Planta (1985) 165:170–184

Planta

The cellular parameters of leaf development in tobacco: a clonal analysis

R.S. Poethig* and I.M. Sussex
Department of Biology, Yale University, New Haven, CT 06511, USA

Abstract. The cellular parameters of leaf development in tobacco (*Nicotiana tabacum L.*) have been characterized using clonal analysis, an approach that provides unequivocal evidence of cell lineage. Our results indicate that the tobacco leaf arises from a group of around 100 cells in the shoot apical meristem. Each of these cells contributes to a unique longitudinal section of the axis and transverse section of the lamina. This pattern of cell lineage indicates that primordial cells contribute more or less equally to the growth of the axis, in contrast to the more traditional view of leaf development in which the leaf is pictured as arising from a group of apical initials. Clones induced prior to the initiation of the lamina demonstrate that the subepidermal layer of the lamina arises from at least six files of cells. Submarginal cells usually divide with their spindles parallel to the margin, and therefore contribute relatively little to the transverse expansion of the lamina. During the expansion of the lamina the orientation and frequency of cell division are highly regulated, as is the duration of meristematic growth. Initially, cell division is polarized so as to produce lineages that are at an oblique angle to the midrib; later cell division is in alternating perpendicular planes. The distribution of clones generated by irradiation at various stages of development indicates that cell division ceases at the tip of the leaf when the leaf is about one tenth its final size, and then ceases in progressively more basal regions of the lamina. Variation in the mutation frequency within the lamina reflects variation in the frequency of mitosis. Prior to the emergence of the leaf the frequency of mutation is maximal near the tip of the leaf and extremely low at its base; after emergence, the frequency of mutation increases at the base of the leaf. In any given region of the lamina the frequency of mutation is highest in interveinal regions, and is relatively low near the margin. Thus, both the orientation and frequency of cell division at the leaf margin indicate that this region plays a minor role in the growth of the lamina.

Key words: Cell lineage – Clonal analysis – Leaf development – Mutation (somatic) – *Nicotiana* (leaf growth) – Somatic mutation.

Introduction

The cellular mechanism of plant morphogenesis is still largely unknown. Although the shape of a plant organ is usually attributed to spatial variation in the rate, duration and orientation of cell division and cell expansion, the morphogenetic significance of these processes has not been clearly defined. This is, at least in part, because there is little detailed quantitative information about cell behavior during morphogenesis. In the absence of such information it is difficult to determine the merit of various hypotheses about the cellular mechanism of morphogenesis, and to devise experimental approaches to this problem.

The value of comprehensive quantitative studies of the cellular parameters of development is particularly evident in the case of leaf morphogenesis. Traditional interpretations of leaf morphogenesis are based almost exclusively on analyses of sectioned specimens. Because this approach restricts the amount of tissue that can be examined and the plane in which it is viewed, it has led to

* *Present address:* University of Pennsylvania, Department of Biology, Leidy Laboratories/G7, Philadelphia, PA 19104, USA

Abbreviation: MF = mutation frequency

a biased view of leaf development. More attention has been given to the behavior of localized regions of the leaf than to the leaf as a whole. In addition there has been a general tendency to ignore events in the paradermal plane of the primordium because it is difficult to section in this plane. It is not surprising, therefore, that more recent quantitative studies of cell patterns in sectioned material (Maksymowych and Erickson 1960; Dubuc-Lebreux and Sattler 1980) and cleared specimens (Fuchs 1966, 1975, 1976; Jeune 1972, 1982; Thomasson 1970), and in particular, Green's (Green and Brooks 1978; Green and Lang 1981; Green and Poethig 1982) in vivo studies of epidermal cell behavior, have provided a view of leaf development that is strikingly different from classical models.

Another approach that has proven useful for studying the cellular basis of leaf morphogenesis is clonal analysis. In a clonal analysis, individuals that are heterozygous for a visible, cell-autonomous mutation (e.g. an albino or pale-green mutation) are X-irradiated during meristematic growth in order to induce chromosomal loss. Cells that lose the wild-type, dominant allele of this mutation have the potential for expressing the recessive mutation and produce a visible (e.g. white or pale-green) clone in tissues in which this mutation is expressed. The number, size and extent of the clones in a mature structure reflects the number of cells present in its primordium at the time of irradiation and their subsequent behavior and fate. For this reason, clonal analysis provides a way of studying the cellular parameters of development in systems that are not amenable to more direct cell-marking techniques.

Although clonal analysis has been exploited extensively by investigators of development in *Drosophila*, it has not been widely used to study plant development. This is somewhat surprising considering that the developmental implications of radiation-induced sectors in plants were recognized as early as 1930 (Stadler 1930). Pioneering clonal analyses of leaf and shoot development in maize were carried out by Stein and Steffensen (1959; Steffensen 1968). More recent clonal analyses of maize development have been carried out by Coe (1978), Coe and Neuffer (1978), Johri and Coe (1983) and Poethig (1984a). These studies primarily concern the number and the developmental fate of initial cells. In addition, radiation-induced sectors have been used to study changes in the distribution of cell division during petal development in *Tradescantia* (Mericle and Mericle 1969) and leaf development in tobacco (Deshayes and Dulieu 1974).

In this paper we report the results of a clonal analysis of the cellular parameters of leaf development in tobacco. Two previous cell-lineage studies of tobacco leaf development have defined the fate of the three fundamental cell layers of the shoot apex, and the behavior of cells in these lineages (Dulieu 1968; Stewart and Burk 1970). However, neither of these studies relates patterns of cell lineage to specific stages in leaf development. By so doing, we have been able to determine the number and fate of the initial cells that give rise to various parts of the leaf, and have characterized the pattern of cell division during leaf expansion. A brief summary of some of this work has been published elsewhere (Poethig 1984a).

Materials and methods

Genetic stock. The stock of *Nicotiana tabacum* L. cv. Xanthi Nc. and the cultural procedures used in this study were as described in Poethig and Sussex (1985). This stock was heterozygous for two non-linked, epistatic chlorophyll mutations, *a1* and *a2*(=*yg*) and is yellow-green in color. Cells that lack either *a1* or *a2* are dark-green in color; those that lack the wild-type allele of either gene are yellow.

Experimental procedure. To obtain sectors representing different stages of leaf development, axillary buds were irradiated at various times after they had been released from growth arrest. Plants were exposed to either 100 R or 200 R of X-rays (250 kVp, 15 mA, 2 mm Al, 36 R/min). To ensure uniform exposure, the leaves of a bud were taped together in an upright position and were irradiated perpendicular to their long axis. Irradiations were carried out in the middle of the light cycle.

The lengths of leaves 8, 9, 10, 11 and 12, counting from the base of the axillary shoot, were measured immediately after an irradiation, and then at daily intervals for a period of up to 14 d. When one or more of these leaves was too small to be measured non-destructively, its length at the time of irradiation was estimated with the aid of a standard growth curve (Poethig and Sussex 1985, Fig. 14a). To do this, the length of such a leaf was measured as soon as it was feasible, and this point was located on the curve. The period of time between this stage and the time of irradiation was then used to estimate the length of the leaf when it was irradiated.

At maturity, leaves were harvested, and then photocopied and photographed with Kodak Technical Pan film 2415 (Eastman-Kodak, Rochester, N.Y., USA). Optimal photographic records were obtained by illuminating leaves from below with fluorescent lamps and from above with incandescent photoflood lamps. The leaves were then wrapped in moist paper towels and stored in plastic bags at 4° C until they could be scored. Under these conditions they remained green and turgid for three to four weeks.

Scoring procedure. Clones in the *a1*/+ *a2*/+ stock are most distinct in the palisade layer, consequently only clones in this layer were scored. For the purpose of obtaining quantitative data on clone number, attention was further restricted to green clones since small white or yellow clones are difficult to identify. The position and number of clones in leaves irradiated early in development could be determined from photographs or photocopies of the leaf because of the relatively large size of the

clones. Leaves containing clones less than 0.5 mm in diameter were scored with a stereomicroscope in the following way. A leaf was placed in a transparent lucite tray containing a small amount of de-aerated water, and was covered with a glass plate inscribed with a grid of 25-mm^2 squares. The grid was carefully positioned so as to correspond to one previously drawn on a photocopy of the leaf. The number of dark-green sectors and twin spots in each square was counted with the aid of an ocular grid and entered in the appropriate square on the photocopy. After recording the number of sectors in a leaf, the entire leaf was vacuum-infiltrated with water and the number of cells in sectors in various regions of the lamina was counted, in situ, under a dissecting microscope. The average sector size in any given region was calculated from a sample of about ten sectors.

