The Plant Cell, Vol. 3, 877-892, September 1991 © 1991 American Society of Plant Physiologists

A Mutation in the Arabidopsis *TFL1* Gene Affects Inflorescence Meristem Development

Susan Shannon and D. Ry Meeks-Wagner[1]

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

We present the initial phenotypic characterization of an Arabidopsis mutation, *terminal flower 1-1* (*tfl1-1*), that identifies a new genetic locus, *TFL1*. The *tfl1-1* mutation causes early flowering and limits the development of the normally indeterminate inflorescence by promoting the formation of a terminal floral meristem. Inflorescence development in mutant plants often terminates with a compound floral structure consisting of the terminal flower and one or two subtending lateral flowers. The distal-most flowers frequently contain chimeric floral organs. Light microscopic examination shows no structural aberrations in the vegetative meristem or in the inflorescence meristem before the formation of floral buttresses. The wild-type appearance of lateral flowers and observations of double mutant combinations of *tfl1-1* with the floral morphogenesis mutations *apetala 1-1* (*ap1-1*), *ap2-1*, and *agamous* (*ag*) suggest that the *tfl1-1* mutation does not affect normal floral meristems. Secondary flower formation usually associated with the *ap1-1* mutation is suppressed in the terminal flower, but not in the lateral flowers, of *tfl1-1 ap1-1* double mutants. Our results suggest that *tfl1-1* perturbs the establishment and maintenance of the inflorescence meristem. The mutation lies on the top arm of chromosome 5 approximately 2.8 centimorgans from the restriction fragment length polymorphism marker 217.

INTRODUCTION

In angiosperms, the floral transition marks the progression from vegetative to reproductive growth, and thus represents a major change in plant development. This transition is regulated by both inhibitory and promotive graft-transmissible factors that are produced in the leaves and roots and transported to the shoot apical meristem (Evans, 1960; Lang et al., 1977); the arrival of these substances at the shoot apex is correlated with the establishment of the inflorescence meristem and its new developmental patterns. Single gene mutations that affect either the signaling process or the response of the apical meristem have been described in several plant species. For example, in pea the production of floral signals involves the *sterile nodes* (*Sn*) and *day neutral* (*Dne*) gene products, whereas the *vegetative* (*veg*) and *late flowering* (*Lf*) gene products influence the sensitivity, or responsiveness, of the apical meristem to these signals (see Murfet, 1990; Poethig, 1990). The signal/response mechanism that regulates the floral transition provides a situation well suited for a molecular analysis of shoot apical meristem function.

The cellular organization of the shoot apical meristem may provide important clues for understanding meristem function. Typically, vegetative meristems have an apical central zone composed of enlarged, slowly dividing cells and a flanking peripheral zone that contains smaller, more rapidly cycling cells. This zonation coincides with the restriction of function to different meristematic regions, with the central zone containing semipermanent initial cells (apical initials) and their recent derivatives (see Wardlaw, 1965; Steeves and Sussex, 1989) and the peripheral zone encompassing the site of organogenesis. Experimental evidence for the existence of semipermanent apical initials comes from analyzing the size, frequency, and persistence of clonal sectors in plants (Stewart and Derman, 1970; Ruth et al., 1985). The occurrence of sectors that span large regions of the shoot (Stewart and Derman, 1970; Jegla and Sussex, 1989), including the inflorescence, suggests that a small number of central zone meristematic cells serve as initials, or stem cells, for shoot development. The presence of apical initial cells is consistent with the reiterative pattern of organogenesis displayed by indeterminate vegetative meristems. Cells derived from these centrally located initials replenish the organogenic peripheral zone of the meristem where cells are being recruited into developing organ primordia (see Steeves and Sussex, 1989).

In many plant species the transition from the vegetative phase to the inflorescence phase is associated with an increase in the mitotic activity of the central zone cells. The entire meristem becomes nearly uniform with regard to cell cycle times (Corson, 1969; Steeves et al., 1969; Bodson, 1975), eliminating the zonation observed in

[1] To whom correspondence should be addressed.

vegetative meristems. The floral transition is also marked by significant changes in cellular metabolism and gene expression. There is an increase in cellular RNA and protein content, particularly in the central zone of the meristem (Bernier et al., 1981), and new patterns of meristem-specific gene expression are observed (Kelly et al., 1990; Melzer et al., 1990). These changes in the organization and activity of the shoot apical meristem are likely to be important for establishing and maintaining the developmental potential of the inflorescence meristem.

The developmental potential of the inflorescence meristem varies greatly among species. In plants that produce a determinate inflorescence, such as tobacco, the inflorescence stage may be short lived, and the shoot apical meristem is transformed into a terminal floral meristem. But in plants producing an indeterminate inflorescence, such as Arabidopsis, the inflorescence meristem may reestablish the cellular organization observed in the vegetative meristem and display a reiterative developmental strategy.

Our genetic analysis of the floral transition has focused on the establishment and maintenance of the inflorescence meristem of Arabidopsis. The Arabidopsis vegetative meristem has a typical central zone/peripheral zone organization. After the floral transition is complete, the central zone is reestablished in the inflorescence meristem (Besnard-Wibaut, 1970). The existence of a central zone of apical initial cells may be crucial for the regenerative functioning of Arabidopsis shoot meristems during the inflorescence phase of development. Initially, during the inflorescence phase, the shoot apical meristem gives rise to stem, cauline leaves, and lateral branches. However, as inflorescence development progresses, the developmental potential of the shoot apical meristem becomes more restricted and the inflorescence meristem gives rise only to stem and flower primordia. Eventually, the inflorescence terminates with the senescence of the shoot apical meristem.

We reasoned that the response of the meristem to the floral signals is controlled by a mechanism(s) identifiable by mutations that alter the timing of the floral transition. Such mutations would be heterochronic in nature (Needham, 1933; Gould, 1977; Raff and Wray, 1989), and thus are likely to dissociate important developmental processes regulating the initiation of flowering. Some of these mutations may represent spatially limited heterochronies (Raff and Wray, 1989) that affect the response of the shoot apical meristem to floral signals and result in abnormal inflorescence development. The study of such mutations may reveal how the temporal and spatial aspects of meristem activity are linked during the floral transition.

To identify genes involved in the coordination of meristem activity during the floral transition, we screened an M2 population derived from ethylmethane sulfonate (EMS)-mutagenized Arabidopsis ecotype Columbia for early flowering mutants, and then analyzed these mutants for defects in inflorescence development. This paper presents our initial characterization of one such early flowering mutation, *terminal flower 1-1* (*tfl1-1*), that identifies a new genetic locus, *TFL1*. Inflorescence development in plants homozygous for the *tfl1-1* mutation is limited by the production of a terminal floral meristem. We have determined that *tfl1-1* is allelic with a mutation (*tfl1-2*) isolated independently by David Smyth, Monash University, Melbourne, Australia (J. Alvarez, C. Guli, X.-H. Yu, and D. Smyth, manuscript in preparation).

RESULTS

Wild-Type Inflorescence Development

When grown during long-day (LD; 16-hr light/8-hr dark) photoperiods, the primary inflorescence of wild-type Columbia plants is first visible to the unaided eye 22 to 26 days after sowing. During the early stages of inflorescence development, the apical meristem initiates several lateral branches (i.e., paraclades) (Weberling, 1989), each subtended by a cauline leaf. Later, lateral branches are no longer produced and solitary flowers are formed. This distal region of solitary flowers is called the main florescence (Weberling, 1989). Floral development ceases after the production of a small number of aborted flowers and the arrest of florescence meristem activity; the wild-type raceme does not produce a terminal flower. Before the cessation of growth, the main florescence will have produced 33 (±5.6) solitary flowers.

The lateral branches reiterate the growth pattern of the main axis, producing secondary branches (secondary paraclades) as well as a distal region of solitary flowers, termed the coflorescence (Weberling, 1989). Because of this similarity in developmental potential, we will use the general term "inflorescence meristem" to refer to both the shoot apical meristem and the lateral branch meristems during all stages of inflorescence development.

Isolation of the *tfl1-1* Mutation

A screen for early flowering mutants was conducted during the summer on a greenhouse-grown M2 population of EMS-mutagenized seed of ecotype Columbia. Of approximately 20,000 plants screened, four early flowering variants were isolated. The heritability of the early flowering phenotype was confirmed for each isolate by comparing flowering time in progeny and wild-type populations during subsequent backcrosses with the wild type. Three of the four variants, *early flowering 1*, 2, and 3(*elf1*, *elf2*, and *elf3*), showed no apparent abnormalities in inflorescence morphology, but the fourth, *tfl1-1*, exhibited a severely truncated inflorescence. Figure 1A shows a wild-type plant and a representative from each of the two morphological

