement of sector boundaries from the apex is that growth is not evenly distributed over the surface of the primordium. When growth is localized to the tip of a structure, such as an apical meristem, the active growth in the center pushes the older cells towards the periphery (Ball, 1972). Thus, a period of localized apical growth during anther development might be expected to increase the likelihood of boundaries being displaced from the tip. Apical growth would probably occupy only a brief period, because ink marking experiments (in lilies) indicate that late in anther development cell division is not localized to the apex (Gould and Lord, 1988). While we have no direct evidence that apical growth occurs, many other aspects of anther development clearly resemble shoot development (see below).

An estimate of four to twelve cells is consistent with scanning electron micrographs of spikelet primordia (Fig. 3), which show roughly twelve cells in the area where stamens are initiated. Electron micrographs provide an upper estimate of the number of initial cells, because meristematic cells may be fated to form an anther well before the initiation event is visible.

Growth in circumference

It is known that the early initiation events of the maize (monocot) and tobacco (dicot) leaves are quite similar (Poethig, 1984). Thus the maize leaf and glume may be considered as representative of angiosperm leaves at the level of organ initiation. However, arguments on the evolutionary relationship between anthers and leaves have generally considered the classic dicot leaf as a standard (e.g. Arber, 1937; Canright, 1952) and, the final form of a maize leaf differs significantly from typical dicot leaves in its basic organization: it has a sheathing base instead of a thin petiole. Evidence suggests that the entire grass leaf is

homologous to a small lower petiole region of the dicot leaf (Kaplan, 1973). Thus, for the purpose of comparing the post-primordial growth of the maize anther to a typical leaf, it is more appropriate to use the data from dicotyledonous leaves.

According to the classical interpretation of the flower, the microsporangia are homologous to the leaf lamina, and the connective homologous to the leaf axis, or midrib (reviewed by Barnard, 1961). It is well accepted that the dicot leaf develops by first forming a central axis which then initiates the lamina in a lateral direction. The initiation of lamina can be visualized in sectioned material as a region of localized growth along the flanks of the central axis. This sequence of events has been verified by clonal analysis in tobacco, where sectors induced prior to leaf initiation are confined to the midrib in the lower portions of the leaf and then shift laterally towards the margins (Poethig and Sussex, 1985).

In contrast, comparative histological studies in many species (e.g. Boke, 1949; Tepfer, 1953; Bonnett, 1961), suggest that anthers lack localized marginal growth and exhibit a uniform radial expansion (for maize, see Kiesselbach, 1949). This suggests that anthers do not develop by a biphasic axis-then-microsporangia sequence, but by a more uniform process. Three aspects of our clonal data support this assertion that anthers exhibit a uniform radial expansion. (i) There were no anther sectors that were confined to the connective, and in all cases where sector boundaries were visible between microsporangia, the connective was either entirely purple or entirely yellow. Leaf sectors, due to the relatively large size of the axis during early development, often have one or both sector boundaries within the midrib. (ii) Anther sectors induced late in development are not directed towards the margins, but in a longitudinal direction (Fig. 5). When sectors are induced late in leaf development, the radial growth of the lamina results in fan-shaped sectors with the long axis oriented towards the margins (Poethig and Sussex, 1985). Although the overall shapes of leaves and anthers differ considerably, the absence of anther sectors with a radial orientation indicates that growth in circumference occurs gradually, and is not localized to a specific stage. (iii) A sector boundary can be continuous and linear in a diagonal course along the microsporangium (Fig. 2F). This very rare anther illustrates that the radial growth of the microsporangium occurs uniformly from a relatively large group of cells. A diagonal boundary would be altered by microsporangium growth in a manner based on the number of cells at the time when radial growth began. This is similar to the effect of changing the number of steps in a flight of stairs: when more steps are added, the incline becomes smoother. This effect is illustrated in Fig. 8. Similarly, only if the number of cells were large at the time of lateral expansion would a boundary be continuous and diagonal across the microsporangium.

