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LEAFY, a Homeotic Gene That Regulates Inflorescence Development in Arabidopsis

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Variation in plant shoot structure may be described as occurring through changes within a basic unit, the metamer. Using this terminology, the apical meristem of Arabidopsis produces three metameric types sequentially: type 1, rosette; type 2, coflorescence-bearing with bract; and type 3, flower-bearing without bract. We describe a mutant of Arabidopsis, Leafy, homozygous for a recessive allele of a nuclear gene *LEAFY* (*LFY*), that has an inflorescence composed only of type 2-like metamers. These data suggest that the *LFY* gene is required for the development of type 3 metamers and that the transition from type 2 to type 3 metamers is a developmental step distinct from that between vegetative and reproductive growth (type 1 to type 2 metamers). Results from double mutant analysis, showing that *lfy-1* is epistatic to the floral organ homeotic gene *ap2-6*, are consistent with the hypothesis that a functional *LFY* gene is necessary for the expression of downstream genes controlling floral organ identity.

INTRODUCTION

Angiosperm shoots can be described as a series of repeating units (metamers) that are formed sequentially by the apical meristem. In the most basic form, a metamer consists of a node with the associated leaflike organ, the lateral meristem in the axil of the leaf, and the internode (White, 1984). According to this concept, much, if not all, of the variation observed in shoot morphology can be accounted for by differences in the number of metameric units produced per shoot, their rotational orientation with respect to each other (determines phyllotaxy, for example), and the metameric unit type. The metamer type can be defined by variations in the constituent parts of the metamer, including internode length, the number and type of leafy organ, and the number and fate of the lateral meristem. Although the developmental significance of the metameric unit concept in plants has not been clearly established (Rutishauser and Sattler, 1985), it offers a convenient means to describe variation in shoot morphology (van Groenendael, 1985).

Figure 1A illustrates the structure of the wild-type shoot of Arabidopsis. The apical meristem forms a rosette of closely appressed leaves during the vegetative phase, followed by a compound raceme typical of the Brassicaceae (Müller, 1961). The first several nodes of the main inflorescence shoot bear lateral flower-bearing shoots (coflorescences, according to the terminology of Troll, 1964; see also Weberling, 1965) in the axils of bracts, whereas nodes produced subsequently bear flowers that are not associated with bracts. Each coflorescence repeats the pattern of development of the main inflorescence, giving rise itself to lateral coflorescence shoots and flowers. Thus, the inflorescence of Arabidopsis should form an infinite series of coflorescences if unlimited growth were possible. We will refer to the lateral shoots (coflorescence or flower) produced from the main (primary) shoot as secondary shoots and those produced from the secondary lateral shoots as tertiary shoots (Figure 1A).

If the metamer concept is applied to the Arabidopsis shoot, several different types of metameric units can be recognized. The Arabidopsis apical meristem sequentially generates at least three distinct metameric forms: "type 1," rosette, consisting of a node with a leaf, a lateral meristem whose development is delayed, and a short internode; "type 2," coflorescence-bearing, consisting of a node with a modified leaf (bract), a lateral meristem that develops into a coflorescence, and an elongated internode; "type 3," flower-bearing, consisting of a bractless node, a lateral meristem that develops into a flower, and an elongated internode. Thus, lateral meristems within the inflorescence may have one of two fates, coflorescence or flower. The coflorescence, like the primary inflorescence, is formed through production of type 2 and type 3 metamers. The determinate flower can be viewed as consisting of at least four novel types of metamers, each having a compressed internode, no lateral meristem, and one of four floral organs (sepal, petal, stamen, and carpel).

The ordered array of different metamers in a mature Arabidopsis plant suggests that mechanisms must exist that specify the type of metamer to be produced at a

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particular time in development. Indeed, a number of Arabidopsis genes that appear to be essential to such mechanisms have been identified. Recessive mutations in several genes cause homeotic transformations of floral organs (Komaki et al., 1988; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989; Irish and Sussex, 1990; Yanofsky et al., 1990). These genes could be considered to be regulators of metamer type within the flower. The late-flowering mutants of Arabidopsis (Hussein and van der Veen, 1965; Martinez-Zapater and Somerville, 1990) fail to produce an inflorescence (type 2 metamers) at the appropriate time and instead continue to produce rosette metamers (type 1). Thus, products of the late-flowering genes appear to be needed to regulate the transition from production of type 1 to type 2 metamers.

We describe here, in detail, a homeotic mutant of Arabidopsis (Leafy; Haughn and Somerville, 1988) in which all metamers of the inflorescence are coflorescence-like metamers (which we will refer to as type 2) because of a mutation in a single nuclear gene, *LEAFY* (*LFY*; *LEAFY*). The nature of the phenotype provides evidence that the transition from production of type 2 to type 3 metamers represents a developmental event that is distinct from the initiation of an inflorescence and suggests a role for the *LFY* gene product in the development of type 3 metamers.

RESULTS

Mutant Isolation and Genetic Analysis

A collection of plant lines (M_3 generation), derived from ethyl methanesulfonate-mutagenized seeds, was screened for those that were segregating plants with an unusual floral morphology. One such line (GH110) contained plants that produced leafy shoots in place of flowers (Leafy) and was rescued by selfing of the phenotypically wild-type siblings that were heterozygous for the mutant allele(s). Although Leafy plants are typically sterile, pistils occasionally develop and can be cross-fertilized. The frequency of such pistils was higher when plants were grown at 16°C compared with those grown at 22°C. Thus, plants were grown at 16°C if cross-fertilization was necessary.