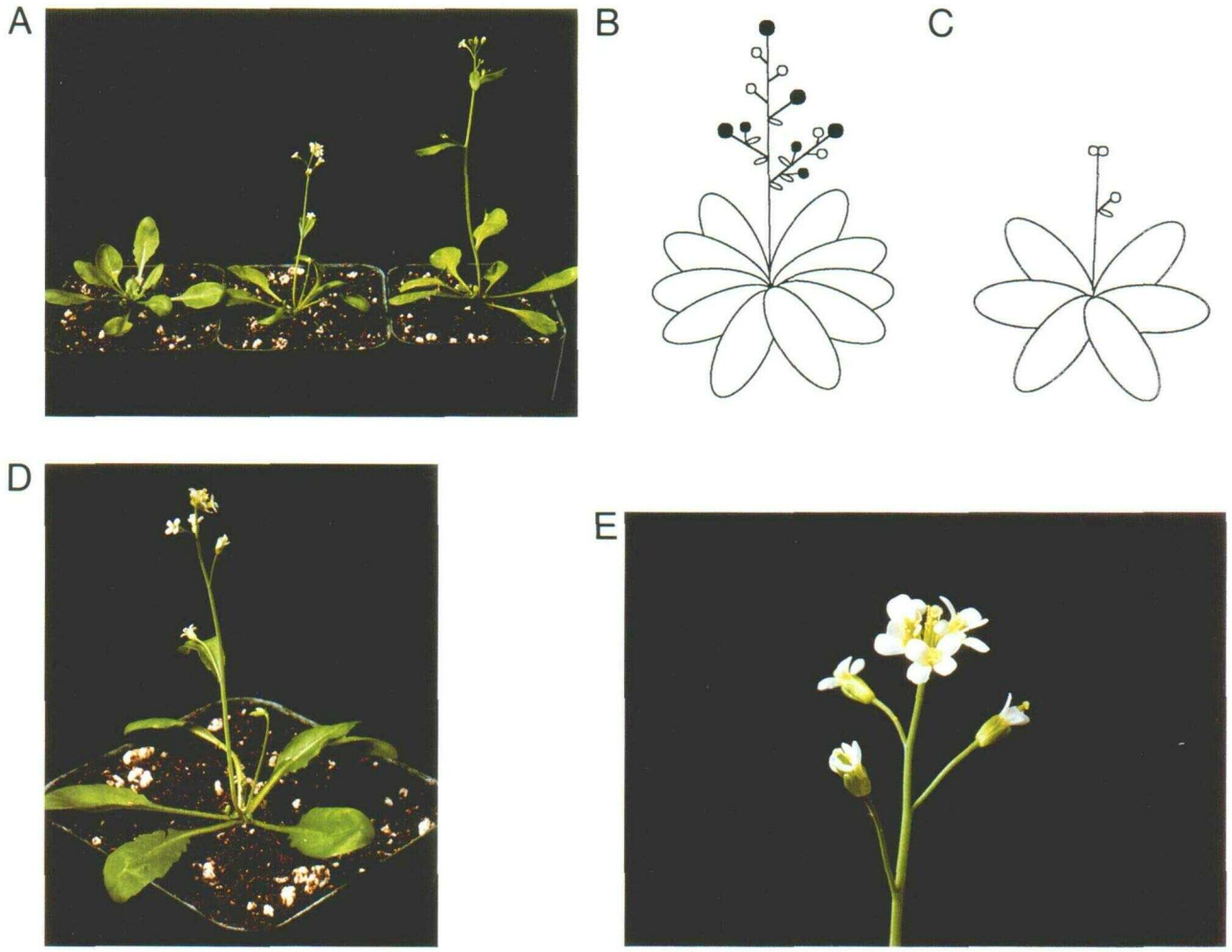

Figure 1. Comparison of Wild-Type and *tfl1-1* Mutant Morphology.

(A) Photograph showing (from left to right) wild-type, *tfl1-1*, and *elf1* plants of the same age. The wild-type plant has not yet bolted.
(B) Diagram of the morphology of a wild-type Arabidopsis plant grown during LD photoperiods. Open circles, floral meristems; closed circles, inflorescence meristems.
(C) Diagram of a *tfl1-1* homozygote grown under identical LD conditions. The adjoined circles represent the flowers of the terminal floral structure.
(D) Photograph of a typical *tfl1-1* homozygote. A lateral branch that terminates in a single flower can be seen on the left of the primary inflorescence.
(E) Photograph of the terminal floral structure of the *tfl1-1* plant shown in **(D)**.

classes of early flowering mutants. Wild-type and *tfl1-1* inflorescence morphology are schematically illustrated in Figures 1B and 1C, respectively.

The regenerative capacity of the *tfl1-1* inflorescence meristem was severely compromised; the mature inflorescence of LD-grown homozygous mutant plants bore from one to five flowers, ending in a terminal floral structure that frequently appeared to be the fusion of two or three flowers (Figures 1C, 1D, and 1E). Occasionally, plants produced one or two lateral branches that terminated in a single flower (see Figures 1C and 1D). However, when grown during very long photoperiods (e.g., 18 or 24 hr of light), the inflorescence of homozygous *tfl1-1* mutant plants lacked lateral branches and distinct solitary lateral flowers. The plant shown in Figure 2A displays this extreme phenotype. The primary inflorescence of this plant produced only the terminal floral structure shown in Figure 2B. The two axillary inflorescences also failed to produce lateral branches and each terminated in a single flower (Figure 2A).

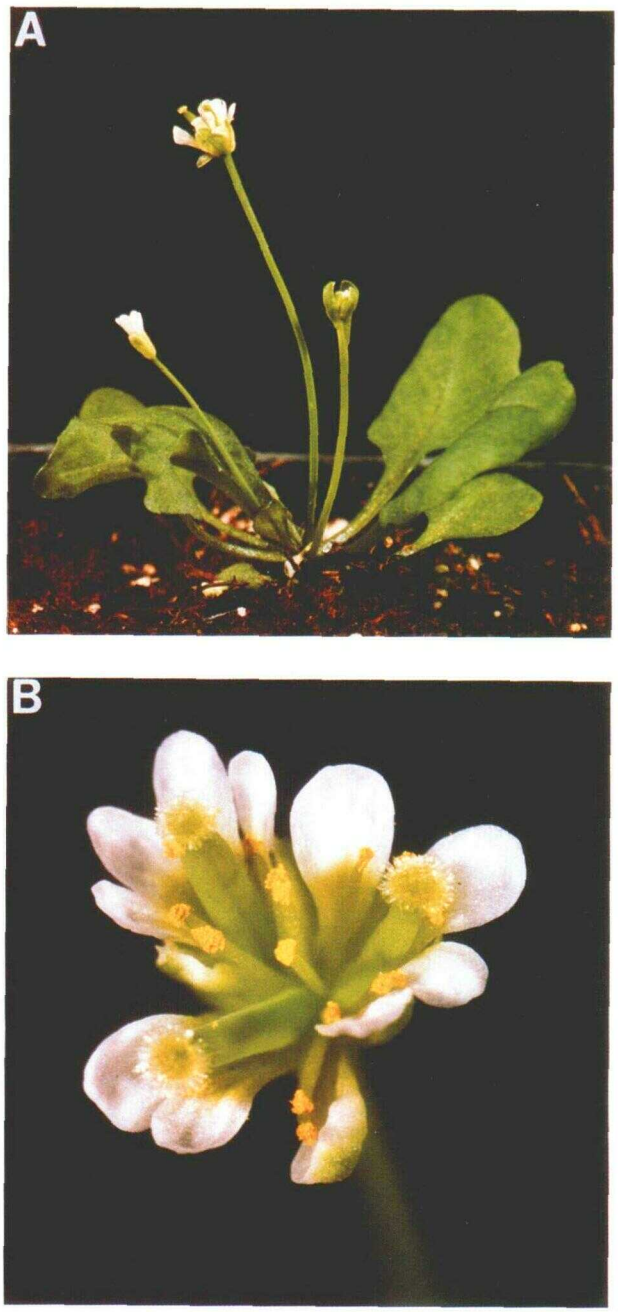

Figure 2. Extreme Phenotype Exhibited by a *tfl1-1* Plant Grown during Very Long Photoperiods (20-Hr Light/4-Hr Dark).

(A) A *tfl1-1* homozygote with a primary inflorescence and two axillary inflorescences that have produced no lateral branches or flowers.

(B) Close-up of the terminal floral structure on the primary inflorescence.

Many of the distal-most flowers on a *tfl1-1* plant contained a small number of completely transformed or, more frequently, chimeric floral organs. However, these aberrations occurred only in the terminal floral structures produced by the plant and were not present in the other flowers. Table 1 summarizes the floral organ transformations associated with the *tfl1-1* mutation. These data were obtained by observing an entire population of plants; any given flower exhibited only a subset of the possible spectrum of abnormalities.

The germination rate and viability of seed were unaffected by the *tfl1-1* mutation. In addition, as shown in Figure 3, the rates of leaf production in *tfl1-1* and wild-type plant populations were virtually identical. Vegetative morphology (i.e., rosette leaf shape and phyllotaxy) was also unaffected by the *tfl1-1* mutation. Despite the apparently normal pattern of vegetative development, Table 2 shows that *tfl1-1* homozygotes flowered prematurely, as measured both by time to flowering and by total leaf production before inflorescence formation. This acceleration relative to flowering in wild-type plants occurred in both LD and short-day (SD; 10-hr light/14-hr dark) photoperiods.

Segregation analysis and mapping data indicated that the *tfl1-1* mutation is confined to a single genetic locus. The wild-type allele of this locus has been designated *TFL1*. *tfl1-1* mapped to a distal region of chromosome 5, 57.5 ± 16.6 map units from *glabra 3* (*gl3*) and 31.4 ± 12.1 map units from *transparent testa*, *glabra* (*ttg*). This position was consistent with results from restriction fragment length polymorphism (RFLP) mapping that placed the locus 2.8 ± 1.1 map units from RFLP marker 217. The *tfl1-1* mutation failed to complement the *tfl1-2* mutation (J. Alvarez, C. Guli, X.-H. Yu, and D. Smyth, manuscript in preparation), indicating that these mutations are allelic.

The *tfl1-1* mutation was semidominant for the early flowering phenotype. Heterozygous *tfl1-1/TFL1* plants flowered at a time and with a leaf number intermediate

Table 1. Floral Organ Identity Conversion Found in the Flowers of the Terminal Floral Structure of *tfl1-1* Plants Grown during an LD Photoperiod

Nature of Floral Organ Transformations	
Sepal to petal	Complete organ conversions
Petal to sepal	
Sepal to petal	Partial organ conversions
Sepal to stamen	
Sepal to carpel	
Petal to sepal	
Petal to stamen	
Stamen to petal	
Stamen to carpel	

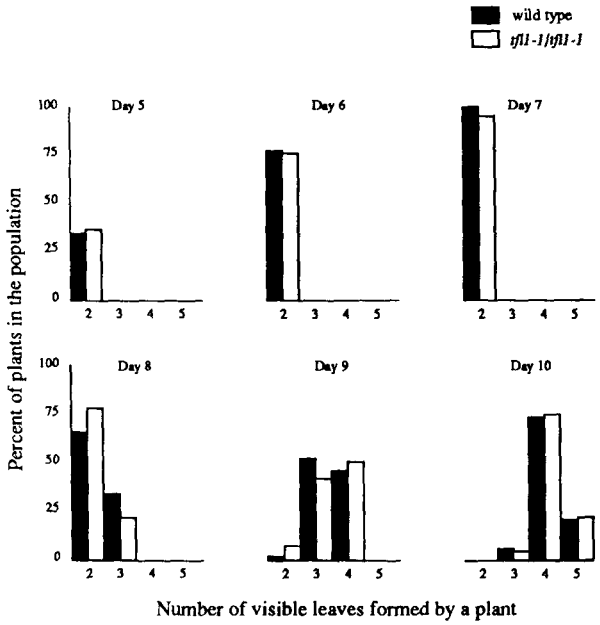

Figure 3. Comparison of Wild-Type and *tfl1-1* Vegetative Growth Rate.

