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Stomatal Patterning in *Tradescantia:* An Evaluation of the Cell Lineage Theory

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The cell lineage theory, which explains stomatal patterning in monocot leaves as a consequence of orderly divisions, was studied in *Tradescantia.* Data were collected to test the theory at three levels of organization: the individual stoma; stomata distributed in one dimension, in linear fashion along cell files; and stomata apportioned in two dimensions, across the length and breadth of the leaf. In an attempt to watch the patterning process through regeneration, stomata in all visible stages of development were laser ablated. The results showed that the formation of stomatal initials was highly regular, and measurements of stomatal frequency and spacing showed that pattern was determined near the basal meristem when the stomatal initials arose. Following the origin of initials, the pattern was not readjusted by division of epidermal cells. Stomatal initials were not committed when first present and a small percentage of them arrested. The arrested cells, unlike stomata, were consistently positioned in cell files midway between a developed pair of stomata. At the one-dimensional level of pattern, stomata in longitudinal files were separated by a variable number of epidermal cells and the frequency of these separations was not random. The sequential spacing of stomata also was not random, and stomata separated by single epidermal cells were grouped into more short and long series than expected by chance. The stomatal pattern across the width of the leaf resulted from cell files free of stomata which alternated with cell files containing stomata, but not with a recurring periodicity. Files lacking stomata were found only over longitudinal vascular bundles. Laser ablations of developing stomata did not disrupt the pattern in nearby cells or result in stomatal regeneration. We conclude that the cell lineage theory explains pattern as an individual stomatal initial arises from its immediate precursor and satisfactorily accounts for the minimum spacing of stomata in a cell file, i.e., stoma–epidermal cell–stoma. However, the theory does not explain the collective stomatal pattern along the cell files, at the one-dimensional level of patterning. Nor does the theory account for the two-dimensional distribution of stomata in which regions devoid of stomata alternate with regions enriched with stomata, but not in a highly regular nor haphazard manner. We suggest that the grouping of epidermal cells and stomata separated by single epidermal cells in cell files may result from cell lineages at a specific position in the cell cycle as they traverse the zone where stomatal initials form. Sister cells at the appropriate position in the cell cycle form stomatal initials while lineages at other positions in the cell cycle yield epidermal cells.

INTRODUCTION

More than 40 years ago Bünning and Sagromsky (1948) put forward the cell lineage theory to explain stomatal patterning in monocot leaves. The theory states that the placement of stomata is wholly determined by ordered series of divisions. These divisions produce the stomatal initials. In contrast, stomatal patterning in dicots is hypothesized to involve inhibitory influences from developing stomata that prevent the origin of stomata close to them (Bünning and Sagromsky, 1948).

Interpretation of the cell lineage theory is problematic. Is the theory intended to encompass all organizational levels of stomatal patterning? Does it explain only the origin of individual stomatal initials? Or does it also include the origin of successive stomatal initials within a longitudinal file of cells? Do all stomatal initials within a file result from repeating sets of these series of divisions? Or can these series of divisions be interspersed with a variable number of epidermal cells? Is the one-dimensional (linear) distribution of stomata also a result of the cell lineage theory? Figures in Bünning's papers following publication of the theory (1952, 1956) show monocot stomata in a checkerboard pattern. Stomata are equally spaced within individual files of cells and staggered with respect to stomata in neighboring rows of cells. Thus, the pattern is ordered in two dimensions, along the length and across the width of the leaf. However, the text of these papers never addresses whether the theory explains the pattern at the one- and two-dimensional levels of pattern or only at the level of the individual stoma.

Since the assertion of this theory, no studies on stomatal patterning in monocots have discussed the organizational levels at which the theory operates. Our goal was to determine the validity of the cell lineage theory for *Tradescantia* stomatal pattern with respect to three

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levels of organization: the individual stoma, stomata distributed in linear series, and stomata distributed across the leaf width. To make these determinations, we examined the development of stomata and made quantitative measurements of their distribution on leaves. Since interfering with cell communication might reveal aspects of patterning through regeneration, developing stomata were irradiated with a laser microbeam and examined at intervals to follow the effects.

The results demonstrated that the cell lineage theory describes the origin and minimum spacing of individual stomata in *Tradescantia*. However, the theory does not account for the pattern at the next two levels of organization. In cell files stomata were separated by a variable number of epidermal cells which occurred in nonrandom frequencies. The sequential spacing of stomata in cell files also was nonrandom. Stomata separated by single epidermal cells were present in longer series than expected by chance alone. Across the leaf width, the stomatal pattern was neither random nor perfectly regular, although longitudinal tracts of cell files containing stomata alternated with tracts devoid of stomata.

MATERIALS AND METHODS

Plant material. Tradescantia plants were clones from an individual shoot cutting provided by Michael Kagan and Tsvi Sachs (Hebrew University, Israel). Cuttings were grown in a greenhouse under ambient conditions in a soil mixture (3 parts sterilized soil, 3 parts peat moss, 3 parts perlite, 2 parts rice hulls), watered, and fertilized. For the laser ablation study, plants were grown in an outdoor garden under ambient conditions in Irvine, California.

Material preparation. Shoot tips and leaves were fixed in FAA, dehydrated in an ethanol series, and then critical point dried. Portions of mature leaves or entire young leaves that were removed from the shoot tip were mounted on stubs with double-stick tape. Material was then coated with a 10-nm layer of gold-palladium before viewing with a JEOL-35C or Philips scanning electron microscope operated at 10 kV.