To determine the genetic basis for the Leafy phenotype, Leafy plants were crossed to wild-type plants. The 29 F_1 progeny examined were all wild type. The F_2 progeny consisted of both wild-type and Leafy plants in a ratio of approximately 3:1 (311 wild type:101 Leafy; $\chi^2 = 0.05$, $P > 0.80$). Thus, the Leafy phenotype appears to be the result of a recessive allele of a nuclear gene we call *LFY*. The chromosomal location of *LFY* was determined by examining 320 F_2 progeny of the cross Leafy × line W100 (Koornneef et al., 1987). *LFY* was assigned to chromosome 5 based on linkage with *TT3* (18.76 centimorgans [cM] ± 5.44). A more accurate map position was obtained by the analysis of F_2 progeny of Leafy × MSU23 (MSU23 carries three genetic markers on chromosome 5; Koornneef et al., 1987). Three hundred ninety-six F_2 progeny were analyzed to determine the linkage of *LFY* to *GL-3* (25.59 cM ± 4.74), and an additional 1019 progeny were analyzed to determine the linkage of *LFY* to *TZ* (9.15 cM ± 2.65) and *CER-3* (0 cM ± 2.67). Figure 2 illustrates the chromosomal

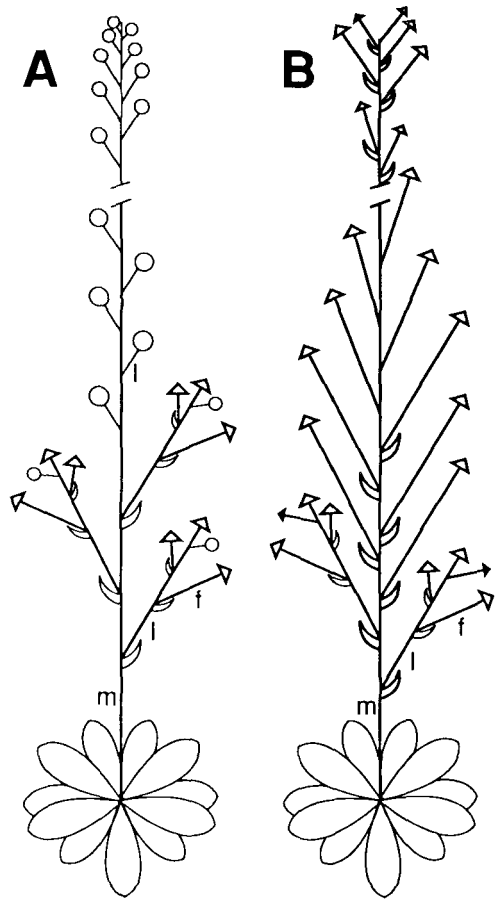

Figure 1. Diagrammatic Representation of Wild-Type and Leafy Plants.

(A) Wild-type plant. ⬭, rosette leaf; ⸝, bract; ⍲, coflorescence; ⌕, flower.

(B) Leafy plant. ⬭, rosette leaf; ⸝, bract or bractlike structure; ⍲, coflorescence-like shoot. The length of the arrow signifies the complexity of the coflorescence-like shoot, such that the longer the arrow, the more complex the shoot. For simplicity, only a small number of the tertiary shoots are represented on the Leafy diagram.

Each individual shoot is represented by a straight line. Lines that deviate from a straight line at inflorescence branch points represent lateral shoots.

m, main inflorescence shoot; l, lateral shoots, secondary; f, lateral shoots, tertiary.

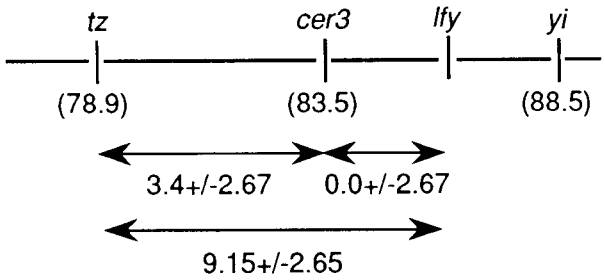

Figure 2. Location of lfy on Chromosome 5 of Arabidopsis Relative to Several Closely Linked Markers.

Arrows span distance between pairs of markers, and the numbers beneath these arrows represent the map distances and standard error in centimorgans, as calculated from our data. Numbers in parentheses are the chromosomal location of the markers as assigned by Koornneef (1990).

position of *LFY* with respect to closely linked markers (Koornneef, 1990).

Rosette and Inflorescence Structure of Wild-Type and Leafy Plants

We analyzed characteristics of both the rosette and the inflorescence of Leafy and wild-type plants to determine the extent of variation between the two phenotypes. Table 1 summarizes some of these results. Figure 3 shows pictures of wild-type and mutant inflorescences, and Figure 1 illustrates the inflorescence structures graphically.

Wild Type

During the rosette stage, plants produce an average of 12.5 leaves (minimum nine, maximum 17). Elongation of the primary shoot to a height of 1 cm (bolting) occurs at about 25 days after sowing (minimum 23, maximum 30). On average, the first three (minimum two, maximum five) metamers produced after bolting are type 2, consisting of an elongated internode, a bract, and an associated lateral meristem that develops into a coflorescence similar in structure to the main inflorescence. The remaining (mean 37, minimum 26, maximum 49) metamers are type 3, having a bractless node, a lateral meristem that develops into a flower, and an elongated internode. Thus, the final form of the wild-type inflorescence is a compound raceme with three coflorescences and approximately 37 flowers arranged in a generative spiral on the main inflorescence (Figures 1A and 3A).