Histogram showing leaf number in wild-type (n = 108) and *tfl1-1* (n = 106) populations on days 5 through 10 after sowing. Leaf number was determined blind to genotype using a ×10 magnifier. Day 5 was the first day that true leaves were visible in either population and day 10 was the last day that no flower primordia appeared in the *tfl1-1* population. The obvious similarity of the two populations is borne out by calculations of the average plastochron duration for the third and fourth leaves: 2.1 ± 0.4 days in the wild-type population and 2.0 ± 0.4 days in the *tfl1-1* population.

between wild-type plants and homozygous mutant plants. The inflorescence of heterozygous plants was morphologically indistinguishable from the wild type and did not generate a terminal flower. However, as a population, heterozygotes showed a 30% reduction in total flower production (data not shown).

The *tfl1-1* Mutation Causes the Production of a Terminal Flower

Using scanning electron microscopy (SEM), we compared the ontogeny of the wild-type and the *tfl1-1* inflorescence. All but the final stages of wild-type inflorescence development in Arabidopsis have been described previously (Smyth et al., 1990). In wild-type Columbia, floral buds emerge from the flanks of the inflorescence meristem and mature acropetally (Bowman et al., 1989). As shown in Figure 4A, the wild-type inflorescence phyllotaxy is helical; the angle between successive buds varies from 130 to 150°. Throughout inflorescence development, the central region of the meristem remains domed and apparently undifferentiated. The terminal dome of a fully developed wild-type inflorescence is shown in Figure 4B.

Figure 5 illustrates the stages of inflorescence development in LD-grown *tfl1-1* homozygotes. During the earliest stages of the inflorescence phase, the *tfl1-1* apical meristem is indistinguishable from the wild type; the terminus is domed and flower primordia emerge in the appropriate helical sequence (Figure 5A). Normal development can continue after the commencement of organogenesis in one or more of the lateral flower primordia (Figure 5B). At some point soon afterward, however, the surface structure of the inflorescence meristem deviates from the wild-type pattern. As shown in Figure 5C, the central region of the inflorescence meristem initiates organ primordia with the whorled phyllotaxy characteristic of an Arabidopsis flower. Figure 5D shows the terminal inflorescence of a *tfl1-1* homozygote at a slightly later developmental time point. Four lateral flowers have been initiated in their proper positions; however, the normally featureless central region of the inflorescence has produced floral organs and appears to be partially fused to an incomplete fifth lateral flower. In this case, both the terminal flower and the fifth lateral flower lie within a single, abnormal whorl of stamens.

As described below, certain environmental conditions, such as a shorter photoperiod or a lower temperature, can mitigate the severity of the inflorescence truncation in the mutant. In *tfl1-1* homozygotes that have been environmentally induced to produce an augmented inflorescence, normal development is prolonged and results in the production of a greater number of lateral flowers. Irrespective of growth conditions, the central region of all *tfl1-1*

Table 2. A Comparison of Flowering Time in Wild-Type and *tfl1-1* Homozygotes during Both LD and SD Photoperiods

Plants	Days to Visible Inflorescence	Number of Rosette Leaves at Time of Visible Inflorescence	Number of Plants in the Population
Wild type[a]	24	10.3 (±1.0)	19
tfl1-1/tfl1-1[a]	14	5.7 (±0.7)	12
Wild type[b]	56	43.2 (±4.6)	21
tfl1-1/tfl1-1[b]	44	26.4 (±3.0)	30

"Days to visible inflorescence" is defined as the time at which 50% of the population possessed flower primordia visible to the unaided eye. A leaf count was taken on the day flower primordia were first observed on a given plant.

[a] LD conditions.

[b] SD conditions.

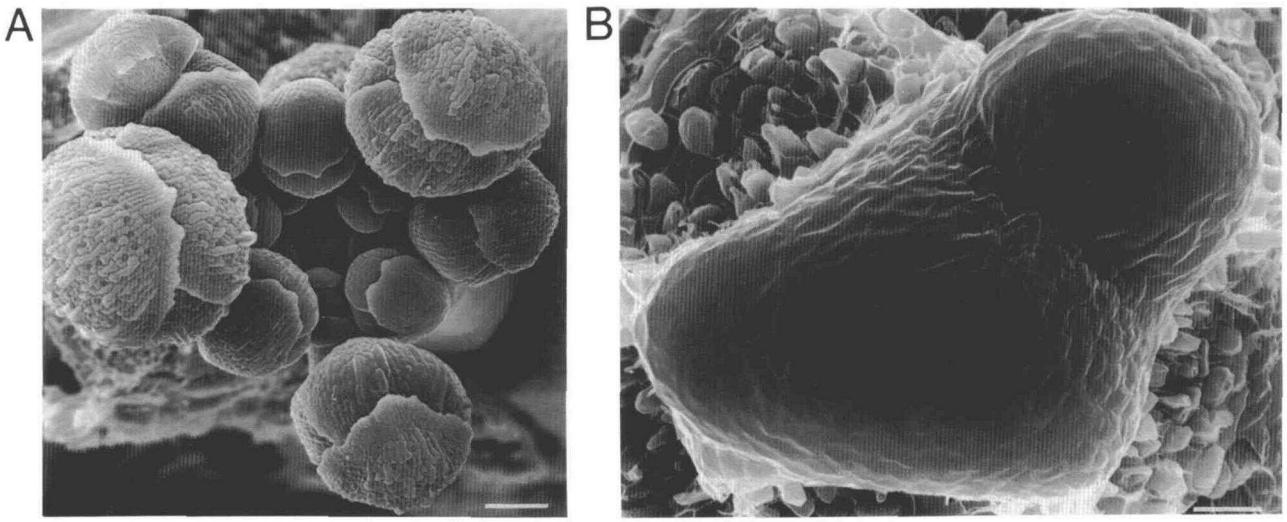

Figure 4. Selected Stages during Wild-Type Inflorescence Development.

(A) Vertical view of an actively growing wild-type inflorescence. Bar = 100 μm.
(B) Vertical view of a senescing wild-type inflorescence after all floral buds older than stage 2 (Smyth et al., 1990) had been removed. This plant had ceased to produce fertile flowers, and seed were ripening on the last fertile flower initiated by this meristem. Bar = 10 μm.

homozygotes eventually gives rise to a terminal flower (Figure 5E).

The *tfl1-1* terminal flower is often morphologically abnormal and frequently lacks complete whorls of sepals and petals. For example, the terminal flower of the plant shown in Figure 5E has only two organs in the first and second whorls. In this instance, closer examination showed them to be normal sepals. Quite often, however, sepals that are associated with the terminal flower exhibit a severe carpelloid transformation. Figures 5F and 5G show different magnifications of the terminal flower of a *tfl1-1* homogygote after the senescence of all noncarpelloid organs. The four organs surrounding the terminal silique are a mosaic of sepal and carpel tissue (data not shown). These sepals emanate from the main axis at the same level as one of the lateral flowers, a common configuration for the first-whorl organs of the terminal flower.

The Cellular Organization of Wild-Type and Mutant Meristems Is Indistinguishable until the Time of Floral Buttress Formation

We have completed a cytological comparison of the cellular organization of wild-type and mutant apical meristems throughout development. Because flowering in *tfl1-1* plants is accelerated, we used well-characterized morphological events to assess developmental stages (e.g., the doming of the apical meristem indicated the beginning of the floral transition). Figure 6 provides a comparison of sectioned wild-type and mutant meristems at two selected developmental stages. Determinations of total cell number, average cell size, total meristem size, and organization into distinct cell layers revealed no anomalies in mutant meristem architecture until the time of floral buttress formation (data not shown). Figures 6A and 6C illustrate wild-type and mutant meristem structure at an early stage of inflorescence initiation.

At a subsequent stage of wild-type development, the flower primordium and the inflorescence meristem are clearly distinct structures, separated by a deep crevice (Vaughn, 1955; Miksche and Brown, 1965) (see Figure 6B). The formation of the first few flower primordia of a *tfl1-1* homozygote may, or may not, follow the wild-type pattern. This is consistent with the variable severity of the inflorescence truncation in isogenic *tfl1-1* populations. In many instances, however, the external separation between the flower primordium and the inflorescence meristem never fully develops and the two meristems remain in close apposition. In these extreme cases, a line of demarcation defined by approximate cell files extends from a shallow crevice at the surface of the meristem into the interior. The L3 cells in these files are elongated, in a direction perpendicular to the surface of the meristem, from twofold to threefold with respect to the dimensions of the average L3 cell. Presumably, this lack of a distinct separation between the two developing structures is reflected later in the existence of partially fused flowers at the terminus. Figure 6D shows a *tfl1-1* inflorescence with two flower primordia: the one on the right flank exhibits relatively normal development, whereas the primordium on the left flank displays abnormal development.