Results

The nature of the *a1* and *a2* mutations and the range of phenotypes resulting from their interaction have been described by Dulieu and Dalebroux (1976). The *a1*/+ *a2*/+ stock was used for this study because its yellow-green phenotype permits the detection of both dark-green and yellow sectors. This feature, and the fact that events involving either locus can give rise to somatic sectors, result in a relatively high frequency of somatic sectors upon irradiation. Three types of sectors were observed: single green sectors, single white sectors, and paired green and white sectors (twin spots). A genetic analysis of plants regenerated from each type of sector indicates a variety of origins (Dulieu 1974, 1975). Single sectors are most often a consequence of deletions. White or yellow sectors arise from a loss of a wild-type allele, whereas green sectors represent a deletion of a mutant (*a1* or *a2*) allele. Twin spots can arise either from somatic recombination between homologous chromosomes, reciprocal translocations between the homeologous chromosomes carrying *a1* and *a2*, or a translocation of one of these genes to a third, unrelated chromosome.

It should be emphasized at the outset that X-ray-induced somatic clones yield reliable information about cell lineage only if the irradiation does not lead to extensive cell death and if mutant cells behave normally. Although it is difficult to determine if this is true in our system, the fact that most clones did not distort the shape of the leaf – even when they occupied a large fraction of it – indicates that mutant cell lineages were fully viable. Moreover, the relatively low dose of X-rays (100 R) used in these experiments did not appear to affect leaf shape; leaves exposed to a higher dose early in development were somewhat elongated.

Spontaneous mutation frequency. One half of the lamina of a control (unirradiated) leaf contained an average of 187 clones in the palisade layer, most of which were less than 0.5 mm in diameter (about 25 cells) and therefore invisible without magnification (Table 1). This size distribution implies that spontaneous mutation is a stochastic phenomenon, so that the frequency of mutational events occuring in the lamina is directly related to the number of cells in the lamina at that point in time. Most spontaneous sectors are small because they occur late in development, when the number of meristematic cells in the lamina is greatest.

Spontaneous clones were distributed in a non-uniform fashion along the length of the leaf. As shown in Fig. 1, clone density was highest at the tip of the leaf and declined in an even gradient towards the base. Evans and Paddock (1977) attributed a similar phenomenon in tobacco leaves heterozygous for the *Sulfur* mutation to variation in cell density within the lamina. But because cell density is relatively uniform throughout the palisade layer in Xanthi Nc. (Poethig and Sussex

Table 1. The frequency of spontaneous dark-green palisade clones in one half of the lamina of an unirradiated leaf of Xanthi Nc. tobacco

Cells/clone	Clone number	Cells/clone	Clone number
1	71.3±4.8	7	3.1±0.4
2	35.9±1.4	8	5.5±0.9
3	21.4±1.7	9–10	3.0±0.7
4	13.9±0.8	14–18	3.8±0.4
5	9.2±1.0	25–40	3.0±0.4
6	7.7±0.6	≥50	2.6±0.5

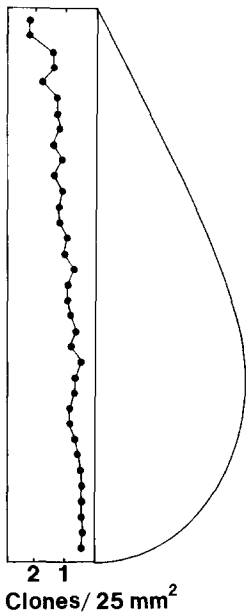

Fig. 1. The average (n = 10) frequency distribution of dark green palisade clones in an unirradiated Xanthi Nc. tobacco leaf

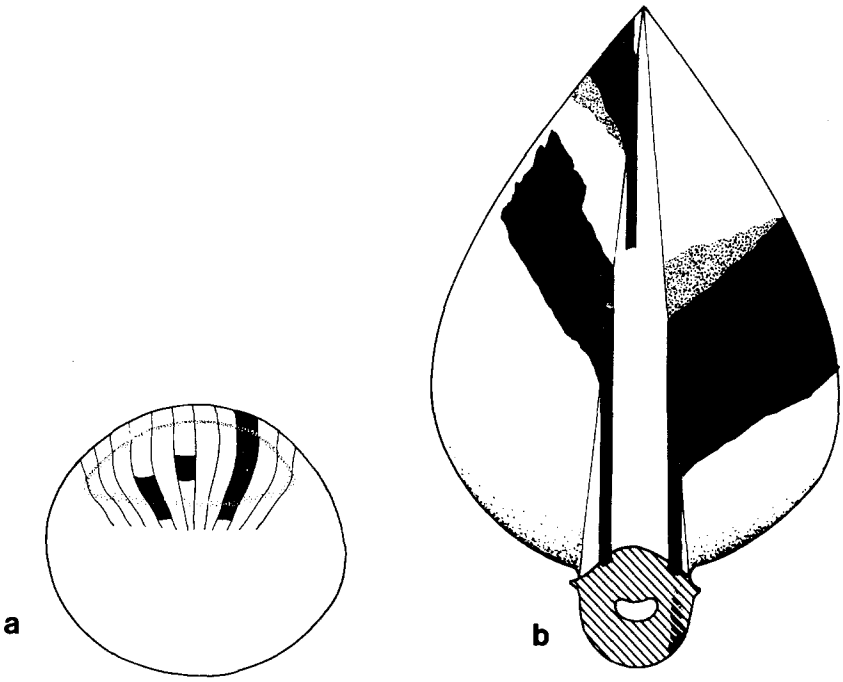

Fig. 2a, b. Clones induced in the shoot meristem of Xanthi Nc. tobacco just prior to the initiation of a leaf. **a** By the time they become incorporated in a leaf primordium, clones present in the shoot meristem may be one or more cells in size. The three possibilities illustrated here are interpretations of the types of clones that give rise to the sectors illustrated in **b. b.** Types of subepidermal clones observed in mature leaves. Clones in the palisade layer are colored *black*; those in the lower spongy mesophyll are *stippled*. Some clones completely envelope the leaf, and end in the internodes above and below it; others end within the subepidermal layer of the leaf. The existence of clones that lie completely within the leaf demonstrates that the leaf primordium encompasses at least three cells in the vertical dimension of the shoot apex

1985), we believe that the observed variation in clone density actually represents variation in the frequency of spontaneous mutation. In tobacco, the frequency of spontaneous mutation varies with the position of leaves on the stem (Deshayes 1973) and with the position of flowers in an inflorescence (Sand 1957), so it is not surprising to find that it varies within an organ as well.

The high spontaneous mutation frequency in this system did not present as great a problem for clonal analysis as might be expected. Although it is impossible to distinguish spontaneous clones from induced clones in leaves irradiated late in development, by this stage spontaneous clones are only a small fraction of the number of clones in the lamina and therefore do not affect the total significantly. Prior to this stage, induced clones could usually be distinguished from spontaneous clones on the basis of size. Since leaves irradiated at early stages were scored without magnification, only clones larger than 0.5 mm in diameter would have been scored. On average, there were only six spontaneous clones of this size per leaf half (Table 1), and in most cases, the vast discrepancy between the size of induced clones and spontaneous clones made it possible to distinguish the two types. As a rule, we ignored clones an order of magnitude smaller than the largest clones in the same region of a leaf.

The cell number of the leaf primordium. The number and fate of the cells that initiate the leaf primordium can be deduced from clones induced in the shoot meristem just before the leaf is initiated. Depending on the subsequent amount of growth, such clones may be one or more cells in size when they become part of a leaf primordium. If one assumes that a leaf primordium encompasses several longitudinal files of cells and is derived from at least three cells in each file, then these clones can intersect the primordium in at least three ways (Fig. 2a). Small clones will fall completely within the primordium; other, larger clones will extend from the shoot meristem into the primordium, and a third class will overlap both the dorsal and ventral boundaries of the primordium. Figure 2b is a schematic representation of the types of clones induced before leaf initiation. All of the clones we observed only occupied a fraction of the leaf. Subepidermal clones induced three or more plastochrons before leaf initiation extend up from the internode below the leaf, out along the underside of the leaf, over the margin into the upper side of the leaf and then up into the internode above. In contrast, clones induced less than 3 d before leaf initiation usually end within the leaf. Some clones extend from the internode to a point within the leaf; others are restricted solely to the leaf. Since all of the conditions illustrated in Fig. 2a have been observed we conclude that a leaf primordium is derived from at least three cells in each of several files of cells in the shoot apex.