Pattern measurements. Frequencies of stomatal initials, stomata, and stomata plus arrested cells were calculated from replicas of 10 leaves. Each stomatal initial and each stomatal complex, although multicellular, was counted as a single stoma. A minimum of 10 stomatal initials or stomata was present in each field used for frequency determinations.

The pattern within cell files was studied by counting the number of epidermal cells between successive stomata or stomatal initials. The frequency (occurrence) of each interval of cells was determined from SEM prints of 75 leaves. Additional measurements between stomata and arrested cells were made within and between cell files by image analysis; all distances are center-to-center. The direction of the leaf tip was known for each SEM print.

The distance from each developed stomata or arrested cell to a randomly selected reference stoma was measured in two-dimensional fields of mature leaves. These determinations were made using SEM prints in conjunction with an image analysis system and random number generators.

Pattern measurements across the leaf width were made from replicas of 10 whole leaves. The number of cell files in tracts containing and devoid of stomata was recorded from the midrib to the margin at the widest section of the leaf.

Laser ablation. Stomatal ablations were performed at the Laser Microbeam Project facility associated with the Beckman Laser Institute at the University of California, Irvine (Irvine, CA). Stomata were viewed with a Zeiss Axiomat inverted microscope equipped with a 40X water immersion objective. A Nd-YAG laser (535 nm) attenuated to power densities ranging from 5 to 50 μJ/pulse was used for irradiations. The laser beam was focused to a spot diameter of 2–5 μm. Selected irradiations were videotaped to provide a record of the cell before, during, and after ablation. Young leaves, approximately 2 mm in length, that remained attached to the shoot were used in experiments. A leaf of this length has mature stomata at the tip and immature stomata at the leaf base, providing the entire developmental range of stomata. Using the leaf midrib as a marker, stomata in an adjacent row were irradiated beginning at the leaf tip and proceeding to the leaf base. Since rows with stomata are more or less continuous along the leaf length, irradiated stomata were easily relocated. Approximately 1000 stomata were irradiated on each of 50 leaves. Following irradiation, the shoot segments were allowed to continue their growth for up to 42 days. Samples were fixed for SEM observation at selected intervals, ranging from immediately following ablation to 42 days later. Daily samples were taken in the first week, and weekly samples thereafter.

RESULTS

Stomata in *Tradescantia* leaves were found only on the lower epidermal surface. The epidermis consisted of linear cell files of uniform width. Some cell files split into two files of cells and then later rejoin. The number of cells within areas bounded by the splitting and joining cell files was variable. Collectively, the epidermal cells in the files were arranged like bricks in a wall. The stomata are studded on the matrix of epidermal cells (Fig. 1).