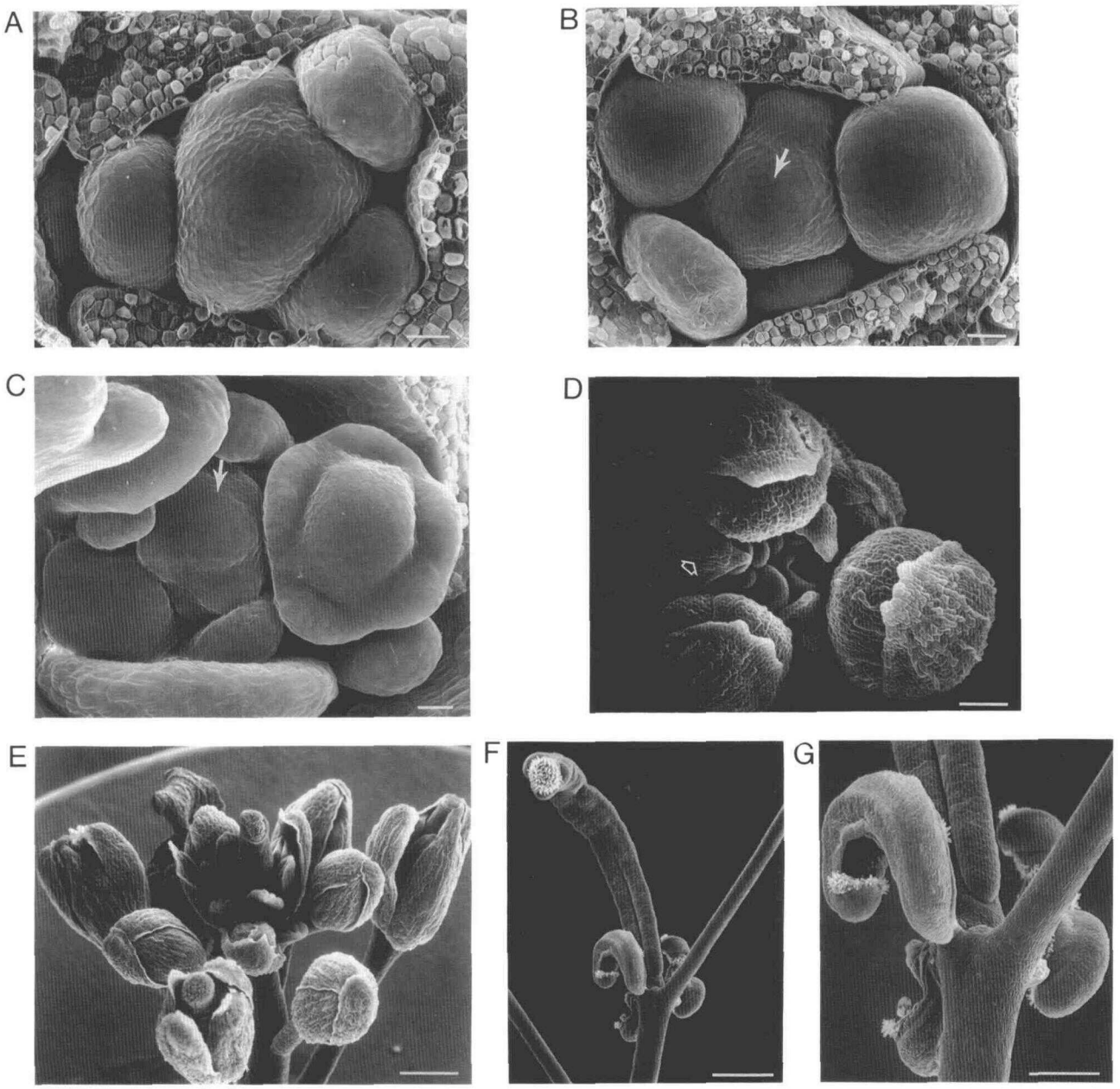

Figure 5. Ontogeny of the *tfl1-1* Inflorescence.

(A) Vertical view of an inflorescence in which the oldest flower primordium has reached stage 2 (Smyth et al., 1990). Bar = 20 μm.
(B) Vertical view of an inflorescence in which sepal primordia are beginning to appear on the oldest flower primordium. As yet, there is no evidence of organogenesis in the terminal region of the inflorescence meristem (arrow). Bar = 20 μm.
(C) Organogenesis is first evident in the terminal flower (arrow) of the inflorescence meristem. The stamen primordia surrounding the gynoecial dome are clearly visible, whereas the petal primordia are still very small. Only three sepals are present; their placement is constrained by the presence of the lateral flower primordia. Bar = 20 μm.
(D) A slightly later stage of inflorescence development. Differentiating floral organs of the terminal flower are visible in the central region. The last lateral flower produced (arrow) remains partially conjoined with the inflorescence terminus. Bar = 100 μm.
(E) A mature *tfl1-1* inflorescence showing the terminal flower. This plant, grown during an LD photoperiod and suboptimal light intensity, has produced an augmented number of flowers. Bar = 500 μm.
(F) Side view of the terminal flower after the senescence of all noncarpelloid organs. Four sepals exhibiting severe carpelloidy can be seen. Bar = 1000 μm.
(G) Close-up of the plant shown in **(F)**. Bar = 500 μm.

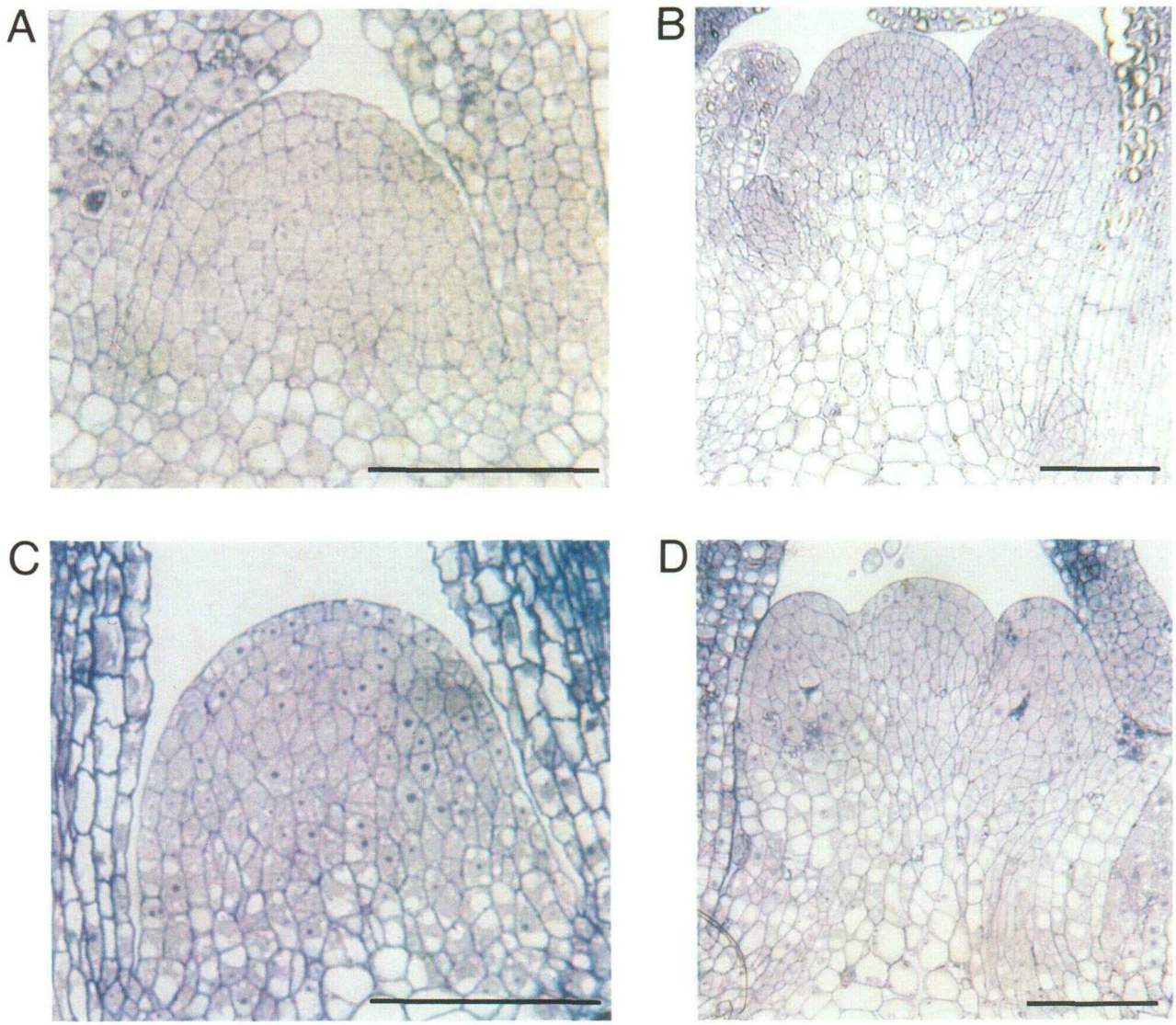

Figure 6. Cellular Organization of Wild-Type and *tfl1-1* Meristems.

(A) Wild-type meristem entering the floral transition.
(B) Wild-type inflorescence meristem (center) with nascent flower primordium (right).
(C) *tfl1-1* meristem entering the floral transition.
(D) *tfl1-1* inflorescence meristem (center) with nascent flower primordia (left and right).
Bars = 50 μm.

Growth of *tfl1-1* Homozygotes in SD Conditions Partially Relieves the Mutant Phenotype

Arabidopsis is a quantitative LD plant. When grown under suboptimal daylength conditions, flowering in wild-type plants is delayed as measured both in time to flowering and in total leaf production before the floral transition (see Table 2). Moreover, the inflorescence of SD-grown plants is prolific, producing many more flowers and branches than under LD growth conditions.