Leafy

Figures 1, 3, 4, 5, and 6 and Table 1 show that the Leafy inflorescence differs dramatically from that of the wild type. Normal flowers are never produced (compare Figure 3A with Figure 3B). In their place are shoots that most resemble coflorescences in that they produce an indeterminate number of metamers with elongated internodes, bract-like organs, tertiary lateral shoots, and spiral phyllotaxy (Figures 3A and 4A). However, these secondary lateral shoots of Leafy cannot strictly be called coflorescences because, as in the Leafy primary shoot, only type 2 metamers develop. The morphology of the lateral shoots produced from the primary axis of an individual Leafy plant varies greatly (Figure 4). The earliest lateral shoots to develop (Figure 4A) are most similar to coflorescences on the basis of the criteria stated above. Shoots produced later in the inflorescence become progressively more flowerlike (Figures 4B and 4C) in that organs with carpel-like or sepal-like cell types develop more commonly than bracts (Figure 4C), tertiary meristems do not develop as frequently at the nodes, and the internodes are often shorter (Figure 4C). It should be noted that an individual shoot may have some characteristics that are more flowerlike, whereas other characteristics are more coflorescence-like. For example, axillary buds may develop even though the internodes are very short and the subtending organ is sepal-like or carpel-like rather than a bract (Figure 4B). Occasionally, shoots end in a pistil-like structure (Figure 4C) that can be cross-fertilized to produce seed. Petals and stamens are never produced even in the most flowerlike shoots, suggesting that the regulatory mechanism controlling the identity of these two organ types (e.g., the *APETALA3/PISTILLATA* pathway; Bowman et al., 1989; Hill and Lord, 1989) is more sensitive to perturbations in floral development than that controlling sepal and carpel development (e.g., *APETALA2* (*AP2*) and *AGAMOUS* developmental pathways; Komaki et al., 1988; Bowman et al., 1989; Kunst et

Table 1. Comparison of Wild-Type and Leafy Primary Shoots[a]

Phenotype	No. of Rosette Metamers	No. of Days to Bolting	No. of Metamers + Bract[b]	No. of Metamers − Bract[c]
Wild type[d]	12.5 ± 2.0	24.9 ± 2.0	3.1 ± 0.7	37.6 ± 4.6
Leafy[d]	10.7 ± 1.4	23.0 ± 1.0[e]	22.0 ± 5.9[e]	27.9 ± 7.0[e]

[a] Values are $n \pm$ SD.
[b] Metamer + bract refers to any metamer having a normal bract, or a structure that is obviously a reduced bract.
[c] Metamer − bract refers to any metamer having no obvious bract.
[d] Twenty-nine wild-type and 26 Leafy plants were analyzed.
[e] Significantly different from wild type at P = 0.05. Data were analyzed using a multiple Student's *t* test.

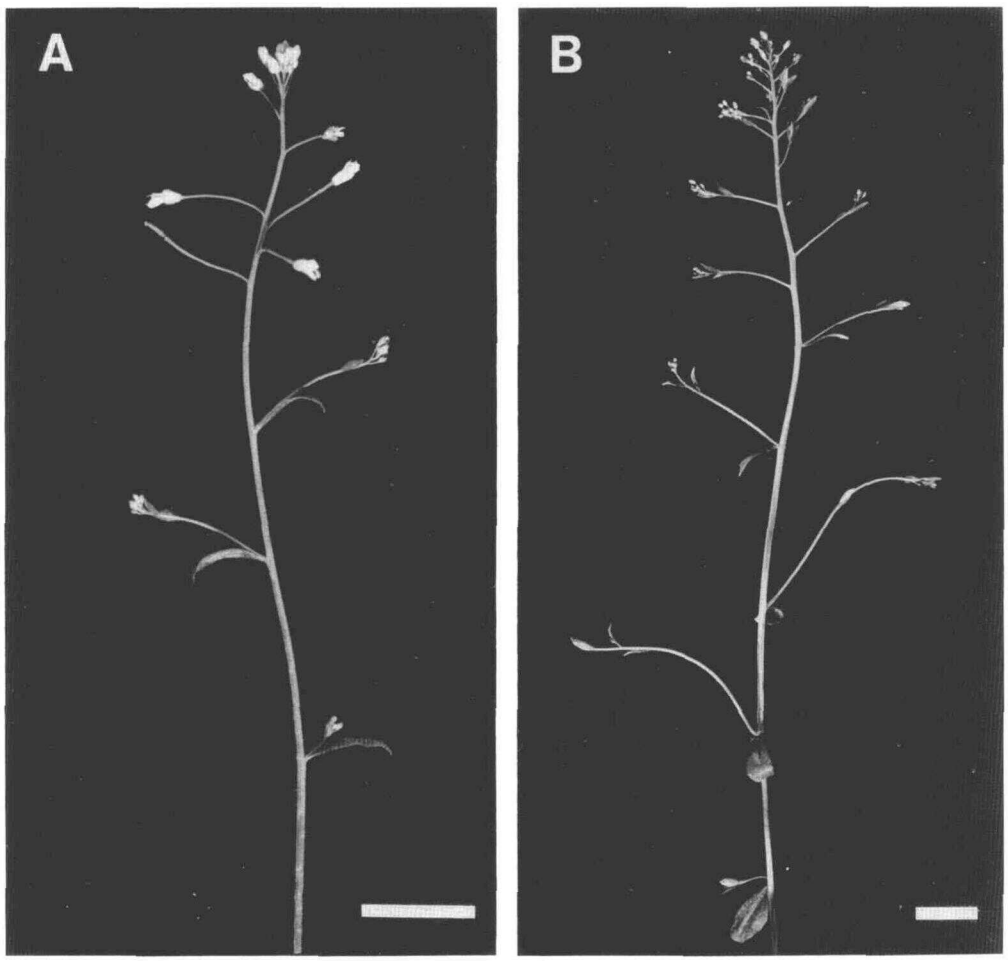

Figure 3. Light Micrographs Comparing Wild-Type and Leafy Inflorescences.

