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LEAFY Controls Floral Meristem Identity in Arabidopsis

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Summary

The first step in flower development is the generation of a floral meristem by the inflorescence meristem. We have analyzed how this process is affected by mutant alleles of the Arabidopsis gene *LEAFY*. We show that *LEAFY* interacts with another floral control gene, *APETALA1*, to promote the transition from inflorescence to floral meristem. We have cloned the *LEAFY* gene, and, consistent with the mutant phenotype, we find that *LEAFY* RNA is expressed strongly in young flower primordia. *LEAFY* expression precedes expression of the homeotic genes *AGAMOUS* and *APETALA3*, which specify organ identity within the flower. Furthermore, we demonstrate that *LEAFY* is the Arabidopsis homolog of the *FLORICAULA* gene, which controls floral meristem identity in the distantly related species Antirrhinum majus.

Introduction

Adult organs of flowering plants develop from groups of stem cells called meristems. The identity of a meristem is inferred from structures it produces: vegetative meristems give rise to roots and leaves, inflorescence meristems give rise to floral meristems, and floral meristems give rise to floral organs such as sepals and petals. Not only are meristems capable of generating new meristems of different identity, but their own identity can change during development. For example, the vegetative shoot meristem is transformed into an inflorescence meristem upon floral induction, and in some species the inflorescence meristem itself will eventually become a floral meristem. Despite the importance of meristem transitions in plant development, little is known about the underlying mechanisms.

We and others are using a molecular-genetic approach to study flower development in two species, the common lab weed, Arabidopsis thaliana, and snapdragon, Antirrhinum majus (for review, Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991). Whereas a number of homeotic genes that specify organ identity within the flower have been extensively characterized (e.g., Klemm, 1927; Bowman et al., 1989, 1991; Hill and Lord, 1989; Kunst et al., 1989; Sommer et al., 1990; Yanofsky et al., 1990; Drews et al., 1991; Schwarz-Sommer et al., 1992; Jack et al., 1992), less attention has been devoted to the earliest step in flower development, namely how the floral meristem itself acquires its identity. Of the genes known to be required for the determination of floral meristem identity, *FLORICAULA* (*FLO*) in Antirrhinum has been studied in most detail. *FLO* is expressed transiently in early flower primordia, and inactivation of the *FLO* gene causes the transformation of flowers into inflorescence shoots (Carpenter and Coen, 1990; Coen et al., 1990). In Arabidopsis, the *leafy-1* (*lfy-1*) mutant exhibits a similar phenotype (Haughn and Somerville, 1988; Schultz and Haughn, 1991).

In this article, we describe in detail a phenotypic series of *lfy* mutant alleles and compare their development to that of wild type. Unlike *flo* mutants of Antirrhinum, complete loss-of-function alleles of *lfy* cause only a partial transformation of flowers into inflorescence shoots. We demonstrate that the transformation of flowers into inflorescence shoots is more complete when *lfy* mutations are combined with the *apetala1-1* (*ap1-1*) mutation (Irish and Sussex, 1990), indicating that the wild-type products of *LFY* and *AP1* act synergistically to determine floral meristem identity. We have cloned the *LFY* gene and show that *LFY* RNA is expressed strongly in young flower primordia, but not in inflorescence meristems, consistent with the proposal that *LFY* controls floral meristem identity. The molecular analysis reveals furthermore that *LFY* is the Arabidopsis homolog of the Antirrhinum gene *FLO*. Although the inflorescence structures of Arabidopsis and Antirrhinum are rather similar, functional comparison of *LFY* and *FLO* reveals that significant genetic and molecular differences exist in the way these two homologous genes control flower development.

Results

lfy Mutations Cause Partial Transformation of Flowers into Inflorescence Shoots

The primary inflorescence shoot of a wild-type Arabidopsis thaliana plant bears a small number of stem or cauline leaves and a potentially indeterminate number of flowers (Figures 1 and 2A). Leaves and flowers arise on the inflorescence in a phyllotactic spiral. In the axils of the cauline leaves, secondary inflorescence shoots develop. In contrast to many other species, Arabidopsis flowers are not subtended by the small leaves commonly referred to as bracts (Figure 2B; Weberling, 1981; Gifford and Foster, 1988). Arabidopsis flowers are composed of four concentric rings of organs, with four sepals in the first, outermost whorl, four petals in the second whorl, six stamens in the third whorl, and two fused carpels forming the gynoecium of the fourth, innermost whorl (Figure 2B; Müller, 1961; Smyth et al., 1990).