The number of cells in the horizontal (i.e. transverse) dimension of the axis primordium was esti-

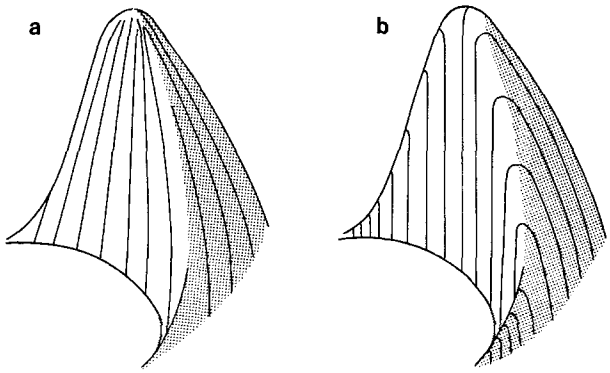

Fig. 3a, b. Alternative models for the cell lineage of the axis of a tobacco leaf. The lines represent the boundaries of cell lineages. **a** If the leaf axis is derived from a group of apical initials, cell lineages will radiate from the apex and will occupy a constant proportion of the circumference of the axis. **b** If the axis is derived from a "line source" of cells – none of which functions as a permanent initial – then cell lineages will occupy longitudinal sections of the axis and only a small number will extend to the tip of the leaf. Futhermore, the size of a sector will change in proportion to the circumference of the axis (see Dulieu 1968)

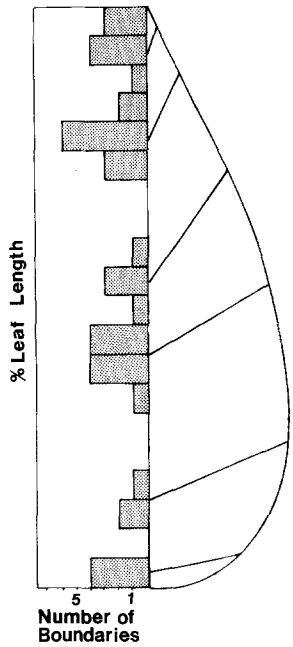

Fig. 4. The position along the length of the leaf at which clonal boundaries in the lamina intersect the midrib (*left*) and the domains defined by these points (*right*). The shape of these domains is typical of the shape of clones located in that part of the lamina. These data were taken from clones induced before leaf initiation, and only represent boundaries that fell in interveinal regions of the lamina. Boundaries that intersected veins were ignored because veins do not expand uniformly

mated by measuring the width of clones relative to the width of the petiole. Assuming that each initial cell contributes equally to the transverse expansion of the leaf axis, the reciprocal of this fraction gives the apparent cell number (ACN) in the transverse dimension of the primordium at the time of irradiation (Coe and Neuffer 1978). Sectors induced one to three plastochrons prior to leaf initiation indicate that the primordium arises from 12.7 ± 0.73 ($n=17$) cells in the circumferential dimension of the shoot apex, a value that is very close to the number of cells in the primordium when it becomes visible at the shoot apex (Poethig and Sussex 1985). Given that a leaf primordium encompasses three cells in its vertical dimension, about 13 cells in its horizontal dimension, and arises from four cell layers (Poethig 1984a), the total number of cells in the primordium is estimated to be about 100.

The fate of leaf initials. If a leaf arose from a group of cells residing at the apex of the leaf axis, one would expect clones to emanate radially from the tip of the leaf, and to occupy a fixed proportion of the axis corresponding to the number of cells in the apex (Fig. 3a). This does not occur. As Dulieu (1968) reported, the position of a clone in the lamina is correlated with its position in the midrib as follows: clones in the median plane of the midrib enter the lamina at the tip of the leaf, clones at the margin of the midrib are restricted to the base of the lamina, and clones located between the median plane and the margin of the midrib occupy the middle of the lamina. Although the width of a clone is uniform throughout the length of the midrib, the diameter of the midrib gradually decreases towards the tip of the leaf. Thus, sectors located near the tip of the leaf occupy a greater fraction of the midrib than sectors near the base of the leaf, indicating that the tip of the leaf is derived from fewer cells than the base. These observations contradict the traditional view of the cell lineage of the leaf, and support the more recent model of Dulieu (1968) (Fig. 3b). Instead of being derived from an apical meristem, the leaf arises from a "line source" of cells, with each cell contributing to a different longitudinal section of the axis. There is no evidence that these cells function as initial cells for the entire lamina. Given the pattern of cell lineage illustrated in Fig. 3b, the most likely position for an initial cell is at the leaf margin because this is the only location in which cells can contribute to both the dorsal and ventral sides of the leaf. However sectors in this region are almost always restricted to one side of the leaf or the other, and are not unusually large in size. Moreover, as will be discussed later, the orientation of submarginal clones demonstrates that submarginal

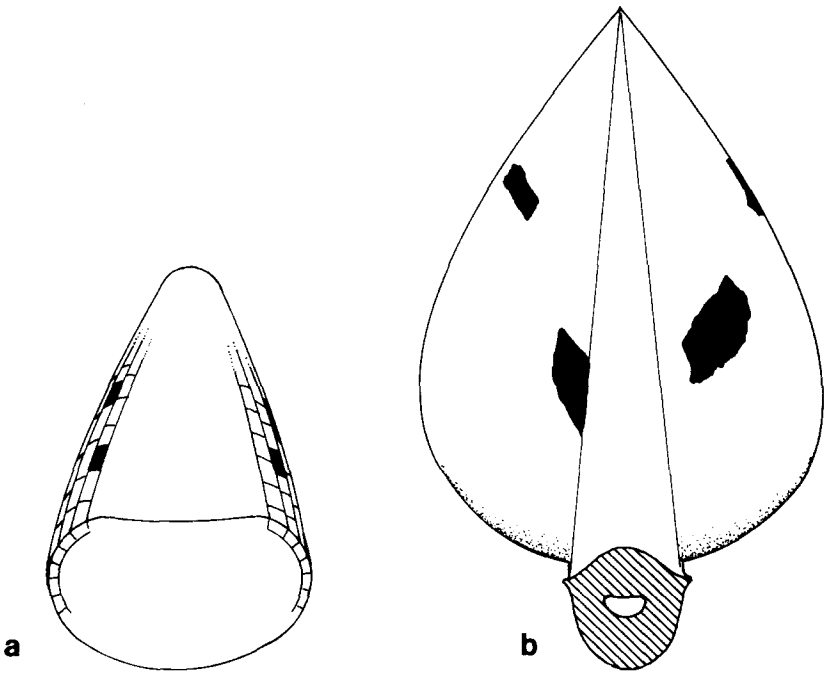

Fig. 5a, b. Clones induced before the initiation of the lamina in Xanthi Nc. tobacco. **a** An interpretation of the location of clones that gave rise to the sectors illustrated in **b**. Note that these clones are actually located in the subepidermal layer; the epidermis is not illustrated. **b** Palisade clones in a mature leaf irradiated prior to the initiation of the lamina. Similar clones occur in the lower spongy mesophyll

cells contribute more to the elongation of the margin than to the transverse expansion of the lamina.

In tobacco, clones generated during leaf initiation occupy well-defined regions of the leaf and have characteristic shapes in each of these regions (Fig. 4). The location of clonal boundaries along the length of the midrib reveals that there are about seven such regions in each half of the leaf. This implies that the lamina is derived from about fourteen cells in the circumferential dimension of the shoot apex, which supports the estimate based on the size of clones in the midrib. As a rule, sectors in the distal part of the leaf are oriented at a more acute angle to the midrib than sectors in the middle of the leaf, indicating that at some point in development cell division is more highly polarized at the tip of the leaf than in the middle. The fact that initial cells have characteristic fates is important because it demonstrates that the amount or orientation of cell division is regulated differently in different parts of the leaf. It must be emphasized, however, that this observation does not mean that the fate of initial cells is strictly determined. Indeed the variability in the size and location of clonal boundaries indicates that the fate of an initial cell lineage is probably an accident of its position in the leaf, not a heritable commitment imposed early in development.

Another striking feature of the cell lineage of the leaf is the relatively high frequency with which clonal boundaries intersect lateral veins. Sixty per cent (35 of 55) of all the anterior and posterior clonal boundaries in leaves irradiated before the initiation of the lamina intersected a lateral vein at the point where the clone entered the midrib. This seems surprising in view of the fact that lateral veins occupy a very small proportion of the tissue in a mature leaf, but becomes less so when one considers the morphology of the leaf at the time of vein initiation (Poethig and Sussex 1985). At this early stage in development the amount of interveinal tissue is about equal to the amount of vascular tissue. Hence the relatively high frequency with which clones intersect veins is a reflection of the number of vein and interveinal initials, rather than evidence of a cell lineage restriction that exists prior to vascular differentiation.