Flowering of *tfl1-1* homozygotes is similarly delayed in SD photoperiods, although still accelerated relative to wild-type plants (see Table 2). As shown in Figure 7A, the inflorescence of SD-grown *tfl1-1* plants is wild type in appearance with ≥20 normal flowers along the length of the main florescence. The multiple lateral branches pro-

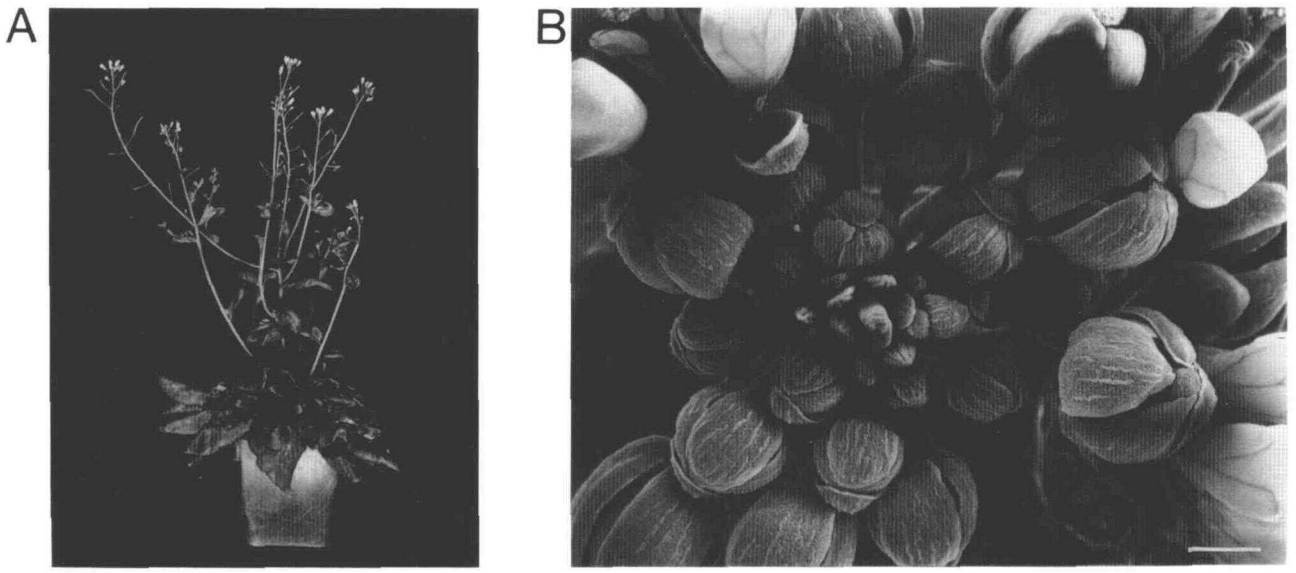

Figure 7. Inflorescence Morphology of *tfl1-1* Plants Grown during an SD Photoperiod.

(A) Photograph of a *tfl1-1* homozygote grown during an SD photoperiod. The robust inflorescence with numerous normal flowers indicates the partial relief of the mutant phenotype.
(B) SEM photograph of the inflorescence showing the centrally located pistil and stamens of the terminal flower. Bar = 500 μm.

duced by the apical inflorescence meristem (and inflorescences arising from axillary buds) show a similar relief of the mutant phenotype. However, both the main florescence and the lateral branches terminate with a carpel and often form unusual floral structures containing a highly variable number of carpels and stamens, but rarely petals and sepals (Figure 7B). When numerous carpels are present, the carpels arise in a helical pattern.

The conspicuous difference in the phenotypes of mutant plants when grown under the two photoperiod regimens suggested that LD to SD shift experiments could be used to determine when the inflorescence meristem of *tfl1-1* plants become committed to the LD phenotype. The effects of the shift on inflorescence development are summarized in Figure 8. The results indicated that by day 8 the developmental potential of the apical inflorescence meristem could not be increased significantly by transfer to an SD photoperiod. This restriction in developmental potential occurred between days 6 and 8. The typical day 8 *tfl1-1* plant grown in LD conditions has an enlarged meristematic dome indicative of entry into the floral transition but shows no evidence of flower primordium initiation (Figure 6C). Wild-type plants produced an augmented number of flowers even when the shift from LD to SD photoperiods occurred after flower primordia were visible to the unaided eye (data not shown).

Frequently, the main florescence of plants shifted from LD to SD conditions exhibited a reduced developmental potential while one or more of the lateral branches displayed the robust development characteristic of growth under an SD photoperiod. We also observed a small number of "airborne" or "perched" rosettes at the base of the lateral branches (see Battey and Lyndon, 1990). These lateral branches were subtended by a normal cauline leaf and produced from three to seven rosette leaves before converting back to reproductive growth (data not shown). This reversion to vegetative growth, although unique to the plants shifted from LD to SD growth conditions, was observed in wild-type as well as *tfl1-1* plants.

Double Mutants of *tfl1-1* with *apetala 1-1* (*ap1-1*), *ap2-1*, and *agamous* (*ag*) Display an Additive Phenotype Except in the Terminal Flower of the *tfl1-1 ap1-1* Double Mutant

To uncover potential functional interactions, we placed *tfl1-1* in double homozygous mutant combination with the floral morphogenesis mutants *ap1-1*, *ap2-1*, and *ag*. These mutations perturb the early stages of flower development. Moreover, the wild-type alleles of these genes have been implicated in promoting the determinate growth pattern of the floral meristem. This role is overtly demonstrated by the indeterminate phenotypes of *ap1-1* and *ag* flowers

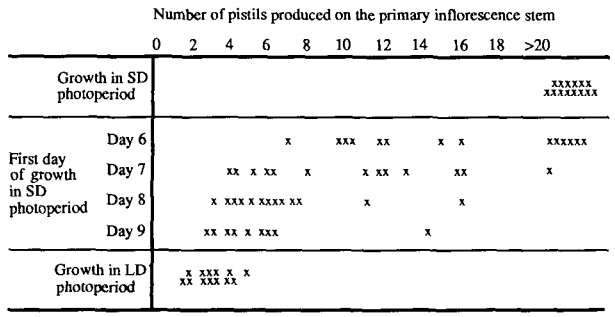

Figure 8. Results of Shifting Homozygous *tfl1-1* Plants from Growth during LD Photoperiods to Growth during SD Photoperiods on Successive Days after Sowing.

Each x represents a single plant and indicates the number of pistils produced along the primary inflorescence. The top row contains data for a population of plants grown entirely during an SD photoperiod. The bottom row contains data for a population of plants grown entirely during an LD photoperiod. Plants that were shifted from LD to SD conditions on day 6, day 7, day 8, and day 9 after sowing produced 11.9 ± 3.2, 7.1 ± 0.6, 6.3 ± 0.5, and 6.5 ± 0.5 rosette leaves, respectively.

(Bowman et al., 1989); it has been suggested for *ap2-1* by a recent analysis of *ap1-1 ap2-1* double mutants (Irish and Sussex, 1990). The gross morphology of double mutants of *tfl1-1* with *ap1-1*, *ap2-1*, and *ag* is illustrated in Figure 9. Figure 10 contains SEM photographs that show aspects of the double-mutant phenotypes in finer detail.

The first-whorl organs of *ap1-1* flowers develop as leaf-like structures and the petals are missing. In the axil of each transformed sepal, a secondary flower with the same first-whorl and second-whorl phenotype as the primary flower is formed. Quite often this process is repeated, leading to the formation of tertiary flowers emanating from the secondary flowers (Irish and Sussex, 1990). As in *tfl1-1* plants, the inflorescence of *tfl1-1 ap1-1* double mutants is truncated and exhibits a terminal floral structure (Figures 9A and 9D). In addition, all flowers exhibit the homeotic transformation of first-whorl organs and the lack of second-whorl organs characteristic of the *ap1-1* mutation (Figure 10A). A tendency toward first-whorl carpelloidy is evident in the terminal flower of the *tfl1-1 ap1-1* double mutant (Figures 10A and 10B); first-whorl carpelloidy is frequently observed in the terminal flower of *tfl1-1* homozygotes (see Figure 5F). In addition, the initiation of secondary and tertiary flowers is preserved in the lateral flowers (Figure 10A) but not in the terminal flower (Figure 10B). Thus, the phenotype of the double mutant is additive except in the terminal flower. In the terminal flower, the *tfl1-1* mutation appears to be epistatic to the indeterminate component of the *ap1-1* phenotype.

The *ap2-1* mutation affects the identity of both first-whorl and second-whorl organs (Bowman et al., 1989). First-whorl organs develop as leaves with stigmatic tissue at the tips. Petals show a stamenoid transformation of variable severity. *tfl1-1 ap2-1* double mutants display an additive phenotype; they exhibit the truncated inflorescence characteristic of the *tfl1-1* mutation (Figures 9B and 9E), and the phenotype conferred by *ap2-1* is evident in all flowers, including the terminal flower (Figures 10C and 10D).

The *ag* mutation causes the indefinite reiteration of the pattern: sepals, petals, petals (Bowman et al., 1989). Plants homozygous for both *tfl1-1* and *ag* also exhibit an additive phenotype. The inflorescence is truncated (Figure 9C), but the flowers show the indeterminate phenotype conferred by *ag* (Figures 9F and 10E). In a few cases, the profusion of petals obscured the precise identity of the terminal flower. Close examination, however, revealed that all flowers possessed the phenotype conferred by *ag*.

DISCUSSION

In this report we describe the initial characterization of an early flowering mutation, *tfl1-1*, in Arabidopsis. Unlike other early flowering mutants that were isolated in the same screen, *tfl1-1* plants exhibit the premature cessation of inflorescence growth after the production of a terminal floral structure. The existence of early flowering mutants with normal inflorescences indicates that the aberrant inflorescence development caused by the *tfl1-1* mutation is not simply a consequence of accelerating floral initiation. A primary objective of the phenotypic description has been to place the action of this highly pleiotropic single gene mutation within the developmental progression: vegetative meristem → inflorescence meristem → floral meristem.

The *tfl1-1* Mutation Does Not Alter Vegetative or Floral Meristem Function

The *tfl1-1* mutation appears to alter shoot meristem functioning throughout the floral transition by restricting the developmental potential of the inflorescence meristem. Based on the results presented in this paper, we have adopted the working hypothesis that *tfl1-1* does not directly affect vegetative or floral meristem development.

In accord with the apparently normal vegetative morphology of mutant plants, light microscopic examination of sections of *tfl1-1* vegetative meristems revealed no obvious structural deviations from the wild type (see Figure 6). Moreover, both the morphology and growth rate of vegetative *tfl1-1* plants are indistinguishable from the wild type (Figure 3).

Several lines of evidence indicate that the *tfl1-1* mutation does not directly affect normal floral meristem function.