Data from a variety of sources suggest that maize anthers are best described as modified shoots. Leaves and glumes are initiated in the outermost cell layers of the shoot apical meristem (Sharman, 1942; Bonnett, 1953), whereas anther and shoots are initiated from internal layers (Bonnett, 1953). Leaves and glumes are initiated from a large number of cells (Poethig, 1984; this report), whereas anthers and shoots are initiated by a small number of cells (Poethig et al., 1990; this report). The cell layers often intermix during leaf organogenesis (Poethig, 1984; Sinha and Hake, 1990), whereas in shoots and anthers the lineages are highly stratified (Dawe and Freeling, 1990). Finally, we argue that the arrangement of anther initial cells is similar to the arrangement shoot progenitor cells, and that the subsequent radial growth of the anther does not follow a leaf-like pattern.

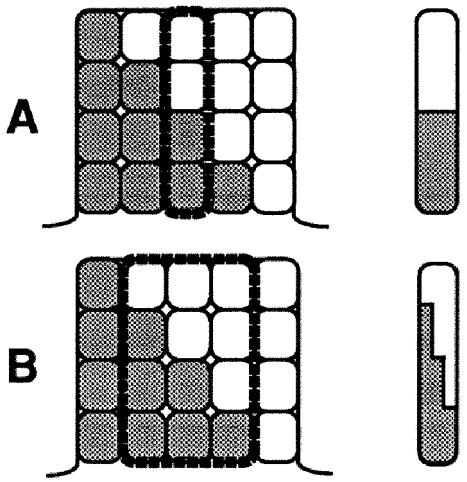

Fig. 8. Hypothetical effect of a small number of microsporangium initials on a diagonal sector boundary. The illustrations on the left depict the anther primordium prior to the formation of microsporangia. The resulting microsporangia are shown on the right. (A) Effect if microsporangia were initiated from a band of initials one cell wide. The diagonal sector boundary on the anther primordium would be transformed into a horizontal boundary. (B) Effect if microsporangia were initiated from a band of initials three cells wide. The diagonal boundary would outline a stepped configuration on the mature anther. This figure illustrates that for a continuous diagonal boundary to exist on a mature anther, the number of microsporangia initials must be large.

The correlation of borders between initial cells and the mature anther's axes of symmetry

A striking aspect of our data is that the majority of sector boundaries intercepted anthers between microsporangia, when in fact the connective represents a very small 'target' (Fig. 6). A similar correlation of initial cells with axis orientation was first described by Steffenson in his studies of maize embryo development (1968: unpublished data cited therein), and verified by Poethig and coworkers (1986). Somatic mutations induced during early embryogenesis were most often restricted to half of the plant, and sector boundaries coincided with the leaf midribs. Further information on the relative orientation of initial cells and the axes of a dicotyledonous plant was provided by Christianson (1986) in his study of cell fate in the cotton embryo. Using a particular cotton strain, it was possible to generate a high frequency of sector boundaries that pre-existed the organization of the shoot apical meristem. In 48% of such sectored seedlings, the total area of the cotyledons was divided into halves or quarters, and in a majority of those (36% of all chimeric plants), the placement of sector bound-

aries fell along the front/back or left/right axes of the embryo.

Based on the animal literature (e.g. Stent, 1985), the possibility that initial cells are genetically committed to form the new axes of an organ primordium has been discussed, and generally discounted (Steffenson, 1968; Christianson, 1986). It is well established that plant cells do not impart to their descendants genetic instructions to perform specific functions (Poethig, 1989; Dawe and Freeling, 1991). In maize anthers, the high incidence of cell invasion between microsporangia during anther growth rules out a commitment process where single initial cells limit their progeny to a specific quadrant of the anther (e.g. Fig. 6, class A no. 2, 4 and 5).

One potential explanation for the concurrence of sector boundaries with microsporangium borders is that the axis, or connective, occupies a large volume of the anther early in development. Tobacco leaf sectors often terminate within veins, and this is thought to be caused by the disproportionately large size of veins in leaf primordia (Poethig and Sussex, 1985). However, the connective of the maize anther cannot be identified histologically until the anther becomes four-lobed, and there is no evidence of the vascular trace within the connective until well after the microsporangia have begun to differentiate (Kiesselbach, 1949; Bonnett, 1953; Cheng et al., 1983; and our unpublished observations). Of 27 sector boundaries we observed at the connective/microsporangium border, *none* fell within the connective, suggesting that the connective is very small early in development. In general, our data indicate that the connective and the microsporangia grow at roughly the same time by a generally uniform radial expansion (see Discussion section on Growth in Circumference).