The cell number of the lamina. The study of periclinal chimeras has demonstrated that the lamina of the tobacco leaf is derived from three or more cell layers of the leaf axis (Dulieu 1968; Stewart and Burk 1970; Poethig 1984a), but has provided no information about how many files of lamina initials are present in each of these layers. In order to estimate this parameter in the subepidermal layer we examined clones induced just prior to the initiation of the lamina (leaf length 100–200 μm). Clones induced at this stage are almost always restricted to either the dorsal or ventral subepidermal layer of the lamina; only rarely do they overlap the margin. The restriction of clones to only one surface of the lamina clearly demonstrates that the upper and lower subepidermal layers are derived

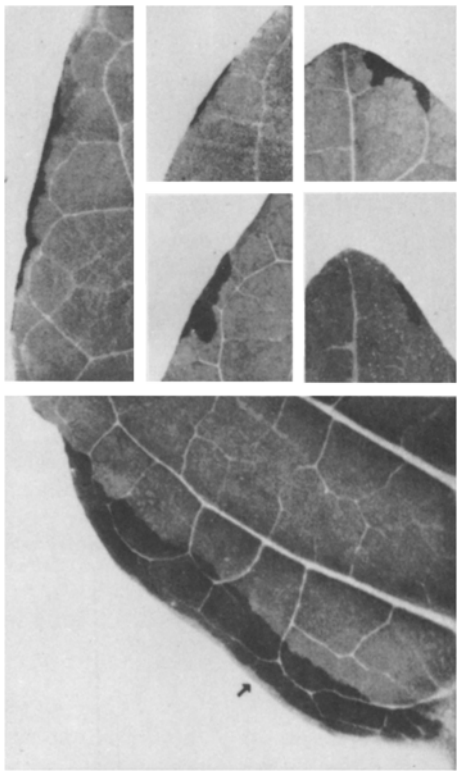

Fig. 6. Marginal palisade clones induced at various times after the initiation of the lamina in Xanthi Nc. tobacco. A narrow strip of normal tissue along the basal margin of the lamina is indicated by an *arrow*

from different files of initial cells, rather than from a single submarginal file as envisioned by Avery (1933). Furthermore the location of clones within these layers demonstrates that each layer arises from at least three files of cells. In leaves irradiated just before the initiation of the lamina, clones are found in one of three places: 1) abutting the leaf margin; 2) abutting the midrib; and 3) isolated in an intercalary region of the lamina (Fig. 5b). Clones isolated in the intercalary region of the lamina (type 3) could only occur if each subepidermal layer was derived from at least three files of cells.

The orientation of cell division in the lamina. The orientation of cell division within the plane of the lamina is highly regulated throughout the course of leaf expansion. Perhaps the most striking feature of this pattern is the orientation of cell division at the leaf margin. As shown in Fig. 6, subepidermal clones are generally elongated parallel to the leaf margin, indicating that submarginal cells more often divide with their spindles parallel to the margin than perpendicular to it. This contradicts Avery's (1933) interpretation of leaf development, which pictures submarginal cells as dividing perpendicular to the margin, and demonstrates that submarginal cells contribute relatively little to the expansion of the lamina. Interestingly, cell division is not as highly polarized in the region immediately adjacent to the margin since clones in this region are usually isodiametric. Nor, for that matter, is it polarized in any intercalary region of the lamina, except during the early growth of the lamina. Evidence for the preferential orientation of cell division early in leaf development is provided by an analysis of twin spots, i.e., adjacent green and white clones. Twin spots result from somatic recombination events that permit the segregation of different alleles of a locus in the subsequent division. The orientation of the members of a twin spot is defined by the line between their centers and is presumed to be parallel to the orientation of the spindle in the division that produced the twin spot (Bryant 1970). Figure 7 shows the orientation of the line between the centers of twin spots in leaves irradiated at a length of 1.3 cm. To facilitate analysis, twin spots were grouped in seven classes according to their orientation relative to the midrib. In every region of the leaf examined,

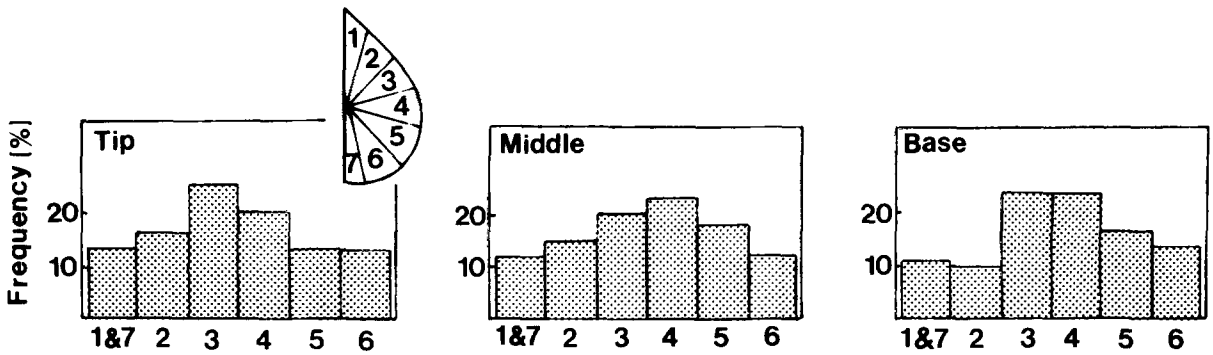

Fig. 7. The orientation of twin spots in different parts of the lamina of Xanthi Nc. tobacco. The orientation of a twin spot is defined by the line running between the center of each spot. There are significantly more spots oriented between 45° and 105° from the tip of the leaf (sectors 3 and 4) than in any other orientation. These data represent a total of 687 sectors in three leaves irradiated at a length of 1.3 cm

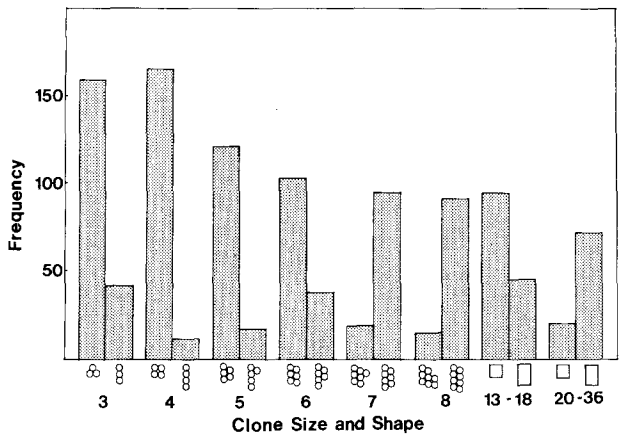

Fig. 8. The frequency of clones of different sizes and shapes in mature X-irradiated leaves of Xanthi Nc. tobacco. Only the most commonly observed types of clones are illustrated. The total number of clones of other shapes was less than the number of clones in the smallest category in each size class

the orientation of twin spots was significantly non-random ($P=0.02$). In general, twin spots tended to be aligned at an angle between 45° and 105° from the tip of the leaf, or approximately parallel to lateral veins. After this stage clones were too small for twin spots to be detected, so the orientation of cell division was studied following the approach of Coe (1978). Clones in various parts of the lamina were classified according to both size and shape, particularly with respect to whether they were elongated or isodiametric (Fig. 8). In any given size class, one shape always occurred more frequently than any other. Four-cell clones, for example, were more often isodiametric than linear, while eight-cell clones were more often linear than isodiametric. Clones around 16 cells in size tended to be isodiametric, while those around 32 cells tended to be linear. This striking pattern indicates