(A) Wild-type inflorescence.
(B) Leafy inflorescence (two basal coflorescence metamers not shown).
Bars = 1 cm.

al., 1989). At the top of the inflorescence, lateral shoots are again more coflorescence-like (Figures 1B and 3B).

We examined secondary lateral meristems from both Leafy and wild-type inflorescences to determine whether, as expected, the early development of Leafy lateral meristems is more similar to that of wild-type coflorescences than to flowers (Figure 5). Wild-type coflorescence meristems and Leafy meristems taken from the lower and middle region of the inflorescence all initiated organs in a spiral manner, with no apparent differences in the relative positions of the organs (compare Figure 5A to 5C). Occasionally, lateral meristems taken from the upper Leafy inflorescence initiate organs in a whorled pattern more typical of wild-type flowers (compare Figure 5B to 5D).

The identity of the lateral shoots is not the only metamer characteristic by which Leafy inflorescences differ from the wild type. On average, the first seven nodes of a Leafy inflorescence have normal wild-type bracts (Figures 1B and 3B; compare Figure 6A with Figure 6C), whereas the wild type typically has three (Table 1, Figures 1A and 3A). Several nodes following these may have bracts that are reduced in size (Figures 6D and 6E) or small filamentous structures (Figure 6F). Only in the middle region of the inflorescence are truly bractless nodes found (as in wild type, Figure 6B). Following the bractless nodes, nodes are again produced that have reduced bracts or filamentous structures. It should be noted that although there was a general trend toward the most coflorescence-like lateral

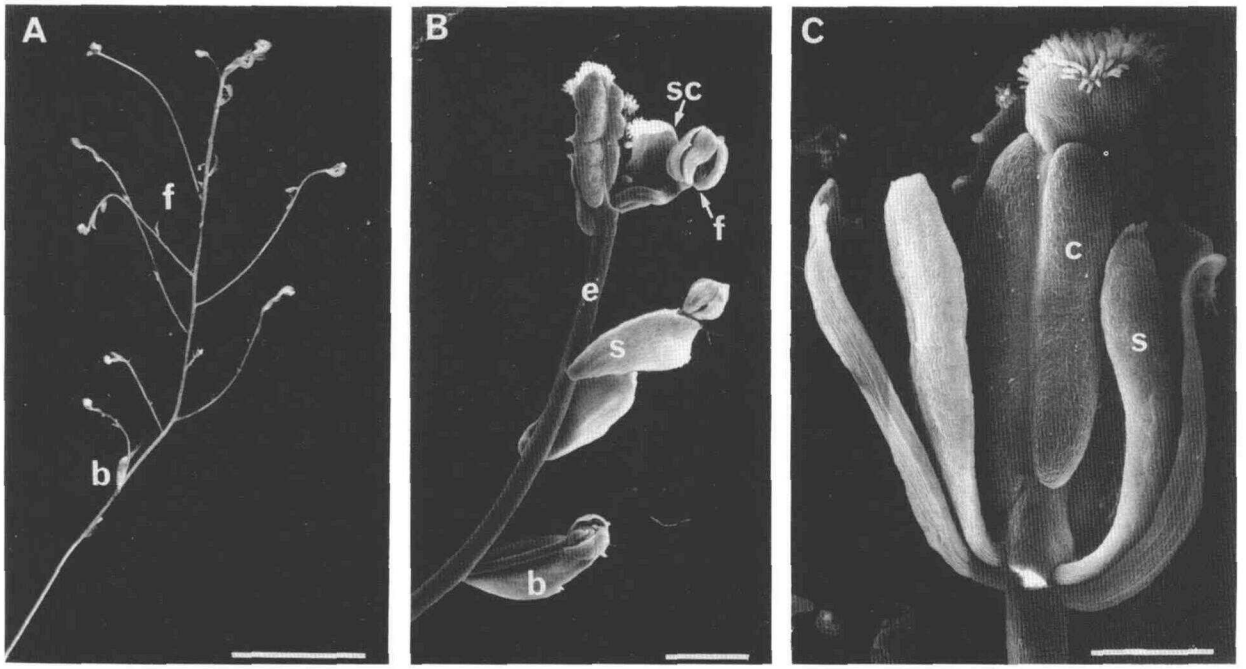

Figure 4. Light Micrograph and Scanning Electron Micrographs Illustrate Variation in Lateral Shoot Type within the Leafy Inflorescence.

(A) Light micrograph of extreme coflorescence-like shoot. Note development of numerous bracts (b) and lateral meristems into tertiary coflorescences (f). Bar = 1 cm.

(B) Scanning electron micrograph of lateral shoot with features intermediate between coflorescence-like and floral shoots. Note elongated internodes (e) and development of lateral shoots (f) in the axils of bracts (b), sepals (s), and sepal carpel intermediate organs (sc). Bar = 1 mm.

(C) Scanning electron micrograph of floral-like lateral shoot. Note that all organs are either sepal-like (s) or carpel-like (c), internodes do not elongate, and no lateral meristems develop. Bar = 0.5 mm.

shoots being subtended by bracts and the most flowerlike lateral shoots having no subtending bract, the correlation was not absolute, and some coflorescences within each inflorescence were not subtended by a bract.

One interpretation of the variation in metamer type of the Leafy inflorescence is that the product of the *lfy* allele is at least partially active and sensitive to changing physiological conditions in the shoot. For this reason, we determined whether the expression of *lfy* is temperature sensitive by growing plants at 16, 22, and 27°C. No major changes in phenotype were noted at any of the temperatures tested. However, carpels within the flowerlike structures produced in the upper inflorescence were often more completely fused and more fertile at 16 than at 22°C.