At least 15 mutant alleles of the *LFY* locus have been

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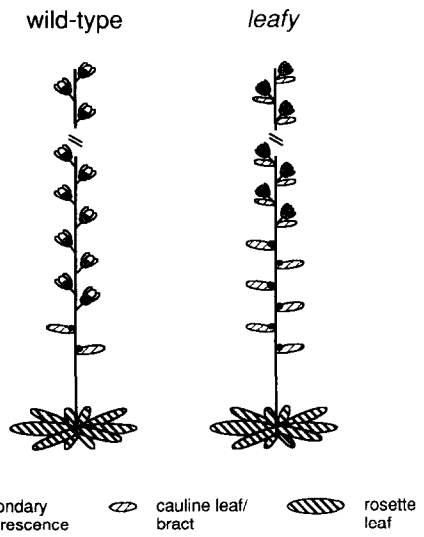


Figure 1. Schematic Representation of a Wild-Type and a Strong *lfy* Mutant Arabidopsis Plant

The inventory of floral organs in *lfy* mutant flowers is different from that of wild type (see text).

isolated, and we have studied 10 of them (Table 1). *lfy* mutants affect the primary inflorescence shoot in two ways (Figure 1). First, the number of secondary inflorescence shoots, which are subtended by cauline leaves, is increased (Table 2; Figures 2C, 2D). Second, the flowers that eventually develop are abnormal and show some characteristics of secondary inflorescences. These two aspects of the mutant phenotype suggest a complete transformation of early-arising flowers and a partial transformation of later-arising flowers into inflorescence shoots. (For a discussion of alternative ways to interpret the *lfy* phenotype, see Discussion). The switch from the production of secondary inflorescences to the production of abnormal flowers is not always very sharp, and structures that are intermediate between secondary inflorescence shoots and flowers may arise in the transition zone (Figures 2E, 2M).

According to the phenotypes of the aberrant flowers, the *lfy* alleles can be arranged in a phenotypic series with three classes: strong, intermediate, and weak (Table 2). In addition to plants homozygous for the different alleles, we have analyzed a number of heteroallelic combinations of *lfy*. All of the tested combinations exhibit phenotypes intermediate between the phenotypes of the parental alleles (data not shown). The phenotypic series of homozygous and trans-heterozygous *lfy* mutants indicates that all of the alleles are partial or full loss-of-function alleles. This was confirmed by our molecular analysis (see below), which showed that the strongest alleles appear to eliminate *LFY* function completely.

Strong Alleles

Flowers of plants homozygous for strong alleles exhibit various characteristics normally associated with secondary inflorescence shoots. The flowers are often subtended by floral bracts (Figures 2F, 2J; Schultz and Haughn, 1991), which are similar in morphology to the cauline leaves subtending secondary inflorescence shoots. The flowers in the axils of the bracts can be absent or aborted, especially later during inflorescence development (Figure 2I).

The outermost organs of a strong *lfy* mutant flower can be very much like cauline leaves (Figure 2G). Most organs in strong *lfy* flowers are sepal-like, or mosaic sepal/carpel organs (Table 2; Figures 2F–2H). The sepal-like organs often bear stellate trichomes, which are characteristic of wild-type cauline leaves produced by inflorescence shoots. Occasionally, secondary flowers arise from the axils of the outer organs (Table 2). Internode elongation, which is not found in wild-type flowers but is typical for inflorescence shoots, is observed in some flowers, especially when the outermost organs are very leaf-like. Internode elongation is more frequent in the early-arising flowers (7 out of 20 flowers in positions 1–4 vs. 10 out of 50 flowers in positions 5–14 in *lfy-6; er*) and in an Erecta $(Er)^+$ background (17 out of 70 flowers in positions 1–14 in *lfy-6;* Er^- vs. 26 out of 67 in *lfy-6*; Er^+). Strong *lfy* flowers are for all practical purposes male sterile, and, since the interior carpels fuse irregularly, female fertility is very much reduced.