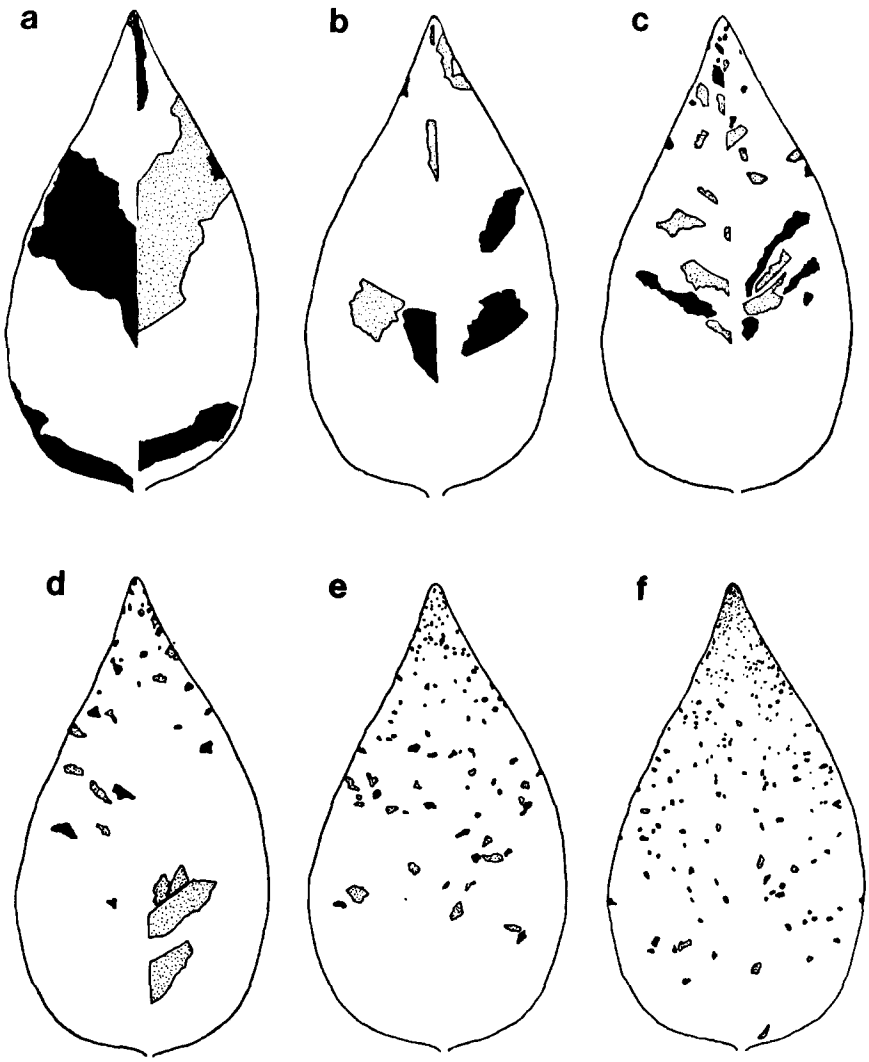

Fig. 9a–f. Composite diagrams of clones in the palisade (*black*) and lower spongy mesophyll (*stippled*) layers of mature leaves of Xanthi Nc. tobacco irradiated at different times in development. The length of the leaf axis at the time of irradiation was extrapolated using the approach described in the Materials and Methods, and is as follows: **a** buttress; **b** 0.1–0.2 mm; **c** 0.4–0.7 mm; **d** 0.8–1.0 mm; **e** 1.5 mm; **f** 3 mm

Table 2. The frequency of mitosis and the frequency of mutation in leaves of Xanthi Nc. tobacco irradiated early in development. The frequency of mutation represents the number of dark-green palisade clones in a mature leaf relative to the number of palisade cells present at the time of irradiation. The number of palisade cells in the lamina was estimated from the product of the area and the average cell density of the lamina, as determined from Feulgen-stained whole mounts. The mitotic frequency represents an average of ten or more measurements taken at various points along the length of the lamina

Leaf length	Palisade cells (No.)	Mitotic frequency	Clone No. (100 R)	Mutation frequency
0.6 cm	29000	$1.7 \cdot 10^{-2}$	270	$9.3 \cdot 10^{-3}$
1.2 cm	267000	$3.5 \cdot 10^{-2}$	980	$3.7 \cdot 10^{-3}$
1.4 cm	353000	$2.9 \cdot 10^{-2}$	1480	$4.2 \cdot 10^{-3}$
2.0 cm	846000	$3.4 \cdot 10^{-2}$	3600	$4.2 \cdot 10^{-3}$

that the final few cell divisions in the lamina occur in alternating perpendicular planes (Coe 1978; Sinnott 1960, p. 50).

The frequency and distribution of cell division in the lamina. As leaves are irradiated at successively later stages of development, the number of clones in the lamina increases and clone size decreases (Fig. 9). The decrease in clone size as a function of age is the result of a decline in the number of cell divisions remaining before maturation. The increase in clone number is more difficult to interpret because it could represent not only an increase in the number of meristematic cells in the lamina but an increase in the sensitivity of cells to irradiation as well. To distinguish between these two possibilities the overall frequency of mutation was calculated by dividing the number of sectors in the palisade layer by the number of cells in this layer at the time of irradiation. This latter figure was estimated from cleared specimens similar in size to irradiated leaves. These data (Table 2) show that the mutation frequency is actually higher early in development than later. Thus the increase in clone number as a function of time is primarily the result of an increase in the number of meristematic cells in the lamina rather than an increase in the sensitivity of the leaf to irradiation.

The change in the number of palisade clones during the course of development is shown in Fig. 10. Clone number increases until the leaf is about one third its final length (6 cm) and then starts to decline. Until the leaf is 2 cm long, clone number increases at an exponential rate of 0.046 h^{-1}, or a doubling time of 15 h. The exponential character of this increase indicates that during this early period virutally all of the cells in the lamina are meristematic. The exponential increase in clone number ends with the cessation of

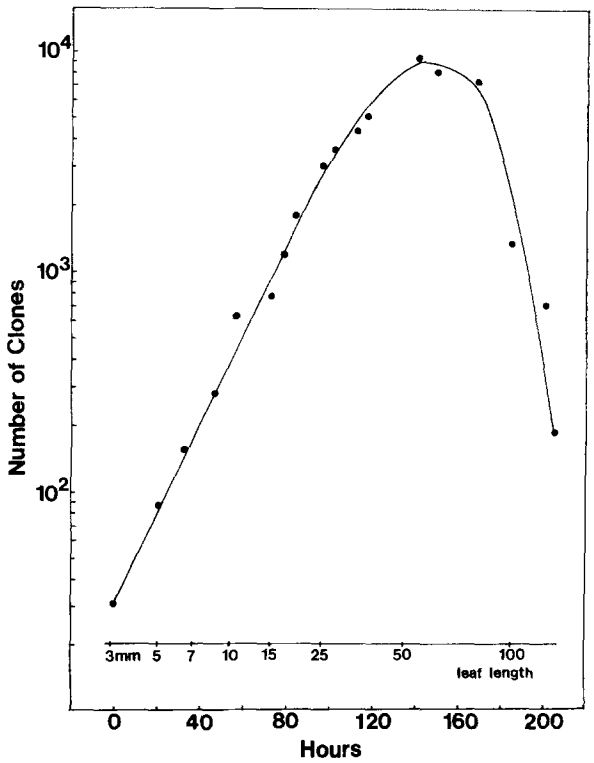

Fig. 10. The number of dark-green palisade clones in one half of the lamina of leaves of Xanthi Nc. tobacco irradiated at various stages in development (dose = 100 R). The length of a leaf at the time of irradiation is indicated on the innermost abscissa. The time between these stages was extrapolated from the growth curves of unirradiated leaves

cell division at the tip of the lamina (Fig. 11). After this point in development the number of meristematic cells in the lamina (i.e. clones) continues to increase, but at a slower rate because of the coincident cessation of cell division at the distal end of the lamina. With time, cell division becomes progressively restricted to more basal portions of the lamina, finally ceasing when the leaf is a little over one half its final length (10–11 cm) (Fig. 11). This basipetal maturation pattern accounts for the gradient in clone size and density in an irradiated leaf. In a leaf irradiated at any stage after the initiation of the lamina, clone size increases and clone density decreases towards the base of the leaf (Fig. 9) because basal cell lineages undergo more cell division than apical lineages over the course of development.

Spatial variation in mutation frequency in the lamina. As previously mentioned, the number of clones induced by irradiation is a function of both the number of meristematic cells present at the time of irradiation and their sensitivity to irradiation (i.e. the frequency of mutation). One of the principal factors determining the sensitivity of a