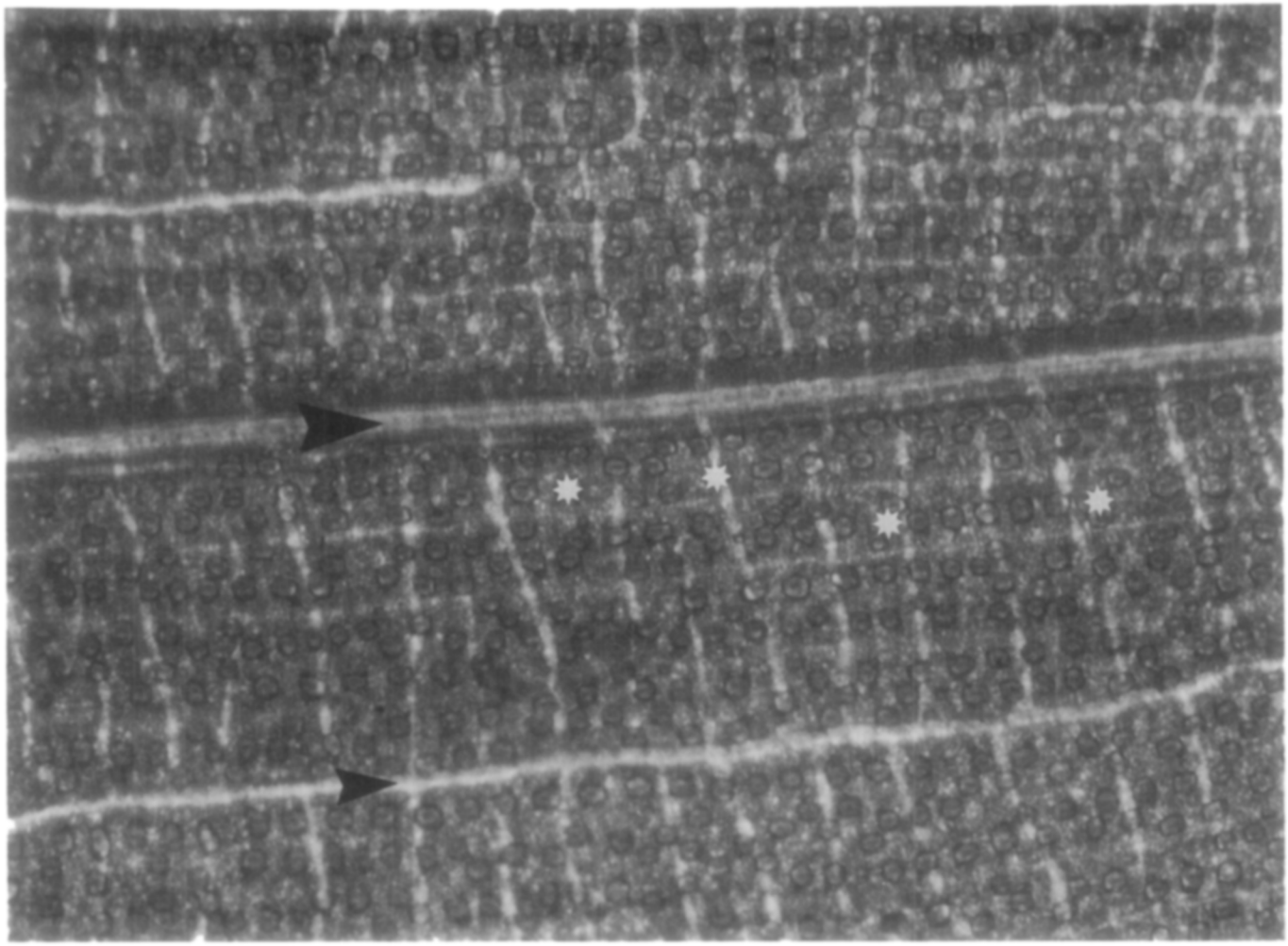

FIG. 1. Photomicrograph (not retouched) of the lower surface of a *Tradescantia* leaf showing the two-dimensional distribution of stomata. Solid arrowheads indicate cell files without stomata, at the midrib (large arrowhead) and atop longitudinal vascular bundles in the blade panel (small arrowhead). Pairs of stars designate linear groups of epidermal cells (upper set) and stomata separated by single cells (lower set).

The basal meristem generates all cells of the leaf by forming derivatives in the distal direction (Fig. 2). As new derivatives arise, cells are displaced distally and some become selected for a specialized function. The first evidence of this selection process was the formation of a stomatal initial by an unequal division in a discrete zone of the leaf (Fig. 2). Additional unequal divisions in cells neighboring stomatal initials took place to create the subsidiary cells of the stomatal complex. These events also occur in a distinct location, although it was broader than the zone where stomatal initials were found.

Stomatal development in *Tradescantia* appeared to take place in the following stages (Fig. 2). An unequal division formed a stomatal initial at the distal end of an epidermal cell (Fig. 2). The formation of an initial took place only once in an epidermal cell. At first the initial was tabular in form and much smaller than its sister cell. As the surrounding epidermal cells expanded, the initial became lenticular in outline (Fig. 2). Then a pair of unequal cell divisions occurred in the epidermal cells of adjacent cell files to form the first set of subsidiary cells. The new cells were positioned neighboring the stomatal initial, one toward the midrib and one toward the margin (Fig. 2). A second set of unequal divisions occurred in epidermal cells next to the stomatal initial, within the same cell file. These divisions formed the final two subsidiary cells of the stomatal complex (Fig. 2).

Each set of unequal divisions was synchronous, except on rare occasions. When asynchrony occurred, the division of the second cell was completed before the next set of divisions began. Shortly after all the subsidiary cells were present, the lenticular stomatal initial became tabular in form and then divided to produce the guard cells (Fig. 2).

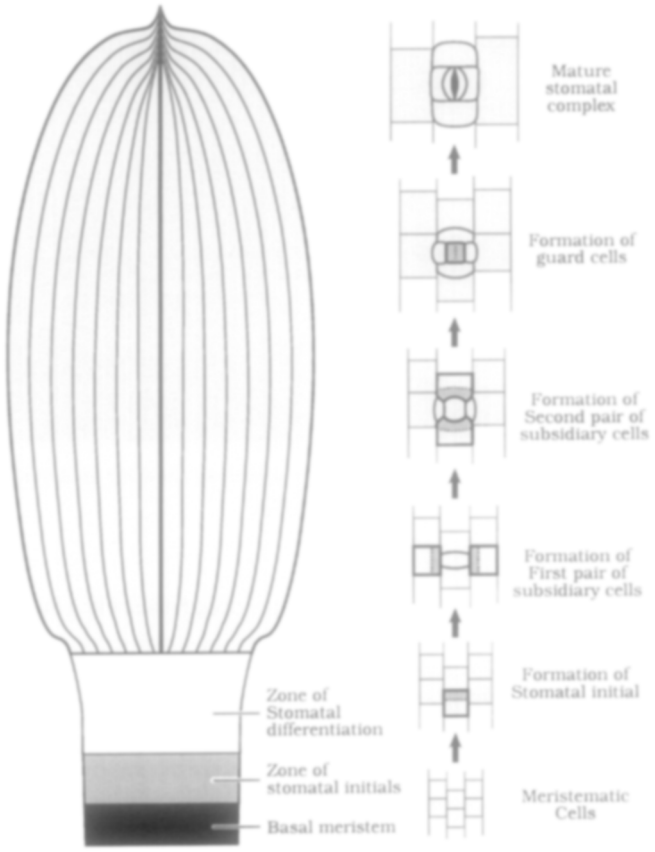

FIG. 2. Schematic diagrams (not to scale) showing the overall organization of the *Tradescantia* leaf (left) and the sequence of developmental events that result in the formation of a stomatal complex (right). The events depicted in this developmental series appear relative to their occurrence in the immature region of the leaf. The lower side of each diagram is proximal and the upper side is distal. The series begins with three files of meristematic cells (vertically oriented) found in the basal meristem region. The remaining events of the series take place in the two zones above the meristem, the zone of stomatal initial origin and the zone of stomatal differentiation. Stomata complete their expansion in the region distal to the zone of stomatal differentiation. The basic sequence of events is as follows: a stomatal initial is formed by an unequal division at the distal end of the meristematic cell; the first pair of subsidiary cells is formed by unequal divisions in adjacent cell files; the next pair of subsidiary cells is created by unequal divisions from cells above and below the initial in the same cell file; the stomatal initial then divides into guard cells; and cell expansion results in a mature stomatal complex.