Aspects of Leafy development other than the inflorescence morphology are indistinguishable from wild type. The only exception to this is that Leafy plants tend to bolt slightly earlier than do wild-type plants (Table 1). However, there is no significant difference in the number of leaves produced in the rosettes (Table 1), suggesting that the early flowering is more likely due to a slightly faster germination or growth rate rather than an early switch from vegetative to reproductive development.

Double Mutant Analysis

Our analysis of the Leafy phenotype suggests that the *LFY* gene is required for flower development at a stage before the determination of floral organ type. We expected, therefore, that *lfy* would be epistatic to the floral organ homeotic genes. To test this hypothesis, we constructed a double mutant homozygous for *lfy* and a recessive allele (*ap2-6*) of the floral organ homeotic gene *AP2* (Kunst et al., 1989). The *AP2* gene was chosen for this analysis because it acts very early in floral development (Bowman et al., 1989; Kunst et al., 1989), and even strong alleles like *ap2-6* are fertile, simplifying confirmation of the genotypes of putative

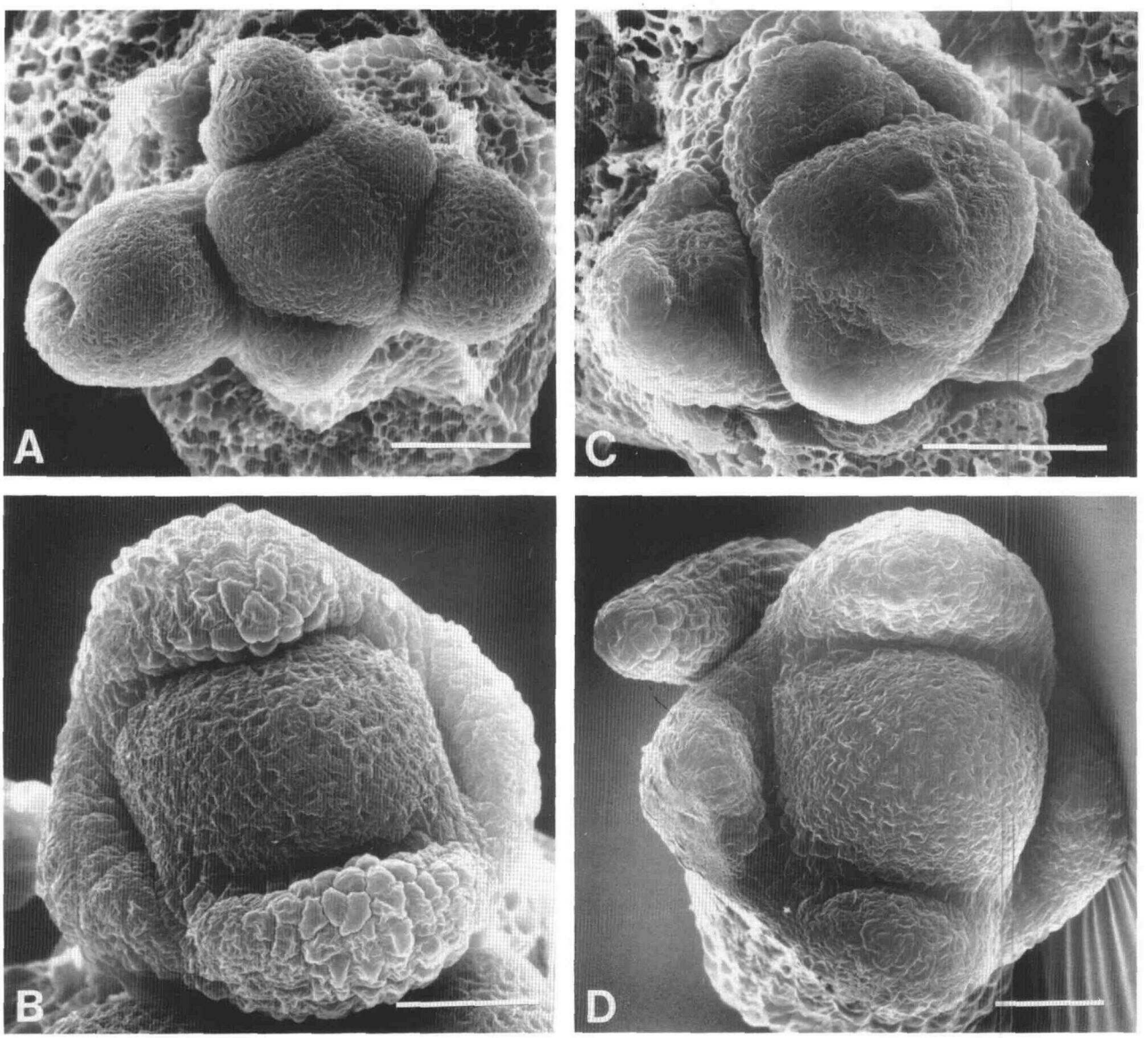

Figure 5. Scanning Electron Micrographs of Wild-Type and Leafy Secondary Lateral Meristems in Early Stages of Development.

(A) Wild-type secondary lateral meristem from type 2 (coflorescence-bearing) metamer. Note that primordia are initiated in a spiral manner. Bar = 0.05 mm.
(B) Wild-type secondary lateral meristem from type 3 (flower-bearing) metamer. Note that primordia are initiated in a whorled manner. Bar = 0.025 mm.
(C) Leafy secondary lateral meristem from midregion of inflorescence. Note that primordia are initiated in a spiral manner. Bar = 0.05 mm.
(D) Leafy secondary lateral meristem from the upper region of the inflorescence. Note that primordia are initiated in a whorled manner. Bar = 0.025 mm.

double mutants. Furthermore, because *ap2-6* affects the identity of sepals, one of two floral organ types produced in the more flowerlike Leafy lateral shoots, we anticipated that we might observe changes to these organs in the absence of *LFY*. Plants homozygous for *ap2-6* were used to cross-pollinate plants homozygous for the *lfy* allele. The F_2 progeny were found to have either a wild-type, Leafy, or Ap2-6 phenotype in a ratio of 138:45:40, respectively.