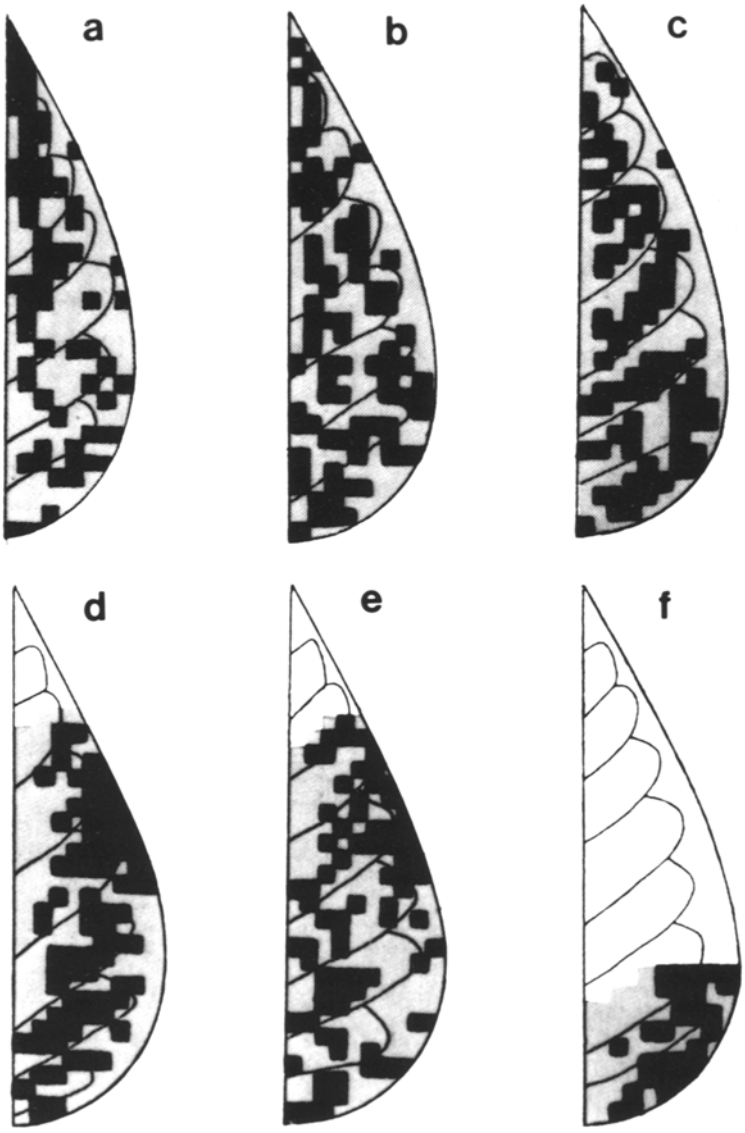

Fig. 11a–f. The relative frequency of palisade clones within transverse sections of mature irradiated leaves of Xanthi Nc. tobacco. The proximal-distal gradient in the duration of meristematic growth obscures variation in clone density that is a consequence of variation in the sensitivity of cells to irradiation. This effect was minimized by comparing the number of clones in scoring units within a narrow (5 mm wide) transverse section of the lamina. In this figure *black squares* represent scoring units with 1.1 or more times the average number of clones in scoring units within that transverse section of the lamina. *Grey squares* had less than 1.1 times the average clone number, and *white regions* were without clones. The length of the leaf at the time of irradiation was: **a** 0.6 cm; **b** 1.3 cm; **c** 3.4 cm; **d** 7.5 cm; **e** 9.7 cm

cell to irradiation is its stage in the cell cycle. A considerable body of research on both animals (St. Amand 1956; Wolff 1968; Dewey et al. 1970) and plants (Sax 1940; Sparrow 1951; Evans and Scott 1964; Scott and Evans 1967; Gudkov and Grodzinsky 1982) indicates that cells are most sensitive to irradiation during late G2 and mitosis. For this reason, variation in mutation frequency (MF) within an organ may be used as an indicator of variation in the frequency of cell division. This assumption is supported by the fact that variation in the frequency of mutation in the tobacco leaf is closely correlated with variation in the frequency of mitosis (Poethig 1984a; Poethig and Sussex 1985).

To study the mutation frequency (MF) in different parts of the lamina, the number of clones within an arbitrary region of a mature leaf was divided by the number of cells in that region at the time of irradiation. Since the number of cells in a region at the time of irradiation could not be directly determined without killing the leaf, this parameter was estimated by dividing the number of cells in an arbitrary region of mature leaf by the average cell number of clones in that region. The MF calculated in this way does not differ greatly from the MF calculated on the basis of actual counts of palisade cell number: using estimates of cell number obtained by counting cells in cleared specimens, the overall MF in leaves irradiated with 100 R at lengths of 1.2 cm, 1.4 cm, and 2 cm was calculated to be about $4 \cdot 10^{-3}$ (Table 2), while calculations based on clonal estimates of cell number give mutation frequencies of $4.8 \cdot 10^{-3}$,

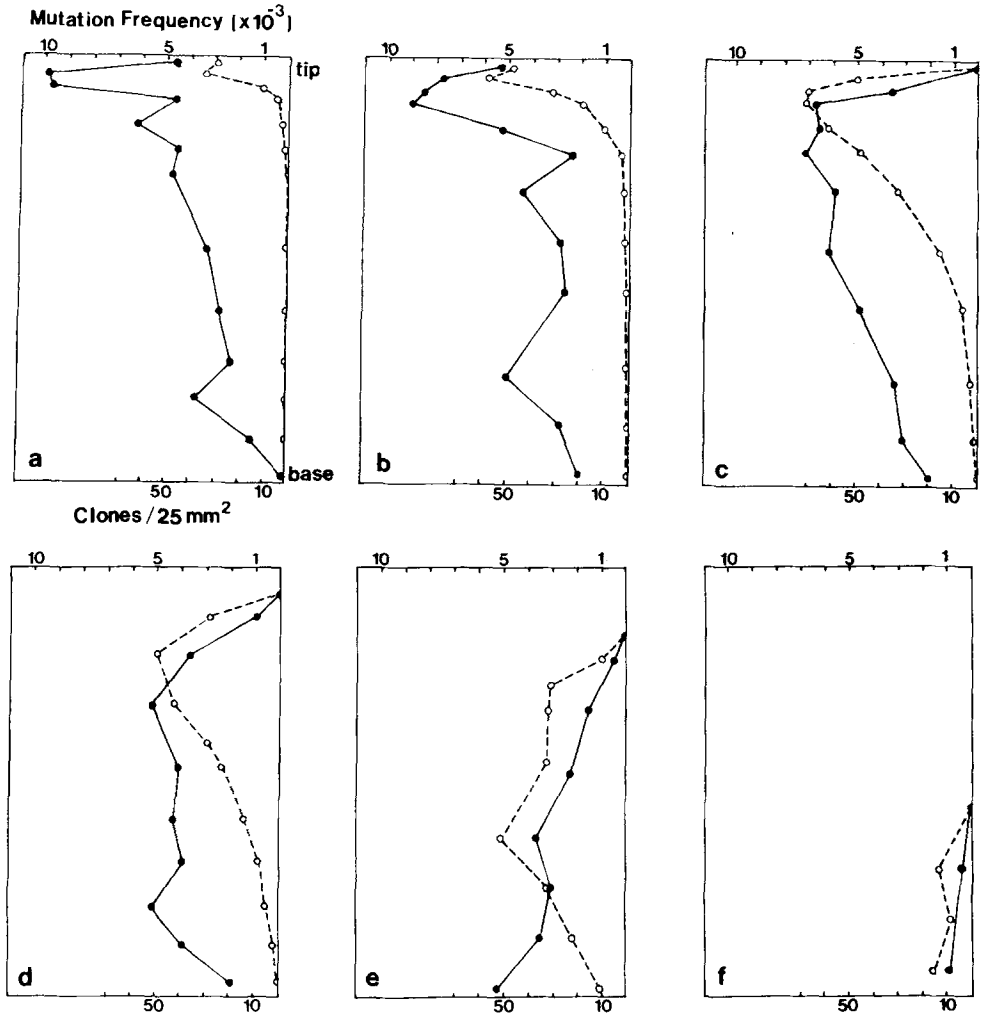

Fig. 12a–f. Spatial variation in clone frequency (No. clones/ 25 mm^2) (o) and mutation frequency (No. clones/No. cells at the time of irradiation) (•) in Xanthi Nc. tobacco leaves irradiated at different times in development. The method used for calculating the number of cells in a local region of the leaf at the time of irradiation is described in the text. The length of the leaf at the time of irradiation was: **a** 0.65 cm; **b** 1.1 cm; **c** 2.5 cm; **d** 3.7 cm; **e** 6.4 cm; **f** 10 cm

$5.3 \cdot 10^{-3}$ and $4.7 \cdot 10^{-3}$ for these particular stages of development.

Leaves irradiated prior to emergence (Fig. 12a) usually have three peaks in MF, two located near the tip of the lamina, and one near the base. At this stage the MF is greatest in the region just behind the tip of the leaf, where it is generally three to four times greater than the frequency in more basal regions. The MF is lowest in the basalmost region of the lamina. Soon after emergence (Fig. 12b) the MF starts to decline at the tip of the lamina because of the cessation of cell division. Coincident with the cessation of cell division at the tip of the leaf, the MF increases in the adjacent basipetal region. This phenomenon occurs along the entire length of the leaf as it matures; in any given region, the MF is maximal just before the cessation of cell division.

Throughout development there is a persistent peak in MF in the basal half of the lamina. Interestingly, the MF in this region remains relatively constant, in contrast to the fluctuations experienced by other parts of the leaf. Although the position of this peak varies slightly in different leaves, it is always located near the widest part of the lamina. Below this peak, in the basalmost region of the lamina, the MF remains relatively low until late in development. In fact, prior to the emergence of the leaf, clones are rarely found in this region. The MF increases at the base of the lamina at about the same time that cell division ceases at the tip of the leaf (2 cm), but remains at a relatively low level until the leaf is about 6 cm long. At this stage MF increases dramatically and then rapidly declines as cell division ceases.

Because the duration of meristematic growth does not vary greatly within a small transverse section of the lamina, variation in clone density within such a section directly reflects variation in MF. In order to examine variation in MF across the lamina, the number of clones in each scoring unit (25 mm^2) was divided by the average number of clones per scoring unit in corresponding transverse sections of the lamina. In Fig. 11 scoring units with

at least 1.1 times the average number of clones are shaded black. Although this way of presenting data obscures variation in MF along the length of the leaf, it demonstrates several otherwise invisible features of the pattern of mutation within the lamina. One of the most interesting of these is the relatively low MF at the margin of the lamina early in development. Until the leaf is 3 cm long, regions with a relatively high MF appear to be randomly distributed in the intercalary region of the lamina, but occur infrequently at the margin. Later, the MF tends to be highest in interveinal regions. Cell division stops first near the midrib, and slightly later at the margin of the lamina. This is apparent not only in that clones disappear from the region near the midrib before they disappear at the margin, but also in the relative density of clones in the region just behind the distal end of the meristematic zone (Fig. 11 d, e, f). Calculations of the actual MF in this region demonstrate that the concentration of clones near the margin is the consequence of a decline in the MF near the midrib rather than of an increase in the MF at the margin.