Stomatal development proceeded rapidly after the stomatal initial appeared. All stages were found within a short distance above the basal meristem. Young, developing stomata were not found among mature complexes in the fully expanded portion of the leaf. To provide a quantitative measure of this observation, stomatal frequency on a per cell basis was determined. In immature leaf regions the frequency of stomatal initials was 0.194, while in mature regions of the same leaves the frequency of stomatal complexes was 0.180. These values

TABLE 1
STOMATAL FREQUENCY[a] IN DEVELOPING *TRADESCANTIA* LEAVES

Leaf region and cell type	Stomatal number per cell $\bar{x}$ (SD)	N
Immature		
Stomatal initials	0.194 (0.022)	10
Mature		
Stomata	0.180 (0.026)	10
Stomata and arrested cells	0.187 (0.022)	10

[a] Frequencies of stomatal initials, stomata, and stomata plus arrested cells were calculated from replicas of 10 leaves. Stomatal initials were counted as stomata in immature leaf regions, while each stomatal complex was counted as a single stoma in the mature leaf regions. These frequencies were compared with paired t tests and found to be not significantly different at a 95% confidence level.

are not significantly different from one another at the 95% confidence level (Table 1).

When the stomatal pattern in one dimension (linear) is evaluated, stomatal initials occurred with a minimum spacing of 9 μm and developed stomata with a minimum spacing of 33 μm. These distances were similar to the average cell diameter in immature (10 $\pm$ 3 μm) and mature (40 $\pm$ 20 μm) areas of the leaves.

While one cell was the minimum separation, the number of epidermal cells occurring between stomata, either initials or mature complexes, varied from one to nine (Fig. 3). Approximately 70% of adjacent stomata were separated by one or two cells, the remaining were separated by three to nine cells (Fig. 3). A χ^2 test showed that the frequency distributions in the young and mature

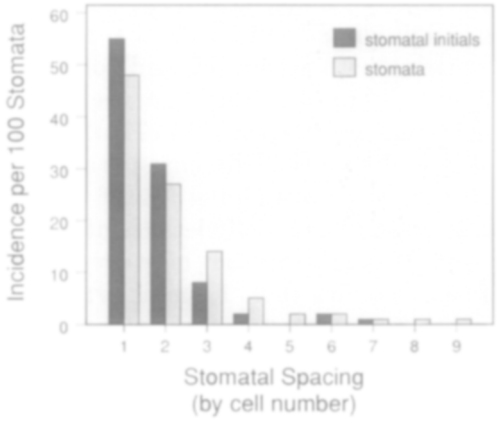

FIG. 3. The frequency distribution of the number of epidermal cells that separate pairs of either stomatal initials or stomata. A χ^2 test shows no evidence to reject a common distribution for the two cell types ($P = 0.14$). Since there were limited data for the spacing classes of six or more epidermal cells, these data were pooled and treated as a single class.

leaf regions were not significantly different from one another. Neither distribution fits a truncated Poisson distribution (a random distribution, but without a "zero-cell class") or a Poisson distribution assuming a minimum stomatal separation of one epidermal cell ($P < 0.001$). (P = probability that the observed data could have occurred by chance if the null hypothesis of randomness were true. Typically, $P < 0.05$ is viewed as significant evidence to reject the null hypothesis in favor of a regular pattern; $P < 0.001$ signifies very strong evidence to that effect). In fact, there were many more instances of only one epidermal cell between stomata or stomatal initials than one would expect by chance.

Stomatal locations along the length of cell files (Fig. 1) appeared to be neither perfectly regular nor random. In fact, stomata sometimes appeared to occur in series (Fig. 1, lower set of stars). In other cases, stomata were widely separated by series of epidermal cells (Fig. 1, upper set of stars). Using data taken from one entire blade panel, the sequence of stomatal spacing was analyzed by categorical data methods. First, we determined the distribution of stomatal separations following stomata separated by a single epidermal cell. We found evidence that this did not follow a truncated Poisson distribution, having more short sequences and more very long sequences than expected ($P < 0.001$).

We then examined the lengths of series of stomata separated by single epidermal cells. This should follow a geometric distribution if there is no interaction between or among stomata along a file. However, we found more long series (five or six consecutive stomata with single cell separations) than expected ($P = 0.026$), indicating that stomata separated by single cells occurred in longer sequences than would be expected by chance.

When we considered stomatal distribution in the second dimension, across leaf width, the pattern was ordered into longitudinal tracts, some that contained stomata alternating with others that did not (Fig. 1). The number of cell files in each tract from the midrib to the margin of the leaf was measured in 10 leaf replicas. The epidermal cells atop the midrib consisted of a wide band of rows free of stomata, ranging from 6 to 15 rows of cells (Fig. 1, large arrow). Cell files free of stomata also occurred in the blade panel, but only over the longitudinal vascular bundles (Fig. 1, small arrow). Each of these stomata-free tracts consisted of 1 to 4 cell files. The number of adjacent cell files containing stomata varied between 2 and 18. Within a given leaf there was no obvious periodicity in the number of cell files with and without stomata proceeding from the middle to the leaf edge. Paired t-tests of these data demonstrated that only tracts without stomata contained similar numbers of cell files. Tracts containing stomata did not have a consistent number of cell files, but the number of cell files per tract tended to decrease progressively toward the leaf edge.