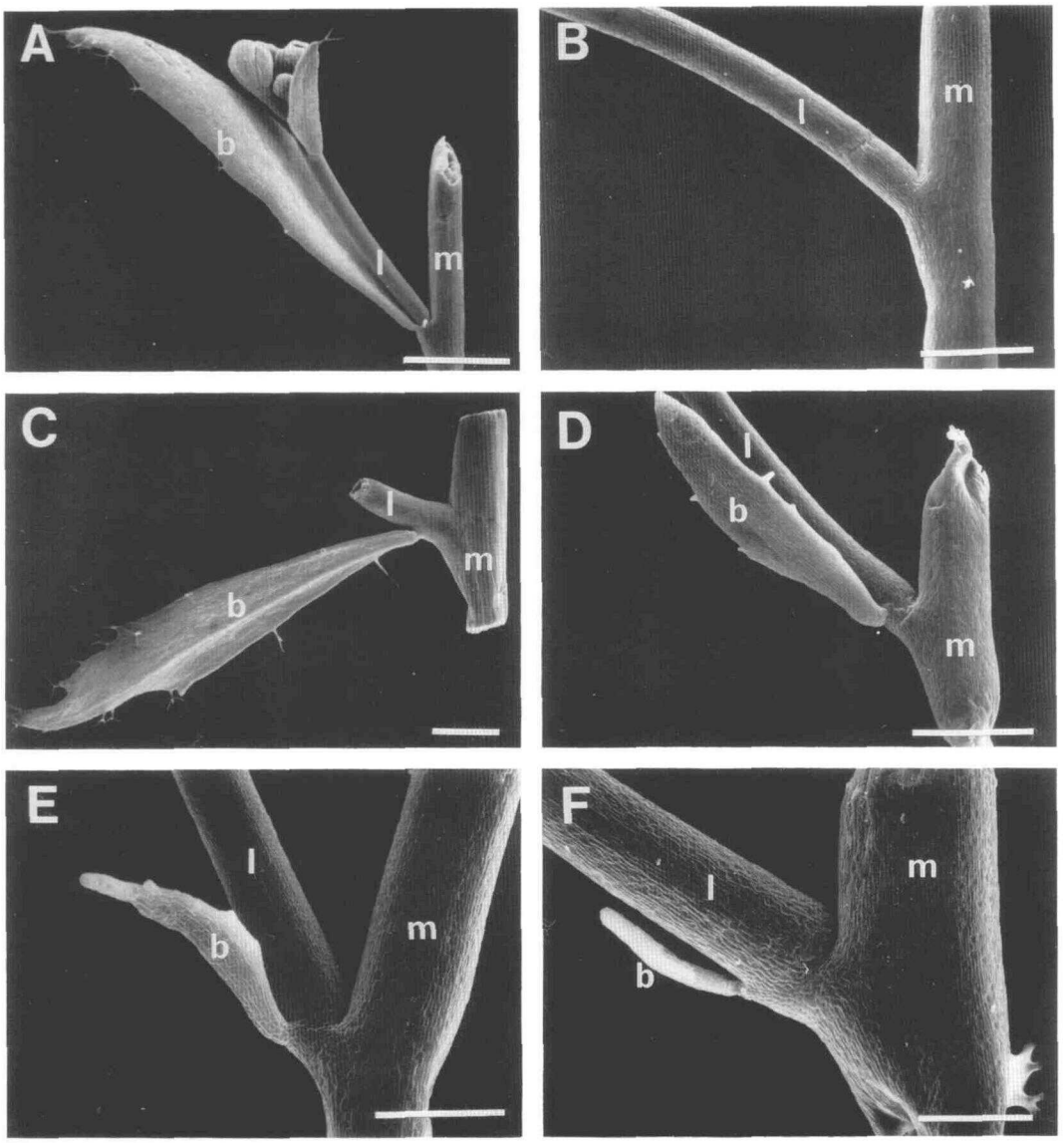

Figure 6. Scanning Electron Micrographs of Bracts or Bractlike Structures (b) Subtending Lateral Shoots (l) on the Main Inflorescence (m) of Wild-Type and Leafy Plants.

(A) Wild-type coflorescence with bract. Bar = 1 mm.
(B) Wild-type flower without bract. Bar = 0.5 mm.
(C) Leafy coflorescence-like shoot subtended by bract. Bar = 1 mm.
(D) Leafy coflorescence-like shoot subtended by a reduced bract. Bar = 0.5 mm.
(E) Leafy coflorescence-like shoot subtended by a reduced bract. Bar = 0.25 mm.
(F) Leafy coflorescence-like shoot subtended by a filamentous structure. Bar = 0.25 mm.

No obvious novel phenotype was detected. Although such a result suggests epistasis, the F_2 phenotypic ratio did not determine unambiguously which of the mutant phenotypes was epistatic. Therefore, we performed the following crosses to determine the epistatic relationship between *lfy* and *ap2-6*. F_2 plants having a Leafy phenotype were testcrossed to the Ap2-6 parent. Only one of these crosses was successful, and it produced only three seeds because of the low fertility of the Leafy phenotype. Of these three plants, two had wild-type phenotypes, and one had an

Ap2-6 phenotype, indicating that the F_2 Leafy parent had been heterozygous for *ap2-6*. The plant with the Ap2-6 phenotype (homozygous for *ap2-6*, heterozygous for *lfy*) was allowed to self-fertilize. Fifty-five of the progeny examined had an Ap2-6 phenotype, and 11 had a phenotype indistinguishable from Leafy (3:1, Ap2-6: Leafy; $\chi^2 = 2.44$, $P > 0.10$). Because all plants were homozygous for *ap2-6*, these data suggest that the Leafy plants are double mutants and, therefore, *lfy* must be epistatic to *ap2-6*.