Discussion

Our results confirm Dulieu's (1968, 1970) interpretation of the cell lineage of the tobacco leaf and provide a more comprehensive picture of the cellular parameters of development than has hitherto been available. In brief, we found that 1) the leaf primordium arises from approx. 100 cells, each of which contributes to a longitudinal section of the axis and an oblique section of the lamina; 2) the orientation and frequency of cell division at the leaf apex and at the leaf margin do not provide any evidence that specialized initial cells reside in these regions; and 3) during the expansion of the lamina the orientation, frequency, and distribution of cell division are highly regulated. These results confirm and extend our observations concerning the developmental morphology and histology of the leaf (Poethig and Sussex 1985), and demonstrate the value of clonal analysis as a tool for studying plant development.

The functional organization of the leaf primordium. A number of conclusions about the functional organization of the leaf can be drawn from its cell-lineage pattern. Firstly, it is noteworthy that the cell number of the leaf prior to initiation is similar to the number of cells in the leaf buttress (Poethig and Sussex 1985). This indicates that determination of a leaf primordium occurs immediately before, if not during, its initiation. If determination took place long before initiation, it would have to involve fewer cells than those present in the leaf buttress simply because it is impossible to "fit" more than one or two leaf buttresses into the region between the shoot apex and the last initiated leaf. This observation supports experimental studies that indicate that the site of a new leaf is determined about half a plastochron before its appearance at the shoot apex (Snow and Snow 1933). Secondly, the observation that the leaf is derived from a relatively large group of cells rather than from one or a few initials in turn implies that leaf morphogenesis is regulated by supracellular factors rather than a cell-autonomous system that directs cells to undergo a precise sequence of genetically programmed steps; it is difficult to imagine how the activity of a large group of cells could be coordinated other than by some type of globally distributed agent. Additional evidence that leaf morphogenesis does not involve a programmed pattern of cell division is provided by the fact that although sectors in analogous parts of different leaves may be similar in size and shape, they are never exactly identical. Furthermore, while it is true that leaf initials have predictable fates, there is no evidence that cell lineages are confined by compartment boundaries like those that exist in *Drosophila* (Garcia-Bellido 1975). The relatively high frequency with which clonal boundaries lie within lateral veins is best explained by the precocious differentiation of veins, and the relatively large size of their primordia relative to interveinal tissue; the position of these boundaries is too variable to conclude that compartment boundaries exist. Thirdly, it should be pointed out that all the major parts of the leaf (midrib, lamina, petiole) arise from the same initial cells because, as a rule, clones induced prior to leaf initiation encompassed all three structures. Hence these structures must become determined some time after leaf initiation. Finally, since both the apex and margin of the leaf contribute relatively few cells to the leaf, it seems unlikely that these sites exercise primary control over leaf shape. This conclusion is supported even more strongly by the observation that the ablation of the apex or margin of the leaf early in development does not prevent the regeneration of a normal leaf (Sachs 1969), whereas later operations only result in localized defects (Sachs 1969; Jeune 1972).

The role of cell division in leaf morphogenesis. As yet we have little information about the morphogenetic significance of the cellular parameters described in this paper. Although preliminary observations indicate that the pattern of cell division

is closely correlated with the dynamics of leaf growth, we have no evidence that cell division directly controls this pattern. Indeed it is unlikely that the rate of cell division directly controls the rate of cell expansion since contiguous cells in the lamina (and in other organs) can have the same relative elemental rate of expansion while having dramatically different rates of cell division. In *Xanthium*, for example, epidermal cells in the lamina actually have a higher rate of increase in cell area than the palisade, even though they cease dividing before the palisade cells (Maksymowych 1963). Furthermore, the cessation of cell division during development has no effect on the polarity of leaf growth in tobacco (Haber and Foard 1963). These observations indicate that coincident changes in the rate of cell division and the relative growth rate may be the result of their dependence on common factors, e.g. nutrients, rather than a cause-and-effect relationship. But, while cell division may not directly affect the relative growth rate of a cell, it may control the shape of an organ by generating spatial variation in cell number, that is, by providing the capital for growth if not the driving force. This possibility can be tested by inhibiting cell division at a stage in leaf development when there is significant variation in cell density within the lamina. If the growth of the lamina is dependent on cell number then the subsequent pattern of growth should be directly related to the initial pattern of cell density. Such an experiment was conducted by Haber (1962) who found that suppressing cell division in a young wheat leaf affects the size of the leaf but not its polarity of growth. Although Haber (1962) interpreted this result to mean that cell division plays a secondary role in leaf morphogenesis, this interpretation can be criticized on the grounds that the polarity of cell division and cell expression had probably already been established in the primordium at the stage it was irradiated. Moreover, variation in the amount of cell division is more likely to affect the shape of an organ when cells expand isotropically – as in the case of broad leaf like that of tobacco – than when their expansion is polarized in one direction as is the case in wheat (Poethig 1984b). Clearly the morphogenetic effect of variation in the amount of cell division depends on the amount and orientation of cell expansion. However, we do not believe that cell expansion determines leaf shape completely independently of cell number because leaf shape is so closely correlated with variation in cell number (von Papen 1935; Bensink 1971; Poethig and Sussex 1985).

Another role for cell division in leaf morphogenesis is suggested by the work of Green and his collaborators (Green and Brooks 1978; Hardham et al. 1980; Green and Lang 1981; Green and Poethig 1982; Green 1984) who have characterized the relationship between the orientation of cell division and the orientation of cellulose microfibrils in developing leaf and shoot primordia of *Graptopetalum paraguayense*. They have shown that during leaf initiation, regionally specific changes take place in the orientation of cell division and cellulose microfibrils. Regions of the primordium whose polarity is inappropriate to the new organ undergo a shift in polarity, while those with the correct polarity maintain the same orientation of cell division and cellulose microfibrils. In the region undergoing a polarity shift, changes in the orientation of cellulose take place in a cell autonomous fashion, and are preceded by a change in the orientation of cell expansion (Green and Poethig 1982; Green 1984). This implies that the orientation of cell division determines the orientation of cellulose deposition, which in turn determines the orientation of cell expansion.

In view of this relationship between cell division and the biophysical polarity of the leaf, it is interesting that the cell lineage of the tobacco leaf is strikingly similar to that of *Graptopetalum*. Because of the bilateral symmetry of the axis, the polarity of its adaxial and abaxial faces is identical to the polarity of the regions of the meristem from which the primordium arises; hence only the lateral margins of the axis would be expected to undergo a change in behavior during leaf initiation. Cell patterns in the epidermis (Poethig and Sussex 1985) and clonal patterns in subepidermal tissue (this paper) indicate that this is indeed the case. Instead of adopting a radial pattern of cell division, leaf initials produce lineages that are essentially extensions of lineages in the stem. Only the leaf margins adopt a pattern of cell division that is at odds with the prevailing transverse orientation of cell division in the stem. There is good evidence, therefore, that the organization of the tissue from which a leaf arises is an important factor in morphogenesis, and that the remodeling of this organization is brought about by changes in the orientation of cell division. The fact that the orientation of cell division is also highly regulated during the initiation and expansion of the lamina (Figs. 7, 8 and Jeune 1972, 1982; Fuchs 1975, 1976) indicates that it plays a similar role in the development of this structure. However, the relationship between the orientation of cell division and the physical organization and growth of the lamina is unknown.

We are grateful to John Duesing (Ciba-Geigy Co., Greensboro, N.C., USA) for criticism and encouragement. R. S. P. was supported by a National Science Foundation Predoctoral Fellowship, National Institutes of Health Training Grant HD07180-01, and a Sigma Xi Grant-in-Aid-of-Research.