Another potential explanation is that there are pre-existing constraints on the arrangement of cells during anther initiation that 'line up' the initial cells with the future axis. Prior to leaf initiation, cells are often organized into distinct longitudinal cell files, and these files can be preserved on the nascent primordia (Green and Lang, 1981; Lyndon, 1982). Such a pre-existing polarity might be expected to restrict the placement of sector boundaries. However, the orientation of cell division on spikelet primordia is highly variable, as evidenced by the chimeric glumes with two sector boundaries, and the unorganized cell pattern in scanning electron micrographs (see Results section on Glume Sectors ; also Lyndon and Cunninghame, 1986). Indeed, the variety in which sector boundaries intercepted anthers (Fig. 6) rules out an ordered pattern of cell division prior to anther initiation.

Nevertheless, sector boundaries that pre-existed organogenesis often delineated the axes of symmetry on the anther. In almost all of the anther sectors (91%), *at least one* sector boundary fell between microsporangia (Fig. 6). Thus, the cell borders at the time of anther initiation are converted with high efficiency into the borders between microsporangia that define axial polarity. The high degree of lineage invasion between microsporangia during later development shows that while anther initial cells may initiate the cell division patterns that ultimately define the axis, the maintenance of axis orientation is under separate control. This is consistent with surgical experiments, which depict plant organs as self-regulating structures (e.g. Feldman and Cutter, 1970). Therefore, we suggest that the initial cells serve as 'templates' for the axes of symmetry by initiating a pattern of polarized cell division that is self-propagated. This effect is undoubtedly mediated, to some extent, by the cytoskeletons of the cells involved. In other cases (Kaplan and Hagemann, 1991), it is conceivable that a complete template could exist within the cytoskeleton of a single cell.

There are numerous precedents for the propagation of pre-existing cell pattern during plant development (reviewed by Lang, 1965). For instance, radial cell walls have been shown to define the placement of specific cell types in the roots of *Sinapis alba*. In this species, root hair cells are formed only over the radial cell walls of the subepidermal tissue. Those epidermal cells that are located over tangential cell walls do not form root hairs. However, Bunning (1951) demonstrated that when the subepidermal tissue was destroyed with a small incision, all the epidermal cells became root hair cells. While it may be a chemical factor that induces root hair differentiation (Lang, 1965), the *pattern* of root hair cells is dictated by the previous placement of cells in the underlying cell layer. The idea that pattern may be inherited or propagated in a non-genetic fashion has been called 'structural inheritance' (Sonneborn, 1970). In various forms, such structure-begets-structure mechanisms are thought to have wide-ranging effects on morphogenesis in both plants (Lang, 1965; Green, 1987; Dawe and Freeling, 1991) and animals (Newman and Comper, 1990).

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Fig. 2. Tassel, glume, and anther sectors. Anther sectors are described by the class designations in Fig. 6. (A) Tassel sector showing two sector boundaries on a single tassel branch. Note purple and yellow glumes and anthers. (B) Outer glume sector. Note that the sector boundaries converge at the tip coincident with the venation of the glume. (C) The smallest outer glume sector. The width of this colorless region was 1/19 of the width of the glume. (D) Class B sector showing that the sector boundary lies at the border between the microsporangium and the connective. (E) Class A sector showing sector boundary at the interface between microsporangium and connective. This sector is one of ten class A sectors that did not show boundary displacement. (F) Diagonal boundary through a microsporangium. The shape of this boundary constitutes evidence against a limited population of microsporangium initials. (G) Class A sector showing boundary displacement. Near the filament attachment point, the back of two microsporangia are yellow. At the top, all four are yellow. (H) Two class B sectors within the floret. At the base of the anther on the right, one microsporangium is completely yellow and three are purple. At the tip, three are yellow and one is purple.