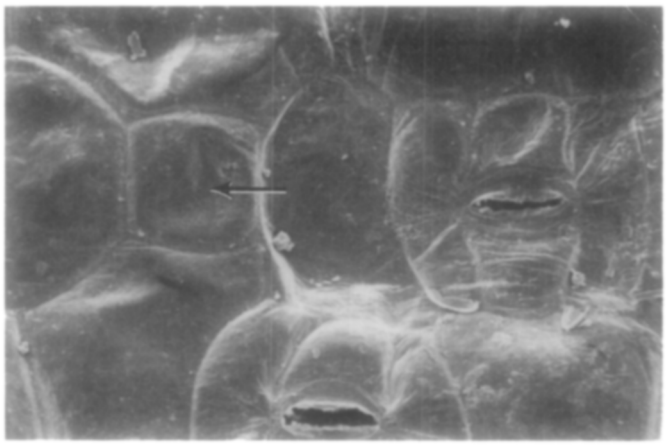

FIG. 4. A mature area of a *Tradescantia* leaf showing an arrested cell (arrow) and a mature, ablated stoma.

Although no developing stomata were found in mature areas of *Tradescantia* leaves, there were cells present that resembled the stomatal initials of the immature leaf region (Fig. 4). The cells were considered to have arrested development beyond the formation of the stomatal initial. The arrested cells were smaller than adjacent epidermal cells (Fig. 4) and constituted about 5% of the stomata we counted (60/1100). Although these cells were originally detected based on size and shape, a series of quantitative measurements was done to validate their designation as arrested stomatal initials.

Stomatal initials and arrested stomatal initials were thought to have formed at the same time. This is supported by frequency measurements that demonstrated stomatal initials formed only in the leaf base region. To test the origin of the arrested cells, the mature stomatal frequency was recalculated to include the arrested cells as stomata and compared to the stomatal initial frequency (Table 1). The frequency of mature and arrested stomatal cells together (0.187) was slightly less than that of the stomatal initials (0.194), but the difference was not significant at the 95% confidence level from the earlier measurements. This supports the assertion that arrested cells were patterned as stomatal initials.

We also compared the two-dimensional distribution of arrested cells to that of differentiated stomata. We assumed that both would have a similar distribution if each had been formed as stomatal initials. The distance of stomata and of arrested cells to a reference stoma was measured in mature leaf areas. Probability plots of these data showed that each sample had a comparable, nonrandom distribution (Fig. 5; Gnanadesikan, 1977, Chap. 6). Arrested cell and stomatal distributions were not different at the 90% level of confidence as shown by

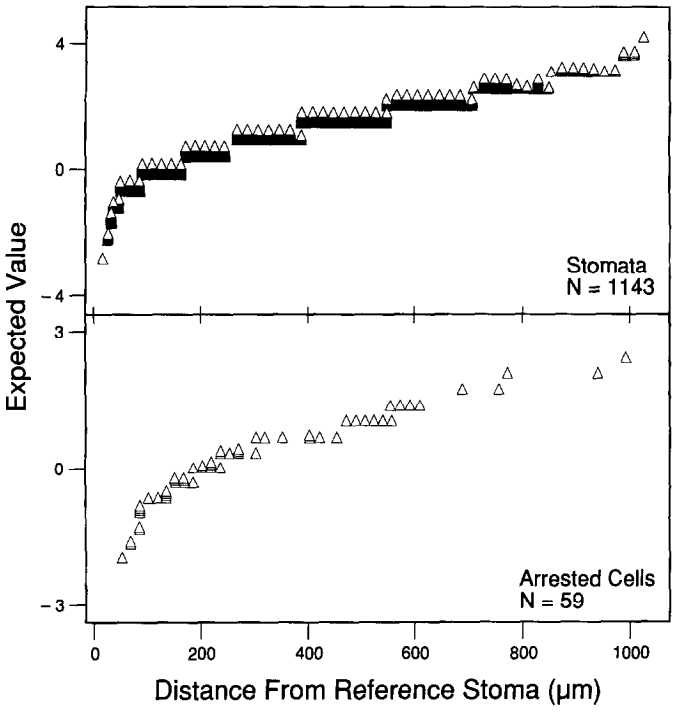

FIG. 5. Normal probability plots of developed stomata and arrested cells as a function of distance from a randomly selected reference stoma.

a Kolmogorov-Smirnov test. A quantile-quantile plot of these data compared the stomata and arrested cell distances by percentiles (Fig. 6). In Fig. 6, the quantiles lie mostly along a straight line, indicating that the two-dimensional distributions of the arrested cells and mature stomata were similar.

Additionally, the positional relationships between arrested cells and stomata were compared through a series of measurements. The average distance from an arrested cell to the nearest stoma in an adjacent row (64 μm) was less than the comparable distance between differentiated stomata (93 μm). These distances are significantly different at the 99% confidence level. The nearest stomata were evenly distributed between the proximal and distal directions for both arrested and developed stomata.

Within cell files, measurements were made to examine the relative distance between a centrally located stomatal complex or arrested cell and the nearest proximal and distal stomata. Analysis was performed on log values of the distance measurements to stabilize the variance; however, similar results were found on the natural scale. Stomatal complexes and arrested cells had positions significantly different from one another ($P < 0.001$) with respect to the nearest stomatal pair within the row. Stomatal complexes have no consistent position between the stomatal pair ($P = 0.16$), but arrested cells do ($P < 0.001$). The average position of an arrested cell between the nearest proximal and distal stomata was not significantly different from the midway point between such a stomatal pair ($P = 0.21$). Regardless of whether an arrested cell or stoma was in the central position, the total distance between the proximal and the distal stomata was approximately the same, 280 μm.