References

Avery, G.S. (1933) Structure and development of the tobacco leaf. Am. J. Bot. **20**, 565–592

Bensink, J. (1971) On the morphogenesis of leaves in relation to light and temperature. Meded. Landbouwhogeschool Wageningen **71**, No. 15

Bryant, P.J. (1970) Cell lineage relationships in the imaginal wing disc of *Drosophila melanogaster*. Dev. Biol. **22**, 389–441

Coe, E.H. (1978) The aleurone tissue of maize as a genetic tool. In: Maize breeding and genetics, pp. 447–459, Walden, D.B., ed. Wiley-Interscience, New York

Coe, E.H. Neuffer, M.G. (1978) Embryo cells and their destinies in the corn plant. In: The clonal basis of development, pp. 113–129, Subtelny, S., Sussex I., eds. Academic Press, New York

Deshayes, A. (1973) Mise en évidence d'une corrélation entre la fréquence de variations somatiques sur feuilles et l'état physiologique d'un mutant chlorophyllien monogénique chez *Nicotiana tabacum* var. Samsun. Mutat. Res. **17**, 323–334

Deshayes, A., Dulieu H. (1974) Étude des variations somatiques de deux mutants chlorophylliens de *Nicotiana tabacum* L., leur nature génétique et les facteurs les favorisent. In: Polyploids and induced mutations in plant breeding, pp. 85–99. International Atomic Energy Agency, Vienna

Dewey, W.C., Furman S.C., Miller, H.H. (1970) Comparison of lethality and chromosomal damage induced by X-rays in synchronized chinese hamster cells in vitro. Radiat. Res. **43**, 561–581

Dubuc-Lebreux, M.A., Sattler, R. (1980) Développement des organes foliacés chez *Nicotiana tabacum* et le problème des méristèmes marginaux. Phytomorphology **30**, 17–32

Dulieu, H. (1968) Emploi des chimères chlorophylliennes pour l'étude de l'ontogénie foliare. Bull. Sci. Bourgogne **25**, 1–60

Dulieu, H. (1970) Les mutations somatiques induites et l'ontogénie de la pousse feuillee. Ann. Amelior. Plant. **20**, 27–44

Dulieu, H. (1974) Somatic variations on a yellow mutant in *Nicotiana tabacum* L. ($a1^+/a1$ $a2^+/a2$). I. Non-reciprocal genetic events occurring in leaf cells. Mutat. Res. **25**, 289–304

Dulieu, H. (1975) Somatic variations on a yellow mutant in *Nicotiana tabacum* L. ($a1^+/a1$ $a2^+/a2$). II. Reciprocal genetic events occurring in leaf cells. Mutat. Res. **28**, 69–77

Dulieu, H., Dalebroux, M.A. (1976) Composantes de variabilité et interactions fonctionnelles aux loci $a1^+/a1$ et $a2^+/a2$ de *Nicotiana tabacum* L. Ann. Amelior. Plant. **26**, 569–577

Evans, D.A., Paddock, E.F. (1977) X-ray induced increase of mitotic crossovers in *Nicotiana tabacum*. Environ. Exp. Bot. **17**, 99–106

Evans, H.J., Scott, D. (1964) Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba*. Genetics **49**, 17–38

Fuchs, C. (1966) Observations sur l'extension en largeur du limbe foliaire du *Lupinus albus* L. C.R. Acad. Sci. Ser. D **263**, 1212–1215

Fuchs, C. (1975) Ontogenèse foliare et acquisition de la forme chez le *Tropaeolum peregrinum* L. I. Les premiers stades de l'ontogenèse du lobe médian. Ann. Sci. Nat. Bot. Biol. Veg. **16**, 321–390

Fuchs, C. (1976) Ontogenèse foliare et acquisition de la forme chez le *Tropeolum peregrinum* L. II. Le développement du lobe après la formation de lobules. Ann. Sci. Nat. Bot. Biol. Veg. **17**, 121–158

Garcia-Bellido, A. (1975) Genetic control of wing disc development in *Drosophila*. In: Cell Patterning, Ciba Foundation Symp. vol. 29, pp. 161–182. Elsevier, New York

Green, P.B. (1984) Shifts in plant cell axiality: histogenetic influence on cellulose orientation in the succulent, *Graptopetalum*. Dev. Biol. **103**, 18–27

Green, P.B., Brooks, K.E. (1978) Stem formation from a succulent leaf: its bearing on theories of axiation. Am. J. Bot. **65**, 13–26

Green, P.B., Lang, J.M. (1981) Toward a biophysical theory of organogenesis: birefringence observations on regenerating leaves in the succulent *Graptopetalum paraguayense* E. Walther. Planta **151**, 413–426

Green, P.B., Poethig, R.S. (1982) Biophysics of the extension and initiation of plant organs. In: Developmental order: Its origin and regulation, pp. 485–509, Subtelny S., Green, P.B., eds. A.R. Liss, New York

Gudkov, I.N., Grodzinsky, D.M. (1982) Cell radiosensitivity variation in synchronously dividing root meristems of *Pisum sativum* L. and *Zea mays* L. during the mitotic cycle. Int. J. Radiat. Biol. **41**, 401–409

Haber, A.H. (1962) Nonessentiality of concurrent cell divisions for degree of polarization of leaf growth. I. Studies with radiation induced mitotic inhibition. Am. J. Bot. **49**, 583–589

Haber, A.H., Foard, D.E. (1963) Nonessentiality of concurrent cell divisions for degree of polarization of leaf growth. II. Evidence from untreated plants and chemically induced changes in the degree of polarization. Am. J. Bot. **50**, 937–944

Hardham, A.R., Green, P.B., Lang, J.M. (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation in *Graptopetalum paraguayense*. Planta **149**, 181–195

Jeune, B. (1972) Observations et expérimentation sur les feuilles juveniles du *Paulownia tomentosa*. Bull. Soc. Bot. France **119**, 215–230

Jeune, B. (1982) Morphogénèse des feuilles et stipules de *Castanea sativa* Miller. Bull. Mus. Natn. Hist. Nat., Paris, Sect. B, Adansonia **4**, 85–101

Johri, M.M., Coe, E.H. (1983) Clonal analysis of corn plant development. I. The development of the tassel and ear shoot. Dev. Biol. **97**, 154–172

Maksymowych, R. (1963) Cell division and cell elongation in leaf development of *Xanthium pensylvanicum* Am. J. Bot. **50**, 891–901

Maksymowych, R., Erickson, R.O. (1960) Development of the lamina in *Xanthium italicum* represented by the plastochron index. Am. J. Bot. **47**, 451–459

Mericle, L.W., Mericle, R.P. (1969) Induced somatic mutations for interpreting floral development and inflorescence aging. In: Induced mutations in plants, pp. 591–601. International Atomic Energy Agency, Vienna

Poethig, R.S. (1984a) Cellular parameters of leaf morphogenesis in maize and tobacco. In: Contemporary problems in plant anatomy, pp. 235–259, White, R.A., Dickison, W.C., eds. Academic Press, New York

Poethig, R.S. (1984b) Patterns and problems in angiosperm leaf morphogenesis. In: Pattern formation, pp. 413–432, Malacinski, G.M. Bryant, S.V., eds. MacMillan, New York

Poethig, R.S., Sussex, I.M. (1985) The developmental morphology and growth dynamics of the tobacco leaf. Planta **165**, 158–169

Sachs, T. (1969) Regeneration experiments on the determination of the form of leaves. Israel. J. Bot. **18**, 21–30

Sand, S.A. (1957) Phenotypic variability and the influence of temperature on somatic instability in cultures derived from hybrids between *Nicotiana langsdorffii* and *N. sanderae*. Genetics **42**, 685–703

Scott, D., Evans, H.J. (1967) X-ray induced chromosome aberrations in *Vicia faba*: changes in response during the cell cycle. Mutat. Res. **4**, 579–599

Sax, K. (1940) An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. Genetics **25**, 41–68

Sinnott, E.W. (1960) Plant morphogenesis. McGraw-Hill, New York

Snow, M. and Snow, R. (1933) Experiments on phyllotaxis. II. The effect of displacing a primordium. Phil. Trans. R. Soc. London Ser. B **222**, 353–400

Sparrow, A.H. (1951) Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Ann. N.Y. Acad. Sci. **51**, 1508–1540

St. Amand, W. (1956) Mitotic inhibition and chromosome breakage induced in grasshopper neuroblasts by X-irradiation at known mitotic stages. Radiat. Res. **5**, 65–78

Stadler, L.J. (1930) Some genetic effects of X-rays in plants. J. Hered. **21**, 3–19

Stein, O.L., Steffensen, D.M. (1959) The activity of X-rayed apical meristems: a genetic and morphogenetic analysis in *Zea mays*. Z. Vererbungsl.. **90**, 483–502

Steffensen, D.M. (1968) A reconstruction of cell development in the shoot apex of maize. Am. J. Bot. **55**, 354–369

Stewart, R.N., Burk, L.G. (1970) Independence of tissues derived from apical layers in ontogeny of the tobacco leaf and ovary. Am. J. Bot. **57**, 1010–1016

Thomasson, M. (1970) Quelques observations sur la répartition de zones de croissance de la feuille du *Jasminum nudiflorum* Lindl. Candollea **25**, 297–340

von Papen, R. (1935) Beiträge zur Kenntnis des Wachstums der Blattspreite. Bot. Arch. **37**, 159–206

Wolff, S. (1968) Chromosomal aberrations and the cell cycle. Radiat. Res. **33**, 609–619

Received 12 October; accepted 21 December 1984