Intermediate and Weak Alleles

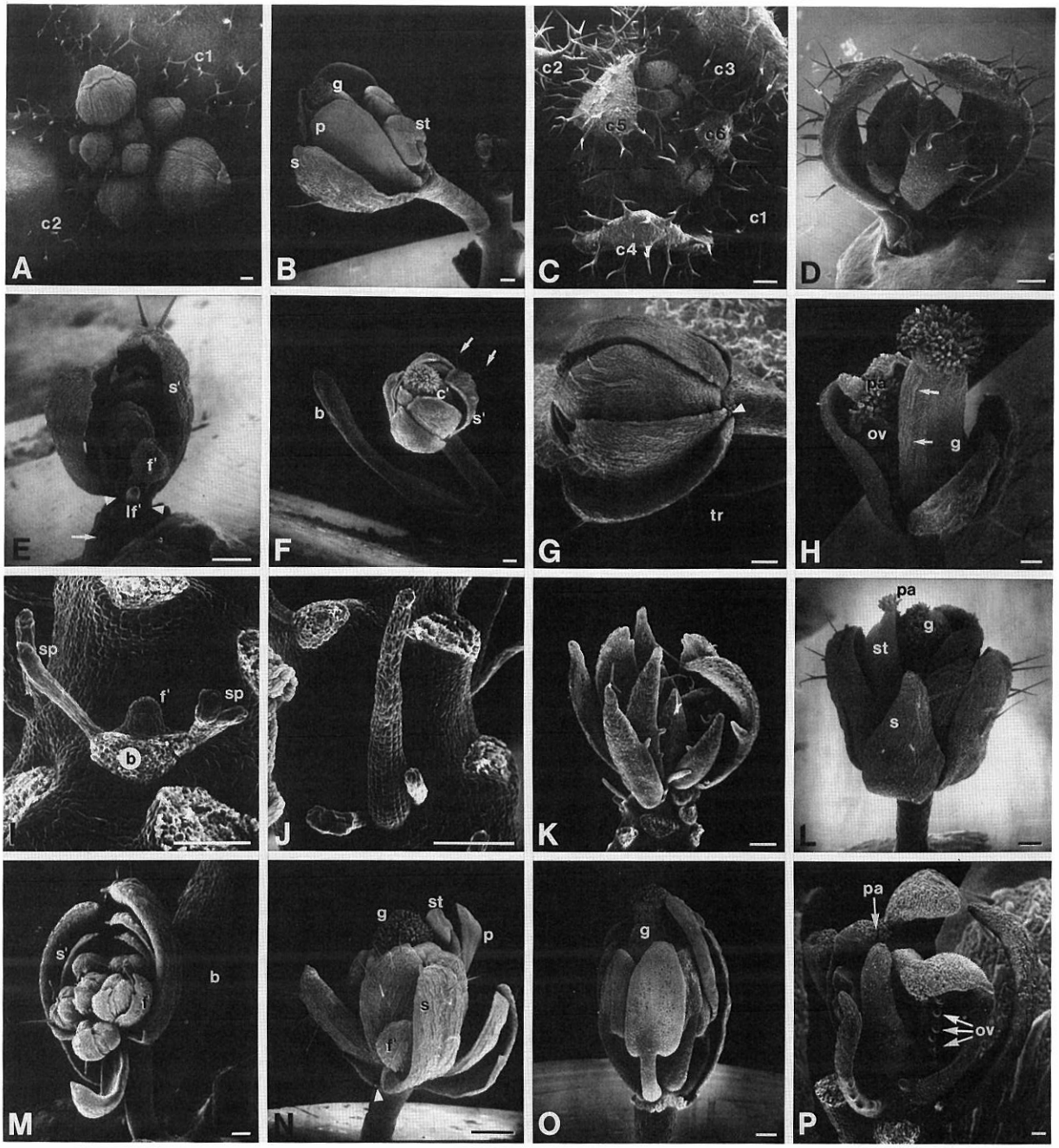
Flowers of intermediate and weak alleles have more organs with petal and stamen sectors, and more nearly normal petals and stamens, when compared with flowers of strong alleles (Table 2; Figures 2L, 2O). The first few flowers, especially the first and second flowers arising on the primary inflorescence, are more abnormal than the later-arising flowers in that they have fewer petaloid and stamenoid organs and more frequently have secondary flowers (Figure 2N), indicating a slow transition from true secondary inflorescence shoots to more flower-like structures. Well-developed floral bracts and internode elongation between floral organs are less frequently observed than in strong alleles. Since well-developed stamens are rare, flowers of intermediate alleles are most often male sterile, but female fertile. Flowers of weak alleles are both