To assess cell communication and its influence on stomatal patterning, *Tradescantia* stomata in all stages of development were laser ablated. We began with the earliest recognizable stage, the stomatal initial. Puncturing of cells by laser microbeam (Fig. 7A) initially caused the loss of cytoplasm (Fig. 7B) and the collapse of the cell wall (Figs. 7C-7D). The ablated cells were easily identified several days later, but the killing of stomatal cells did not result in division of neighboring cells to compensate for the ablated cells (Fig. 7D). Occasionally, expansion throughout the leaf was altered following irradiation and epinastic curvature of the entire leaf resulted. Examination of samples allowed to grow up to 42 days following ablation showed no division in the irradiated or adjacent cell files. However, ablation did not prevent cell division associated with stomatal development in nearby, untreated rows.

The physical configuration of the laser microscope frequently prevented access to the most basal regions of the leaf. In a few samples, however, cells in the meristematic region were ablated. SEM of these samples 1 week after ablation also showed no evidence of compensatory division.

DISCUSSION

The cell lineage theory explains stomatal patterning as wholly the result of ordered series of divisions. We

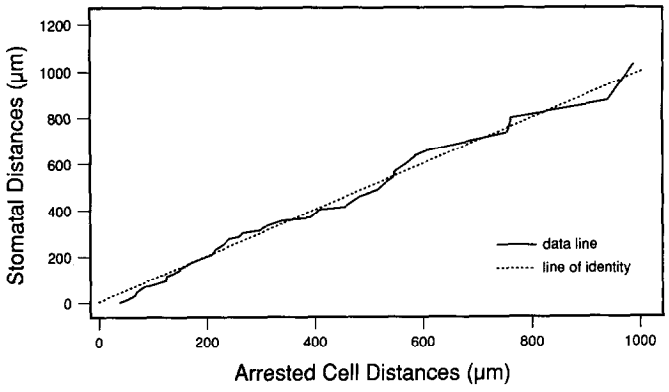

FIG. 6. A quantile-quantile plot of the stomata and arrested cell distances in two dimensions to a reference stoma by percentiles. The line of identity serves as a reference to indicate where the data sets are the same. A Kolmogorov-Smirnov test of the two data sets demonstrated that the distributions were not significantly different at the 90% confidence level.