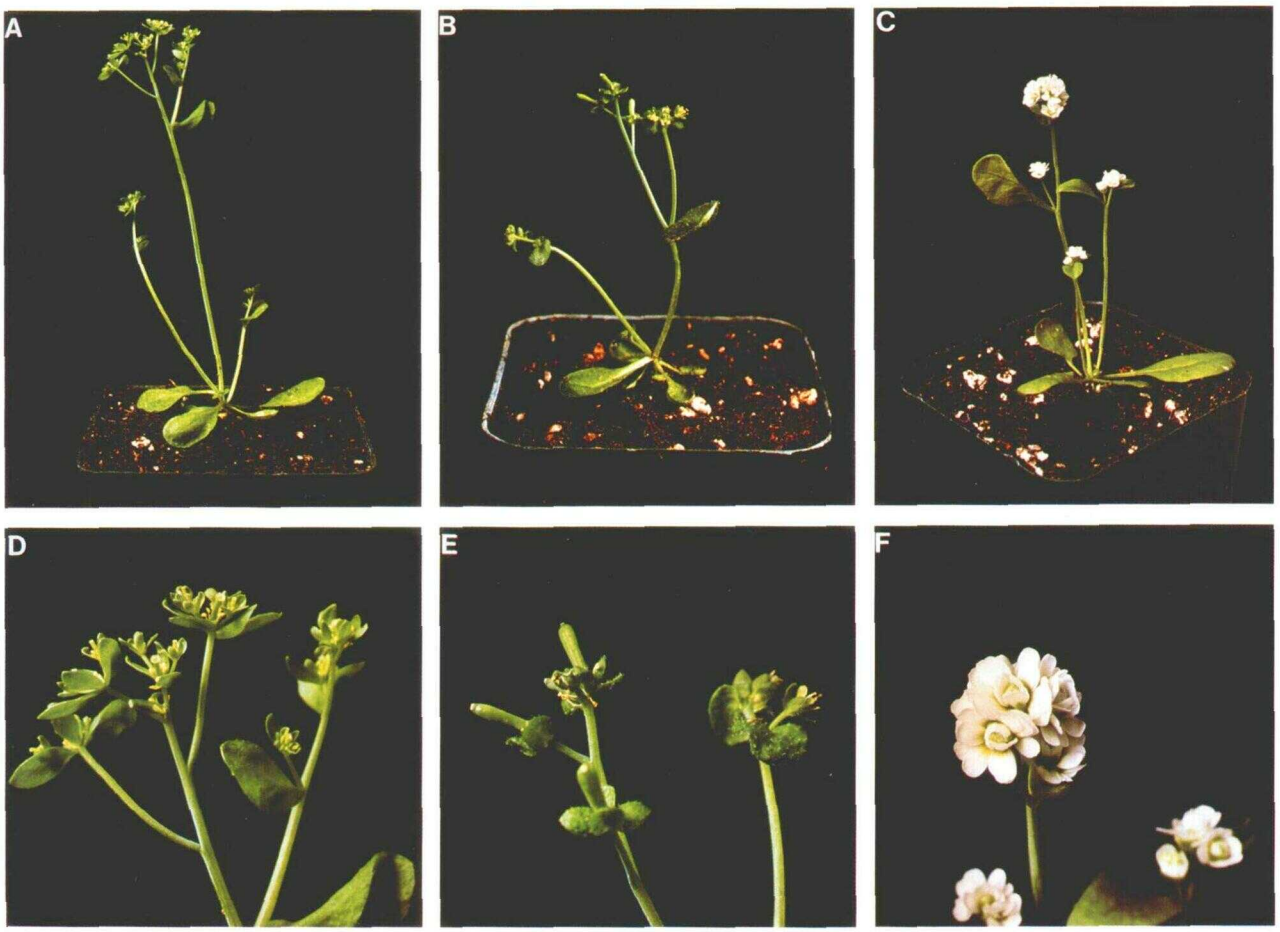

Figure 9. Double Mutant Combinations of *tfl1-1* with *ap1-1*, *ap2-1*, and *ag*.

(A) A *tfl1-1 ap1-1* double mutant.
(B) A *tfl1-1 ap2-1* double mutant.
(C) A *tfl1-1 ag* double mutant.
(D) Close-up of the *tfl1-1 ap1-1* inflorescence.
(E) Close-up of the *tfl1-1 ap2-1* inflorescence.
(F) Close-up of the *tfl1-1 ag* inflorescence.

Sections of unfused lateral flowers observed at stages up to the time of the initiation of carpel primordia showed an apparently normal pattern of organogenesis, as described by Hill and Lord (1989). The additive phenotypes observed in the lateral flowers of the double mutants suggest that the action of the *tfl1-1* mutation is independent of the actions of *ap1-1*, *ap2-1*, and *ag* in the floral meristem. These three mutations were chosen for the double mutant studies because the corresponding wild-type gene products are required to establish the determinate growth pattern of the floral meristem. Given that the *tfl1-1* mutation appears to promote the switch to determinate growth in the inflorescence meristem, they were thought to be likely candidates to participate in a common developmental pathway with *tfl1-1* (Bowman et al., 1989; Irish and Sussex, 1990). Because *ap1-1*, *ap2-1*, and *ag* are known to perturb early stages of flower development (Bowman et al., 1989; Irish and Sussex, 1990), the double mutant phenotypes support the hypothesis that *tfl1-1* does not disturb the ontogeny of regularly produced floral meristems. The phenotype of double homozygous *tfl1-1*, *pistillata* (*pi*) mutants is also additive (data not shown).

The only phenotypic symptom suggestive of perturbed floral meristem function is the homeotic conversions of floral organ identity (see Table 1). However, the mosaic floral organs are found only in those flowers that appear to be conjoined at the inflorescence terminus. We assume that these terminal floral structures arise from the abnormal

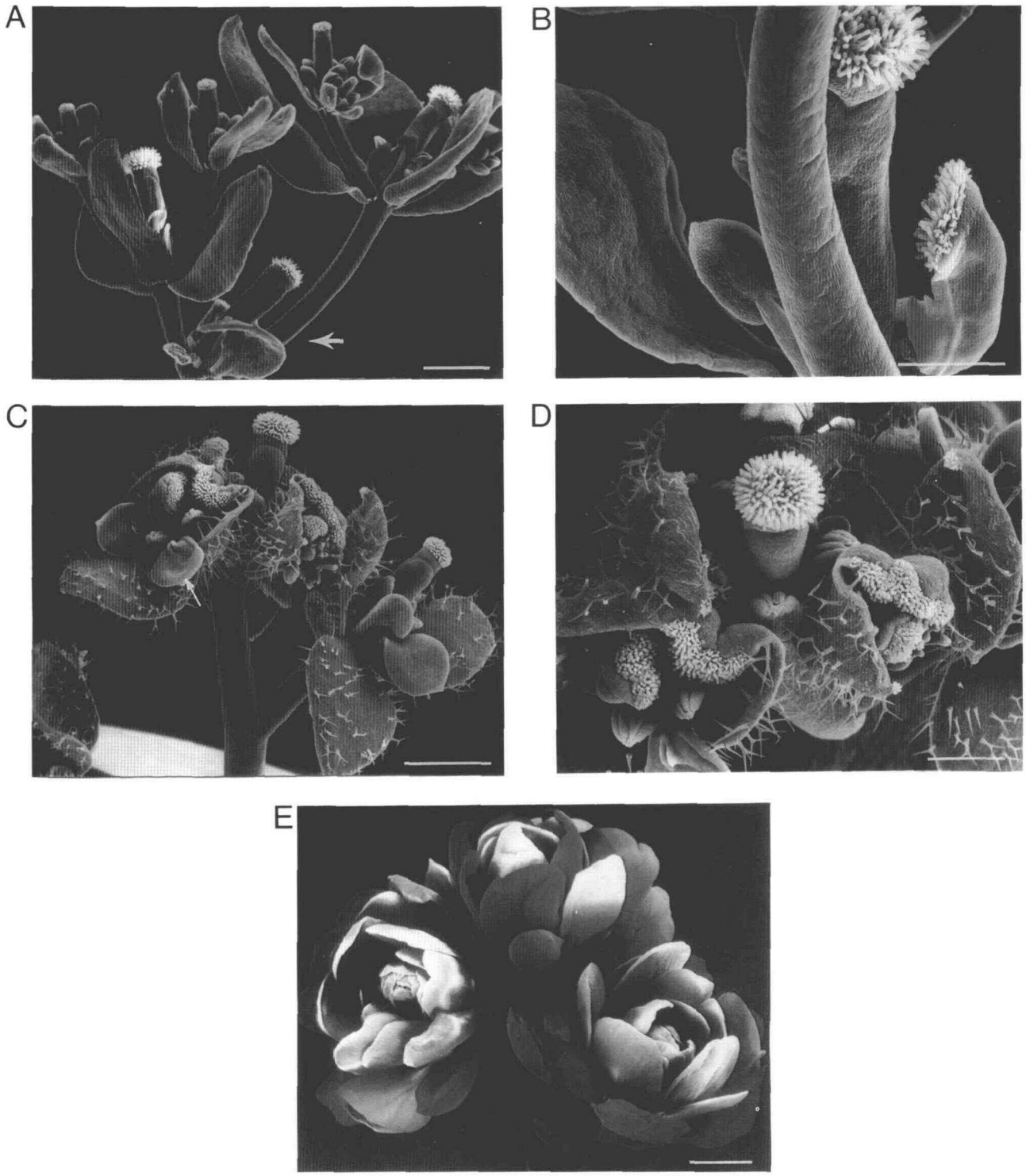

Figure 10. SEM Photographs of the Inflorescence Terminus of *tfl1-1 ap1-1*, *tfl1-1 ap2-1*, and *tfl1-1 ag* Double Mutants.