Figure 2. Phenotype of Wild-Type and *lfy* Mutant Plants As Seen in the SEM

Bars represent 100 μm.

(A–B) L-*er* wild-type. (A) A primary inflorescence before bolting. There are two cauline leaves (c1, c2) followed by immature flowers of acropetally decreasing age. (B) A nearly mature flower (stage 12; Smyth et al., 1990) with sepals (s), petals (p), stamens (st), and gynoecium (g). Two sepals and two petals have been removed.

(C–K) Strong *lfy* mutants, all of the *lfy-6* allele, except (E), *lfy-7*. (C) A primary inflorescence before bolting. There are six cauline leaves (c1–c6) followed by abnormal flowers. (D) An immature secondary inflorescence removed from the axil of a cauline leaf. It repeats the pattern of the primary inflorescence. (E) A structure produced by the primary inflorescence, with a phenotype intermediate between a secondary inflorescence (D) and a flower (F). Note a secondary flower (f'), which is subtended by a reduced leaf (lf') flanked by stipules (arrowheads), and another filamentous



structure corresponding to a reduced leaf (arrow). The other visible organs (s') have epidermal cells characteristic of sepals. (F) First flower produced by an inflorescence. The flower is subtended by a cauline leaf-like bract (b). Most organs are sepal-like (s'), but they occasionally bear stellate trichomes (arrows), which are typical for cauline leaves. In the center, carpelloid organs (c') with stigmatic papillae are found. (G) The first flower produced by a different inflorescence, with one outer organ removed. The outer organ that is lowest on this panel is very leaf-like. It is thicker than a normal sepal, has a stellate trichome (tr) and stipules at its base (arrowhead), both characteristics of leaves. (H) The fourteenth flower produced by an inflorescence. The number of organs is reduced, and some of the outer organs are carpelloid with stigmatic papillae (pa) and ovules (ov). Arrowhead points to long cells typical for sepals on the central gynoecium (g). (I) An aborted flower primordium (f') in the axil of a bract (b), which *has been removed. Note abnormal stipules (sp). (J) A reduced, filamentous bract flanked by stipules. The corresponding flower has not developed.* (K) An old apex with numerous carpelloid bracts.

(L) Intermediate *lfy-3* allele; fifth flower produced by an inflorescence. There are more than four sepals (s), and the central gynoecium (g) is unfused. Some of the interior organs are chimeras with cells characteristic of stamens (st) as well as stigmatic papillae (pa).

(M–P) Weak *lfy-5* allele. (M) Structure produced by an inflorescence that has developed at 16°C. This structure, which is taken from position 9 above the true secondary inflorescences, is intermediate between a flower and a secondary inflorescence, and is subtended by a bract (b). The visible organs are leaf/sepal intermediates (s'). Numerous secondary flowers (f') develop. (N) First flower of an inflorescence that has developed at 25°C. One of the outer, sepal-like organs has stipules (arrowhead), which are typical for leaves. There are more than four sepals (s), and a secondary flower (f'). (O) Eighth flower, with three of the four sepals removed. The central gynoecium (g) is largely normal. It is surrounded by stamen/petal mosaic organs (compare with [B]). (P) An old apex with numerous carpelloid bracts, which have fused. Note stigmatic papillae (pa) and ovules (ov).