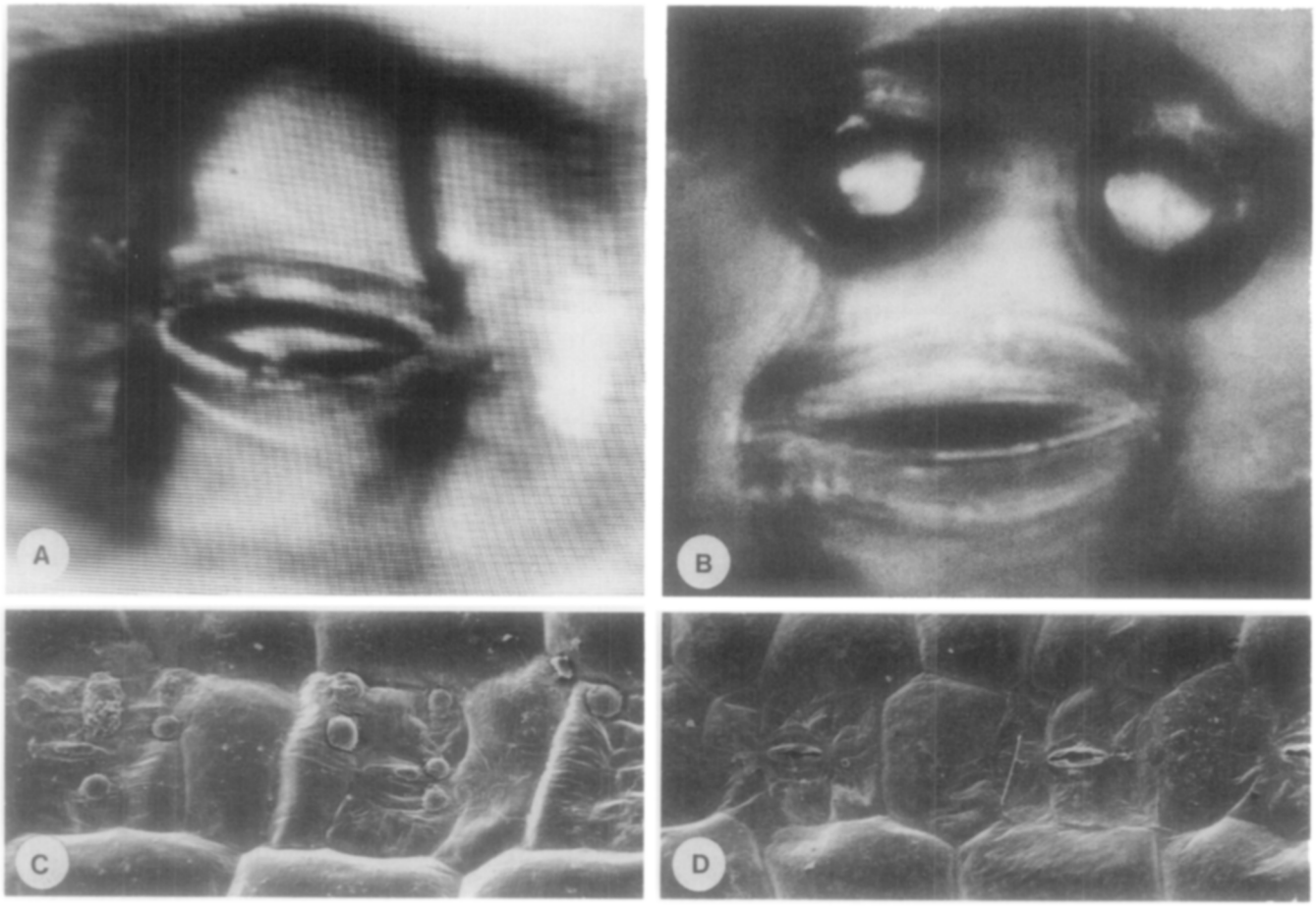

FIG. 7. Videotape and SEM images of developing stomatal cells taken after ablation. Video images are blurred because of the depth of field limitations of the 40X water immersion lens used on the contoured leaf surface. Raster lines are visible in A because the videotape was replayed to a monitor and photographed from the screen by pausing the tape. No raster lines are present in B because it was produced from an internegative taken from a positive slide produced directly from the videotape. The recording system used one-quarter inch videotape. (7A) A mature stoma just irradiated in the lower guard cell (dark area on the left of this cell). (B) An ablated stoma with cytoplasm exuding through the subsidiary cell walls just after ablation. (C) A series of stomata immediately after ablation with spheres of cytoplasm on the outer walls which have collapsed. (D) The epidermal surface near the stomata 30 days after ablation.

tested the validity of this theory for *Tradescantia* by observation, measurement, and experimentation. We found that the theory explains the origin of individual stomata, but not the sequential placement of stomata in cell files nor the position of stomata across the leaf width.

At the first level of organization, the origin of individual stomatal initials, the pattern of *Tradescantia* stomata was consistent with the cell lineage theory. The divisions that produced the initials were highly regular and their origin was determined by the pattern of divisions. The predictive power of stomatal placement in this species is restricted, however, because only a single division preceded the origin of the stomatal initial, rather than a set of divisions.

Initial origin was found near the leaf base and occurred only while the cells traversed this zone. Observation of the epidermis along the entire leaf length and the quantitative measures of pattern support this conclusion. The measures included stomatal frequency and stomatal spacing, both of which were the same in immature and mature leaf regions. The constancy of the pattern values also showed that stomatal spacing is not adjusted by additional epidermal cell divisions after the specialized cells originated.

Although only a single division consistently preceded the formation of the stomatal initials, development continued with an additional five divisions which were highly regular. Four of these divisions form the subsidiary cells and the last division produces the guard cells. However, it is not these divisions associated with stomatal development that the cell lineage theory addresses, but only those divisions giving rise to the stomatal initial. Further development of the stomatal complex in *Tradescantia* was like that previously reported by Campbell (1881) and in *Rhoeo* by Stebbins and Jain (1960). However, in *Tradescantia* subsidiary cells form before the stomatal initial divides, while in *Rhoeo*

guard cells may form in as many as 50% of the stomatal complexes while the second set of subsidiary cell divisions is still taking place.

The pattern at the next level of organization, along cell files, was not in accord with the basic tenet of the cell lineage theory, that is, regular sets of divisions. The linear distribution of stomata was not perfectly regular nor was it random. The actual number of cells between stomatal pairs varied between one and nine and their frequencies were not random. The sequential spacing of stomata separated by single epidermal cells also was not random. There were more long and short sequences of stomata separated by single cells than expected by chance alone. Long sequences have been reported also in *Chlorophytum, Galanthus,* and *Schizostylis* (Charlton, 1988). In these species, the probability of a new stoma forming with a single epidermal cell increases with the number of stomata already in series (Charlton, 1988).

Since the cell lineage theory proposes sets of divisions, resulting in highly ordered placement of stomata when the sets repeat, the theory cannot account for the linear distribution of *Tradescantia* stomata separated by different numbers of epidermal cells. Only if one allows the patterning mechanism of the cell lineage theory to be interrupted by nondividing, nonpatterned epidermal cells could the theory account for the linear distribution of stomata.

The pattern across the width of the blade also did not meet the basic principle of the cell lineage theory. In two dimensions, stomata were not present in a checkerboard pattern. Instead stomata-containing cell files alternated with stomata-free files from the midrib to the margin of the leaf. Even in tracts containing cell files with stomata, the stomata were not regularly ordered in two dimensions. There was no apparent periodicity to the number of cell files with and without stomata. Although all cells originate from the meristem at the leaf base, the resulting cell files do not have the same potential for stomatal patterning. Files free of stomata, which only overlie vascular bundles, might result from interactions between cell files and tissues (epidermis, mesophyll, vascular tissue). It is also possible that a patterning event has already taken place to divide the cell files into those that will contain stomata and those that will not.

The two-dimensional placement of stomata in *Tradescantia* is at odds with data from *Crinum* in which, beyond a minimum distance of one epidermal cell, stomata in a two-dimensional field occur by chance (Sachs, 1974). However, the evidence—the uniformity of stomatal frequency at selected distances from a reference stoma—is not a statistical test of randomness. The stomatal frequency curves are consistent with spatial patterns described as "soft-core" models (Ripley, 1987) or a simple inhibition process (Matérn, 1960; Diggle, 1981). Rasmussen (1986) reported similar results in *Anemarrhena asphodeloides* using the same methods. We found no evidence of randomness in stomatal pattern along the length or the width of *Tradescantia* leaves. Neither the frequency of stomatal spacing in one dimension nor the occurrence of stomatal spacings in series follow Poisson distributions.