(A) View of the *tfl1-1 ap1-1* inflorescence. Secondary and tertiary flowers can be seen emanating from the lateral flower. The terminal flower is indicated (arrow). Bar = 100 μm.

flower primordia observed in the cytological studies. During floral morphogenesis, the cells of these abnormal primordia remain in an unusually intimate association with cells of other lateral flower primordia, as well as the cells of the developing terminal flower. We imagine that the differentiating cells come under the influence of multiple "floral fields" (Bowman et al., 1989) and therefore must interpret ambiguous or conflicting positional information. Mosaic patches of cells arise because a few cells in one or more organ primordia adopt an inappropriate fate.

The *tfl1-1* Mutation Affects Both the Establishment and Maintenance of the Inflorescence Meristem

SEM analysis of early inflorescence development in *tfl1-1* homozygotes demonstrated that the normally undifferentiated terminal region of the inflorescence meristem gives rise to floral organs and therefore functions as if it were converted into a floral meristem. This disrupts further activity of the inflorescence meristem, as is shown by the fact that the last one or few flower primordia to emerge from the flanks of the inflorescence meristem often fail to form an entire, separated flower (see Figures 5D and 5E). Presumably, they are incorporated into the compound *tfl1-1* terminal floral structure.

Light microscopic examination revealed that homozygous mutant plants display aberrations in the cellular organization of the apical meristem before any overt evidence of organogenesis in the terminal region of the inflorescence meristem. The first notable abnormal feature is the occurrence of the files of elongated cells that project from the axil of a developing flower primordium into the meristem interior (see Figure 6D). The ontogeny of flower primordium emergence in the Cruciferae has been most completely described in *Sinapis alba* (Bernier, 1962). In Sinapis, flower primordium initiation begins with periclinal divisions in the interior cell layers of the meristem flanks. The resulting expansion is accommodated by both periclinal and anticlinal divisions in surrounding cells. Subsequently, anticlinal divisions that are concentrated in the axil of the developing primordia function to regenerate the meristem. These anticlinal divisions are responsible for the formation of the cleft that develops to separate the nascent floral meristem from the inflorescence meristem.

If the details are similar in Arabidopsis, the abnormal morphology of the terminal floral structure observed in mutant plants could be caused by an inadequate number of anticlinal divisions during the early stages of flower primordium initiation. A lack of anticlinal divisions would prevent the formation of a cleft and force cells adjacent to those that have divided periclinally to stretch in a direction perpendicular to the surface of the meristem; this would account for the files of elongated cells observed in *tfl1-1* apical meristems just before floral organogenesis. Such a relative deficiency of anticlinal divisions could be caused by the presence of periclinal divisions in two regions (i.e., in both lateral and terminal flower primordia), as opposed to in a single region. Alternatively, the terminal region of the meristem, which encompasses the axil of the developing lateral flower primordium, might not retain the capacity to perform the necessary regenerative anticlinal cell divisions because it is committed to floral organogenesis.

This hypothesis is consistent with the results of the daylength shift experiments. Eighty-five percent of plants shifted from LD to SD photoperiods on day 8, when the meristem is clearly domed and enlarged but flower primordia are not yet evident, produce no more than eight pistils along the primary inflorescence (Figure 8). This represents an increase of at most three pistils, or one turn of the inflorescence helix, over the number of pistils produced by a typical *tfl1-1* plant grown entirely during an LD photoperiod. This also represents many fewer pistils than the absolute minimum of 20 produced by *tfl1-1* plants grown under SD conditions. Thus, whereas wild-type and mutant apical meristem structures are indistinguishable during the earliest stages of the floral transition when the inflorescence meristem is being established (Figures 6A and 6C), the daylength shift experiment indicates that the apical inflorescence meristem becomes committed to producing a phenotypically LD inflorescence during this initial stage.

Although we cannot infer anything about the time of action of the *tfl1-1* gene product from this experiment, one interpretation of these results is that the terminal region of the apical inflorescence meristem is committed to form a floral meristem by day 8 of growth in LD conditions. Wild-type plants showed no evidence of commitment to an LD phenotype, even when shifted to SD photoperiods after the inflorescence was visible to the unaided eye, which is compatible with this interpretation. Similar daylength shift experiments were performed by Gebhart and McDaniel (1987) with the SD tobacco *Nicotiana* cv Maryland Mammoth. In tobacco it was found that the commitment of the apex to form a terminal flower occurs very early during the floral transition, when the apical meristem was in the

Figure 10. (continued).

(B) Close-up of the terminal flower of the plant shown in **(A)**. The terminal flower failed to produce the secondary flowers characteristic of the *ap1-1* mutation. Bar = 500 μm.

(C) View of the *tfl1-1 ap2-1* inflorescence. A stamenoid petal is indicated (arrow). Bar = 1000 μm.

(D) Vertical view close-up of the terminal flower of the plant shown in **(C)**. Bar = 500 μm.

(E) View of the *tfl1-1 ag* inflorescence. Bar = 1000 μm.

domed and enlarged prefloral state (Gebhart and McDaniel, 1987). To prove unequivocally that the terminal region, or even the apex, of the *tfl1-1* mutant is stably committed to floral meristem formation would require demonstrating that it could undergo floral organogenesis when grown in isolation. These experiments will be technically difficult in Arabidopsis.

We speculate that after primordia comprising the first few turns of the inflorescence helix deplete the meristem flanks, no new primordia arise because the now committed central zone of the apical meristem no longer replenishes the organogenic peripheral zone. In the daylength shift experiments, the existence of a few day 8 plants with an intermediate number of pistils could indicate either a gradual (or unstable) commitment of the central zone of the meristem or an asynchronous plant population. The similar numerical distribution of total carpel production in the day 8 and day 9 populations argues that the commitment process is gradual or unstable. The occurrence of plants in which the main inflorescence axis ceases growth after the production of a small number of pistils, whereas lateral branches exhibit the prolific SD phenotype, suggests that the commitment process that occurs in the apical inflorescence meristem is independent of the processes involved in inflorescence meristem development in lateral branches.

The Terminal Floral Meristem in *tfl1-1* Homozygotes Differs from the Lateral Floral Meristems

The homeotic conversions of floral organ identity and the reduced number of first-whorl and second-whorl organs frequently seen in the *tfl1-1* terminal flower could be a consequence of the constraints imposed by the peculiar geometry of the inflorescence terminus (i.e., the partial fusion with lateral flowers or the existence of lateral flowers' pedicels emanating from within the sepal whorl). The geometry alone cannot easily explain the observation that production of secondary flowers is suppressed in the terminal flower of *tfl1-1 ap1-1* double mutants because these plants often produce multiple sepals, as well as the correct number of apparently normal third-whorl and fourth-whorl organs. The fact that *tfl1-1* is epistatic to the indeterminate component of the phenotype conferred by *ap1-1* could imply that the terminus of the inflorescence meristem is not converted into a normally functioning floral meristem. Alternatively, the epistasis might be indicative of some underlying function of the *AP1* gene in the inflorescence meristem. This would be consistent with the reversion of *ap1-1 ap2-1* floral meristems to the indeterminate growth pattern and phyllotaxy of the inflorescence meristem (Irish and Sussex, 1990).

The Role of the *tfl1-1* Mutation in the Study of Meristem Function

Many examples exist in which the floral transition is regulated by signals originating outside of the meristem (Evans, 1969; Bernier, 1988). However, it is also clear that the differential sensitivity of the meristem to these promotive and inhibitory substances influences the response of the shoot apex (Murfet, 1990). Thus, changes in the time of floral initiation could be effected by mutations that alter any functional interactions involved in either the signal or the response. For example, the late flowering mutant of Arabidopsis (Redei, 1962; Hussein and van der Veen, 1965; Koornneef et al., 1983) may cause aberrations in the putative signal transduction pathway leading to flowering, rather than defects in the developmental coordination of meristem activity. These mutants produce both a greater number of rosette leaves before the floral transition and inflorescences that bear an augmented number of cauline leaves and flowers. This phenotype is a virtual phenocopy of wild-type plants grown under minimal daylength conditions. Similarly, one class of early flowering mutations that we isolated (represented by *elf1*) may also identify components of the floral signal transduction pathway. These mutations promote early floral initiation, yet produce morphologically normal inflorescences, suggesting that the coordination of meristem activity during the floral transition is not perturbed.

Because the coordination of spatial and temporal aspects of meristem function is obscured during normal plant development by a pattern of organogenesis that is both sequential and directional (Poethig, 1988; Hill and Lord, 1989), we chose to focus our research on mutants, such as *tfl1-1*, that develop morphologically abnormal inflorescences as well as flower prematurely. The study of mutations with both morphological and heterochronic features may contribute to an understanding of the relative importance of spatial and temporal factors in the regulation of meristem function throughout development. We believe that the *tfl1-1* mutation perturbs the normal developmental activity of the shoot apical meristem early in the floral transition. Although we have not demonstrated that the altered terminal region in *tfl1-1* mutants corresponds to the cytohistologically defined central zone, this remains a formal possibility. By analogy to vegetative meristem function, the proposed stem cell activity of the cells of the central zone may be critical for the maintenance of the organogenic regions during the extended period of reiterative growth observed in indeterminate inflorescence meristems. The mitotic activation of the central zone invariably occurs at the inception of the floral transition, and this activation may be essential for the transition. One hypothesis that would account for the effects of the *tfl1-1* mutation on both flowering time and inflorescence morphology is that the cells of the meristematic central zone are

developmentally accelerated. Initially, this developmental acceleration manifests as a precocious floral transition. Subsequently, it causes the normally undifferentiated cells of the meristematic central zone to adopt the fate of a terminally differentiated structure, the flower.

In summary, we believe that the analysis of pleiotropic mutations like *tfl1-1* may elucidate the relationship between meristematic structure and activity during development. Such investigations should be very powerful if the molecular functions of the genes identified by these mutations can be determined. To this end, we are in the process of cloning the *TFL1* locus to understand how this gene regulates meristem activity and why the *tfl1-1* mutation results in a pleiotropic phenotype.

METHODS

Plant Material and Growth Conditions

Seeds were sown in Fison's Sunshine Mix after 2 days of vernalization at 4°C. All plants were grown in growth cabinets at 22°C under cool-white fluorescent lights. Plants were grown during either a 16-hr light/8-hr dark photoperiod (LD conditions) or a 10-hr light/14-hr dark photoperiod (SD conditions).