The phenotype of the 11 Leafy-like double mutants was examined carefully to see if it differed from that of Leafy, especially with respect to organ identity within the secondary lateral shoots. As in Leafy, coflorescences, which have elongated internodes and produce bracts and tertiary shoots, are formed in the place of flowers (data not shown). The number of metamers having bracts is increased as it is in Leafy. The most coflorescence-like lateral shoots produce only bracts as organs. In the more flowerlike coflorescences, bracts, sepals, carpels, and organs intermediate among them are formed. We anticipated that in these more flowerlike coflorescences, there might be increased carpelloidy of the organs compared with those in the Leafy single mutant. Although there may have been a slight increase in carpelloidy in such shoots, the changes were not obvious amid the lateral shoot variability, and sepal-like organs with no carpel tissue were still observed.

DISCUSSION

The Leafy Mutant Identifies a Novel Gene

We have described the abnormal inflorescence morphology of an Arabidopsis mutant, Leafy, and have shown that its phenotype is due to a recessive allele (*lfy-1*) of a nuclear gene that we designate *LFY*. Two independently isolated mutants with phenotypes similar to but less severe than Leafy, one recently isolated by ourselves and one by Dr. D. Smyth (Monash University, Clayton, Australia; unpublished results), have been found to be *LFY* alleles by *trans*-complementation analysis (E.A. Schultz and G.W. Haughn, unpublished results). We designate our second allele *lfy-2* and Smyth's allele as *lfy-3*. The similarity in phenotype of these randomly induced alleles suggests that the Leafy phenotype represents a loss of function of the *LFY* gene.

LFY does not map near any gene known to affect inflorescence morphology, suggesting that it represents a novel locus. However, our data do not rule out the possibility that *lfy-1* is allelic to the closely linked gene *YI* (yellow inflorescence), a recessive allele (*yi*) of which affects the color of the inflorescence (Koornneef et al., 1983).

LEAFY Is Required for Development of Type 3 Metamers

In plants homozygous for the mutant *lfy-1* allele, coflorescence-like lateral shoots and bracts, both characteristics of type 2 metamers, are produced at most nodes of the inflorescence. Bractless flowers, characteristic of type 3 metamers, are never observed. Thus, the Leafy phenotype can be considered to be homeotic (type 3, flower-bearing metamers transformed to type 2, bract and coflorescence-bearing metamers) or heterochronic (Ambros and Horvitz, 1984) (the developmental switch from type 2 to type 3 metamers is delayed) at the level of the inflorescence metamer. These data suggest that the *LFY* gene product positively regulates morphogenesis of type 3 metamers and/or negatively regulates morphogenesis of type 2 metamers.

In the wild-type Arabidopsis inflorescence, coflorescence shoots are always subtended by bracts. In contrast, within all Leafy inflorescences, some of the coflorescence-like lateral shoots lack subtending bracts. This observation suggests that bract formation and lateral shoot type are independent developmental decisions regulated by *LFY*.

Because bolting occurs at the normal time in Leafy plants, it appears that the *lfy-1* allele does not affect the initiation of the inflorescence. Therefore, the switch from the production of type 2 to type 3 metamers, which requires the *LFY* gene product, represents a developmental step subsequent to, yet distinct from, the metameric switch defined by the late-flowering genes (Martinez-Zapater and Somerville, 1990).

The variability observed in the inflorescence metamers of Leafy-1 can be explained in at least two ways. The *lfy-1* allele might encode a partially active product that is sensitive to physiological differences within the inflorescence. Alternatively, *lfy-1* may represent a null allele. If the *LFY* gene product represents only one component of the regulatory mechanism controlling metamer type, the mechanism lacking *LFY* may be partially active and sensitive to physiological changes.

LEAFY Exerts Positive Control over Floral Morphogenesis

Because in the absence of *LFY* activity, coflorescences develop instead of flowers, *LFY* must have a positive role in specifying that lateral meristems produce a flower as distinct from a coflorescence shoot. Developmental decisions required to be made in this respect include organ type and arrangement, internode length, production of lateral meristems, and shoot determinacy. Genes known to be required for such decisions include *APETALA1*, *AP2*, *APETALA3*, *AGAMOUS*, and *PISTILLATA* (Komaki et al., 1988; Bowman et al., 1989; Kunst et al., 1989; Irish and

Sussex, 1990). One would expect, therefore, that these genes are positively regulated, either directly or indirectly, by *LFY*. The epistatic interaction between *AP2* and *LFY*, shown here, is consistent with this hypothesis because in the absence of *LFY* gene product, *AP2* would not be expressed.

Control of Inflorescence Structure

Through its role in the regulation of metamer type, *LFY* helps to establish the form of the inflorescence of Arabidopsis. We predict that any alteration in the timing of expression of *LFY* would result in a novel inflorescence structure. For example, expression of *LFY* earlier in development might eliminate type 2 metamers entirely, resulting in a simple raceme rather than the compound raceme of the wild type. Alternatively, expression of *LFY* later in development would result in a more highly branched inflorescence. It is easy to imagine that at least some of the great diversity of inflorescence structure observed among species of angiosperms (Rickett, 1944; Weberling, 1965; Foster and Gifford, 1974) may have evolved simply through variation in the timing of expression of genes like *LFY*. A similar hypothesis has recently been forwarded by Coen (1991) on the basis of his analysis on the snapdragon *flo* gene.