Table 1. Origin, Classification, and Sequence Analysis of *lfy* Mutant Alleles

Allele	Isolation #	Reference	Background[a]	Mutagen	Class	Sequence
1	–	1,2	Col-0	EMS	Strong	Gln32 > stop
2	–	2	NA	EMS	NA	NA
3	S192	3	L-*er*	EMS	Intermediate	Thr244 > Met
4	S679	3	L-*er*	EMS	Intermediate	Glu238 > Lys
5	S1251	3	L-*er*	EMS	Weak	Pro240 > Leu
6	S1339	3	L-*er*	EMS	Strong	Gln32 > stop
7	993	4	Ws-0	T-DNA transformant	Strong	Gln187 > stop
8	188	5	L-*er*	EMS	Strong	Gln32 > stop
9	278	5	Col-0	EMS	Intermediate	Arg331 > Tyr
10	S2380	3	Col-0	EMS	Weak	ND
11	S2143	3	L-*er*	EMS	Strong	ND
12	–	6	Col-0	EMS	ND	ND
13	–	6	Col-0	EMS	ND	ND
14	–	6	Col-0	EMS	ND	ND
15	T1	7	Col-C24	T-DNA transformant	Strong	Rearrangement after Gln196

NA, not available; ND, not determined.

References: 1, Haughn and Somerville, 1988; 2, Schultz and Haughn, 1991; 3, this work; 4, Feldmann, 1991; 5, S. Poethig, personal communication; 6, E. Huala and I. Sussex, personal communication; 7, P. Perez and D. Gerentes, personal communciation.

[a] Col, Columbia; L-*er*, Landsberg *erecta*; Ws, Wassilewskija.

Table 2. Mean Number of Secondary Inflorescences Arising from the Primary Inflorescence, and Mean Number of Organs in Wild-Type and in *lfy* Mutant Flowers

	25°C								16°C[a]		
Allele	(L-*er*)	*lfy-5*	*lfy-10*	*lfy-9*	*lfy-4*	*lfy-3*	*lfy-6*	*lfy-6*	(L-*er*)	*lfy-5*	*lfy-6*
Classification	Wild-type	Weak	Weak	Intermediate	Intermediate	Intermediate	Strong	Strong	Wild-type	Weak	Strong
Erecta phenotype	–	–	+	+	–	–	–	+	–	–	–
n[b]	10/150	6/60	6/56	5/43	5/50	6/51	6/60	6/44	20/300	6/60	6/50
Second-order inflorescences[c]	2.2	5.5	5.3	10.2	4.2	6.0	6.5	7.5	3.9	12.2	9.9
Flowers with Second-order Flowers	0.0	0.0	0.1	<0.1	0.0	0.0	<0.1	0.2	0.0	1.0	0.1
Floral Organs:											
Leaves or Sepals	4.0	3.9	3.9	3.8	4.4	4.8	8.9	9.5	4.0	9.9	15.7
Petals	4.0	1.3	0.6	0.4	<0.1	<0.1	0.0	0.0	4.0	<0.1	0.0
Stamens	5.8	2.6	1.7	0.7	<0.1	<0.1	0.0	0.0	5.6	<0.1	0.0
Other petaloid/stamenoid organs[d]	0.0	1.6	1.7	2.2	3.1	2.2	0.2	<0.1	0.0	0.2	<0.1
All petaloid/stamenoid organs	9.8	5.5	4.0	3.3	3.2	2.3	0.2	<0.1	9.6	0.3	<0.1
Carpels or sepal/carpels	2.0	2.1	2.1	2.7	3.0	2.9	5.4	4.9	2.0	5.2	4.9
Sum of organs	15.8	11.7	10.0	9.9	10.8	10.0	14.5	14.6	15.6	15.4	20.6

[a] See Experimental Procedures for a discussion of the data obtained at 16°C.

[b] The first number indicates the number of individuals, the second number the total number of flowers scored. Only the fifth to fourteenth flowers produced by the primary inflorescences of *lfy* mutants were scored, since the first few flowers often exhibited a stronger phenotype (see text).

[c] Includes all second-order inflorescences arising on the primary inflorescence, regardless of whether they were subtended by well-developed cauline leaves or not.