The mutant screen was performed on an M2 population derived from EMS-mutagenized seed of *Arabidopsis thaliana* ecotype Columbia obtained from Guhy's Specialty Nursery (Tucson, AZ). This seed was homozygous for the *gl-3* mutation. All other mutant strains were in the Landsberg ecotype and contained the *erecta* mutation. The lines carrying *ap1-1*, *ap2-1*, *ag*, and *pi* were provided by Vivian Irish (Yale University, New Haven, CT). The marker lines used for the genetic mapping were a gift from Maarten Koornneef (Wageningen Agricultural University, The Netherlands).

The W9 marker line used for genetic mapping contained the *ttg* and *yellow inflorescence* (*yi*) mutations. The conversion from centimorgans to map units was made using the Kosambi mapping function (Koornneef et al., 1983). Crosses were performed manually using *tfl1-1* homozygotes as the pollen recipient. Except for crosses with *ag* and *pi*, which can only be propagated in the heterozygous condition, the pollen parent was also homozygous mutant. To generate the double mutant strains, the F_1 plants were allowed to self-pollinate, and double mutant plants were selected from the F_2 population.

For the comparison of wild-type and vegetative growth rates, all plants that had not germinated by day 3 were removed from consideration. The germination rates were 92% and 97% for the wild-type and the *tfl1-1* populations, respectively. For the day-length shift experiment, growth under LD and SD conditions was performed under conditions of equivalent light intensity. On day 5 of this experiment, any plant whose development was noticeably accelerated or retarded was removed.

RFLP Mapping

F_3 families from an original cross between the Columbia ecotype carrying the *tfl1-1* mutation and the Landsberg ecotype were scored for their genotype with respect to the *tfl1-1* mutation by the presence or absence of a terminal flower. Total DNA was extracted from F_3 plants, digested with EcoRI, run on 0.6% agarose gels, and blotted onto nylon membranes. A phage λ clone containing the RFLP marker 217 (Chang et al., 1988) provided by Elliott Meyerowitz (California Institute of Technology, Pasadena) was radiolabeled with α-^{32}P-dCTP. Hybridizations, washes, and autoradiographic exposures were carried out as described by Kelly et al. (1990).

Histological Analysis

Dissected specimens for light microscopy were placed in *n*-heptane for 15 min, followed by overnight fixation in 4% paraformaldehyde, 0.5% glutaraldehyde, and 50 mM Pipes buffer (pH 7) at 4°C. This was followed by postfixation with 2% osmium tetroxide in 50 mM Pipes buffer for 2 hr. After rinsing, specimens were dehydrated in a graded ethanol series and embedded in Spurr's resin. Sections of 1- to 2-μm thickness were made with glass knives on an LKB ultramicrotome, affixed to glass microscope slides, and stained with methylene blue/azure II. Finished specimens were photographed under bright-field illumination.

For SEM, plants were fixed in 3.7% formaldehyde, 50% ethanol, and 5% acetic acid for 4 hr and dehydrated in a graded ethanol series. Specimens were critical point dried in liquid CO_2. The dried material was mounted and coated with gold-palladium in a Technics Hummer I sputtercoater. Specimens were examined in an AMR 1000A scanning electron microscope with an accelerating voltage of 20 kV.

ACKNOWLEDGMENTS

Susan Shannon was a Howard Hughes Medical Institute Predoctoral Fellow. Support for this research was from the HHMI fellowship to S.S., Grant 70108-501601. We wish to thank David Smyth and the members of his laboratory for communicating unpublished results. We are indebted to Carolyn Jacobs and Genevieve Reid for their help with several aspects of this project, and to Scott Givan for his assistance with the construction of the *tfl1-1 ap1-1* double mutant. Also, we wish to thank Maarten Koornneef and Vivian Irish for providing Arabidopsis strains, and Elliot Meyerowitz for providing RFLP markers. Jerry Gleason, Eric Schabtach, and Tracie Bork provided excellent help with photography and SEM. We also thank Michelle Zagotta and Alan Kelly for their useful suggestions during the course of this work, and for their critical readings of the manuscript. Finally, we wish to thank the anonymous reviewers for their comments on this manuscript.

Received May 10, 1991; accepted July 5, 1991.

REFERENCES

Battey, N.H., and Lyndon, R.F. (1990). Reversion of flowering. Bot. Rev. **56,** 162–189.

Bernier, G. (1962). Evolution of the apical meristem of *Sinapis alba* L. (long-day plant) in long days, in short days and during the transfer from short to long days. Caryologia **15,** 303–325.

Bernier, G. (1988). The control of floral evocation and morphogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 175–219.

Bernier, G., Kinet, J.M., and Sachs, R.M. (1981). The Physiology of Flowering, Vol. II (Boca Raton, FL: CRC Press).

Besnard-Wibaut, C. (1970). Action comparée de la photopériode et de l'acide gibérellique sur la fonctionnement apical de l'*Arabidopsis thaliana*. Etude histoautoradiographique des synthèses nucléiques et de leur modification lors du passage à l'état reproducteur. Rev. Cytol. Biol. Veg. **33,** 265–288.

Bodson, M. (1975). Variation in the rate of cell division in the apical meristem of *Sinapis alba* during the transition to flowering. Ann. Bot. **39,** 547–554.

Bowman, J.L., Smyth, D.R., and Meyerowitz, E.M. (1989). Genes directing flower development in *Arabidopsis*. Plant Cell **1,** 37–52.

Chang, C., Bowman, J.L., DeJohn, A.W., Lander, E.S., and Meyerowitz, E.M. (1988). Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **85,** 6856–6860.

Corson, G.E., Jr. (1969). Cell division studies of the shoot apex of *Datura stramonium* during the transition to flowering. Am. J. Bot. **56,** 1127–1134.

Evans, L.T. (1960). Inflorescence initiation in *Lolium temulentum* L. II. Evidence for inhibitory and promotive photoperiodic processes involving transmissible products. Aust. J. Biol. Sci. **13,** 429–440.

Evans, L.T. (1969). The nature of flower induction. In The Induction of Flowering: Some Case Histories, L.T. Evans, ed (Ithaca, NY: Cornell University Press), pp. 457–480.

Gebhart, J.S., and McDaniel, C.N. (1987). Induction and floral determination in the terminal bud of *Nicotiana tabacum* L. cv. Maryland Mammoth, a short-day plant. Planta **172,** 526–530.

Gould, S.J. (1977). Ontogeny and Phylogeny. (Cambridge: Belknap Press, Harvard).

Hill, J.P., and Lord, E.M. (1989). Floral development in *Arabidopsis thaliana*: A comparison of the wild type and the homeotic pistilla mutant. Can. J. Bot. **67:** 2922–2936.

Hussein, H.A.S., and van der Veen, J.H. (1965). Induced mutations for flowering time. Arabidopsis Inf. Serv. **2,** 6–8.

Irish, V.F., and Sussex, I.M. (1990). Function of the *apetala-1* gene during *Arabidopsis* floral development. Plant Cell **2,** 741–753.

Jegla, D.E., and Sussex, I.M. (1989). Cell lineage patterns in the shoot meristem of the sunflower embryo in the dry seed. Dev. Biol. **131,** 215–235.

Kelly, A.J., Zagotta, M.T., White, R.A., Chang, C., and Meeks-Wagner, D.R. (1990). Identification of genes expressed in the tobacco shoot apex during the floral transition. Plant Cell **2,** 963–972.

Koornneef, M., van Eden, J., Hanhart, C.J., Stam, P., Braaksma, F.J., and Feenstra, W.J. (1983). Linkage map of *Arabidopsis thaliana*. J. Hered. **74,** 265–272.

Lang, A., Chailakhyan, M.K., and Frolova, I.A. (1977). Promotion and inhibition of flower formation in a day neutral plant in grafts with a short-day and a long-day plant. Proc. Natl. Acad. Sci. USA **74,** 2412–2416.

Melzer, S., Majewski, D.M., and Apel, K. (1990). Early changes in gene expression during the transition from vegetative to generative growth in the long-day plant *Sinapis alba*. Plant Cell **2,** 953–961.

Miksche, J.P., and Brown, J.A.M. (1965). Development of the vegetative and floral meristems of *Arabidopsis thaliana*. Am. J. Bot. **52,** 533–537.

Murfet, I.C. (1990). Flowering genes in pea and their use in breeding. Pisum News. **22,** 78–86.

Needham, J. (1933). On the dissociability of the fundamental process in ontogenesis. Biol. Rev. **8,** 180–223.

Poethig, R.S. (1988). Heterochronic mutations affecting shoot development in maize. Genetics **119,** 959–973.

Poethig, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. Science **250,** 923–930.

Raff, R.A., and Wray, G.A. (1989). Heterochrony: Developmental mechanisms and evolutionary results. J. Evol. Biol. **2,** 409–434.

Redei, G.P. (1962). Supervital mutants of *Arabidopsis*. Genetics **47,** 443–460.

Ruth, J., Klekowski, E.J. , Jr., and Stein, O.L. (1985). Impermanent initials of the shoot apex and diplontic selection in a juniper chimera. Am. J. Bot. **72,** 1127–1135.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in *Arabidopsis*. Plant Cell **2,** 755–767.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development. (Cambridge: Cambridge University Press).

Steeves, T.A., Hicks, M.A., Naylor, J.M., and Rennie, P. (1969). Analytical studies on the shoot apex of *Helianthus annus*. Can. J. Bot. **47,** 1367–1375.

Stewart, R.N., and Derman, H. (1970). Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Am. J. Bot. **57,** 816–823.

Vaughan, J.G. (1955). The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa-pastoris* (L.) Medic. and *Anagallis arvensis* L. J. Linn. Soc. Lond. Bot. **55,** 279–300.

Wardlaw, C.W. (1965). The organization of the shoot apex. In Encyclopedia of Plant Physiology, Vol. XV, W. Ruhland, ed (New York: Springer-Verlag), pp. 966–1079.

Weberling, F. (1989). Morphology of Flowers and Inflorescences. (Cambridge: Cambridge University Press).