The *LFY* gene is required for lateral shoots to develop as flowers in Arabidopsis. Other genes must specify the development of coflorescences. We have recently identified a mutant of Arabidopsis, Terminator, in which coflorescences are replaced by flowers and the apical meristem itself differentiates as a flower after producing only a few (five or six) metamers (E.A. Schultz and G.W. Haughn, unpublished results). The phenotype of Terminator suggests that it is mutated in a gene (*TERMINATOR [TRM]*) expressed in both the primary apical meristem and lateral meristems of type 2 metamers and required for formation of inflorescence shoots. The phenotype of plants doubly homozygous for *lfy* and *trm* is intermediate between that of the two single mutants. Lateral shoots produced are more flowerlike than those produced in Leafy but more shootlike than those produced in Terminator. The apical meristem terminates prematurely in a flowerlike structure, but the termination occurs after more metamers (10 to 12) than in Terminator. The nature of the double mutant phenotype suggests that in the absence of *LFY*, *TRM* determines that meristems form coflorescence-like structures, whereas in the absence of *TRM*, *LFY* determines that meristems form flowers. Thus, in wild-type shoots, *TRM* and *LFY* not only direct the development of coflorescences and flowers, respectively, but also must regulate negatively the expression of each other within their respective domains.

We would like to note that the Terminator mutant is similar to descriptions of the Premature Termination of Inflorescence mutant (S. Shannon, C. Jacobs, and D.R. Meeks-Wagner. Analysis of the shoot apical meristem during the transition to flowering. Fourth International Conference on Arabidopsis Research, University of Vienna, Vienna, June 2–5, 1990) and of the Triple-Flower mutant (J. Alvarez, C.L. Guli, and D.R. Smyth. Mutations affecting inflorescence development in *Arabidopsis thaliana*. Fourth International Conference on Arabidopsis Research, University of Vienna, Vienna, June 2–5, 1990).

Developmental Decisions Made by *LEAFY* Are Not Unique to Arabidopsis

Variants or mutants having an abnormal inflorescence structure analogous to that of Leafy have been observed in a variety of plant species including snapdragon (Floricaula mutant, Coen et al., 1990; Squamata and Squamosa mutants, Schwarz-Sommer et al., 1990), tomato (Cauliflower mutant, Paddock and Alexander, 1952), and ribwort (variant, van Groenendael, 1985). Thus, similar mechanisms may regulate inflorescence development in a wide variety of species.

Of the mutants mentioned above, the Floricaula mutant of snapdragon is the best characterized (Coen et al., 1990). The snapdragon inflorescence is a simple raceme consisting of flowers subtended by bracts. In the Floricaula mutant, the bracts remain, but the flowers are replaced by coflorescence-like shoots, suggesting that the gene mutated in Floricaula (*flo*) regulates the identity of the lateral shoot but has no role in the development of the bract. Recently, the *flo* gene was cloned and used as a probe for in situ hybridizations to developing floral shoots (Coen et al., 1990). Surprisingly, *flo* was found to be expressed at the level of transcription not only in the floral meristem but also in the subtending bract primordia. It is possible that, like *LFY*, the ancestral *flo* gene regulated both lateral shoot development and bract development but *flo* has lost the ability to regulate the latter.

METHODS

Plant Material

The mutant line GH110, segregating the Leafy phenotype, was isolated from an ethyl methanesulfonate-mutagenized population of *Arabidopsis thaliana*, ecotype Columbia (Haughn and Somerville, 1988). Before phenotypic analysis was done, Leafy was back-crossed two times to the wild type, and individuals with the mutant phenotype were reselected from segregating populations. Line Ap2-6 has been described previously (Kunst et al., 1989). The lines W100 (*an*, *ap1*, *er*, *py*, *hy2*, *gl1*, *bp*, *cer2*, *ms1*, *tt3*;

Koornneef et al., 1987) and MSU23 (*cer3*, *er*, *gl3*, *tz*) of the Landsberg background were gifts from Maarten Koornneef (Department of Genetics, Wageningen Agricultural University, The Netherlands).

Normally, plants were grown at 22°C under continuous fluorescent illumination supplemented with incandescent light (100 to 150 μE m^{-2} sec^{-1} PAR) on Tera-lite Redi-earth (prepared by W.R. Grace & Co. Canada Ltd., Ajax, Ontario, Canada) in 6-inch-diameter plastic pots. Crosses were done on plants grown at 16°C.

Genetic Mapping

Recombination frequencies were determined by analyzing F_2 progeny using the method of Suiter et al. (1983). All values were corrected for double cross-overs with Kosambi mapping function $D = 25 \ln (100 + 2r)/(100 - 2r)$, where D = distance in centimorgans and r = estimated recombination percentage (Koornneef et al., 1983).

Light and Scanning Electron Microscopy

Morphological characterization of mutant phenotypes was performed on at least 10 coflorescence-like structures from each of approximately 30 plants. Structures were removed from various positions within the inflorescence at different stages of inflorescence development and examined with a dissecting microscope to determine trends in lateral meristem development within the inflorescence.

Rosette leaves, bracts, and coflorescence-like structures produced from the primary shoot were fixed for scanning electron microscopy. Samples were first vacuum infiltrated with 3% gluteraldehyde in 0.02 M sodium phosphate buffer (pH 7.2) and fixed overnight at 4°C. They were then rinsed in buffer and dehydrated on ice through a graded acetone series, followed by critical-point drying in liquid carbon dioxide. Finally, the specimens were mounted on stubs and coated with gold in an Edwards S150B sputter coater before examination in a Philips 505 scanning electron microscope at an accelerating voltage of 30 kV.

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