From observation and measurements of stomatal pattern in *Tradescantia* we cannot detect evidence of a patterning mechanism that results only from sets of cell divisions. If there is a lineage of divisions responsible for stomatal pattern, it would have to be a progressive, changing lineage, such as those described by Lindenmayer systems (Prusinkiewicz and Hanan, 1989) or by triangular lattices of cellular automata (Cocho *et al.*, 1987).

The pattern of *Tradescantia* stomata along leaf length and width is enigmatic because sometimes stomata occur in groups, e.g., the long series of stomata separated by single epidermal cells and the tracts of cell files containing stomata, and other times they occur more widely spaced, e.g., stomata separated by as many as nine epidermal cells and tracts free of stomata. Interactions of cells and interior tissues may influence the patterning process. The presence of stomatal files only at the juncture between two hypodermal files in the Pandanaceae (Tomlinson, 1965; Pant and Kidwai, 1966) is an indication that the leaf interior may regulate cell types produced in the epidermis. In *A. asphodeloides* leaves, the papilla-containing cell files never bear stomata and nearly always are separated from them by at least one cell file of epidermal cells, evidence that short-range interactions affect cell type. Epidermal cell length also is greater when papilla cell files are adjacent. Thus, in this species too, there is evidence of regulated stomatal placement beyond their minimum separation (Rasmussen, 1986). Nevertheless, in chimeric tomato plants made from genetic stocks with different stomatal frequencies (Szymkowiak, 1990), the interior leaf tissue does not influence stomatal patterning by a change in frequency. However, in a graft between potato and tomato (Heichel and Anagnostakis, 1978), stomatal response to light was controlled by the genetic stock of the interior tissue, although stomatal number was not.

We attempted to learn if patterning might be influenced by communication between cells of the epidermal layer in a laser microbeam study. Ablation of linear files of stomata in all stages of development did not result in new cell division. No new stomata appeared, either in the irradiated file or in adjacent files of cells. The normal signals responsible for stomatal patterning may be active only very close to the basal meristem and thus below (proximal to) the region ablated or the cells may no longer be competent to respond. Under the con-

ditions employed, cell ablation does not reactivate patterning nor do any compensatory divisions occur.

Cells arrested as stomatal initials represent another example of regulation. These cells were not committed when first present and their development was arrested at the initial stage. Their position within files, which was approximately midway between the proximal and the distal stomata, indicates no obvious cell–cell interaction that would check their development. However, the arrested cells were closer to stomata in adjacent files than were the developed stomata. Halting stomatal development in monocots and dicots is a common means of modulating stomatal number and in some cases pattern. In certain dicotyledons (Bünning and Sagromsky, 1948), inhibition from developing stomata is thought to prevent the formation of new initials nearby and therefore affects the patterning process. On the other hand, reversion of stomatal precursors to epidermal cells, which has been reported in pea (Sachs, 1978), interferes with the continuation of stomatal development rather than affecting the process of patterning. The means of change (inhibition, cell reversion, and developmental arrest) involve only the development of stomatal cells, rather than a change in the epidermal cells. If agents of arrest operate because stomata would be too close if all completed their development, as has been suggested, it is curious that additional division in epidermal cells does not occur to modify the spacing of existing stomata. Physical constraints associated with a growing leaf may prevent this means of adjusting stomatal spacing.

We conclude that the cell lineage theory (Bünning and Sagromsky, 1948) accounts for the origin of stomatal pattern at the level of individual initials and the minimum spacing between stomata in *Tradescantia* leaves. However, it does not account for the origin of successive stomata within cell files because stomata are separated by a variable number of cells. Ordinary epidermal cells occur between the meristematic cells that generate stomatal initials. Sometimes as many as nine cells separate a stomal pair and in other cases stomata separated by single epidermal cells occur in series longer than expected by chance alone. Therefore, repeating sets of orderly divisions do not wholly determine pattern in one dimension. Nor does the theory account for the distribution of stomata in the second dimension, across the leaf. Although tracts of cell files containing stomata and devoid of stomata alternate with one another from the midrib to the leaf margin, the recurrence has no regular periodicity.

Series of epidermal cells and of stomata separated by single epidermal cells may result from a mechanism suggested for the sequential series of stomata in *Chlorophytum* (Charlton, 1990). In this case, the formation involves sister cells and their position in the cell division cycle. Since sister cells maintain a degree of cell synchrony for several division cycles (Webster, 1979), groups of sister cells near mitosis could proceed through the zone of stomatal formation, divide unequally, and produce a series of stomata. Thus, a linear series of stomata may result from a single lineage of cells. Similarly, a group of contiguous cells in an earlier phase of the cell cycle would not form any stomatal initials as they were displaced through the zone.

The linear sequence of stomata and epidermal cells in *Tradescantia* also might be explained by this mechanism. In keeping with this mechanism, the maximum length of the epidermal cell and the stomatal sequences is about the same. Each type of sequence would have consisted of the same number of sister cells in the lineage before cell programming occurred. Such a mechanism would also explain by a single means the dilemma of understanding how stomata sometimes seem to occur close together and other times seem to be widely separated. Thus, the linear distribution of stomata may result from a progress zone type of pattern formation (Wolpert, 1984) that operates in concert with the cell cycle.

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