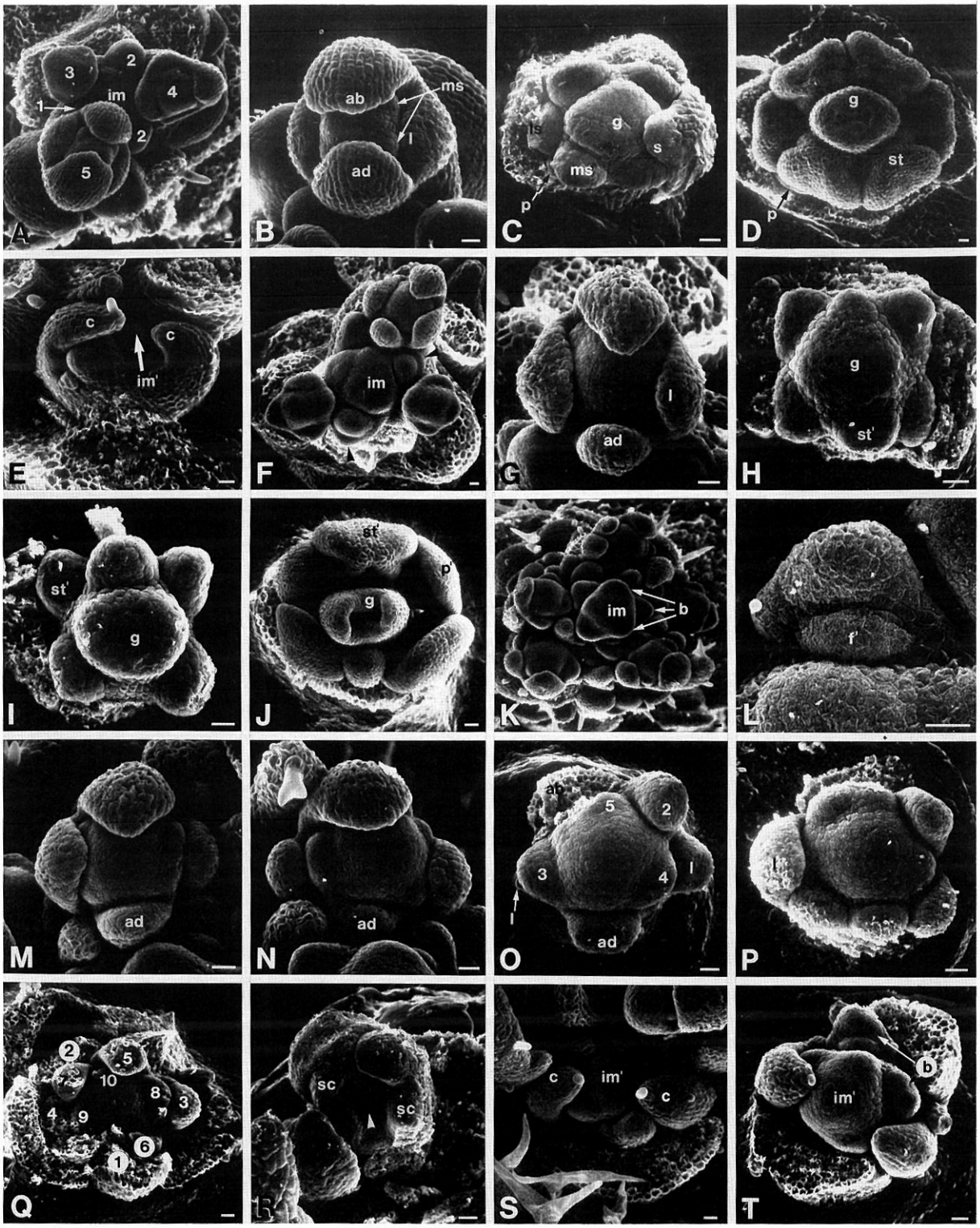
[d] Includes petal/stamen mosaic organs as well as all other mosaic organs that showed some stamen/petal characteristics, even if they had mostly sepal or carpel characteristics.

Figure 3. Development of L-*er* Wild-Type and *lfy* Mutants

Adaxial, abaxial, medial, and lateral refer to the positions of floral organs relative to the inflorescence meristem. Bars represent 10 μm. Stages of flower development are according to Smyth et al. (1990).

(A–E) Wild type. g, gynoecium; ls, lateral stamen; ms, medial stamen; p, petal. (A) Inflorescence apex with flower buds of stages 1 through 5. im, inflorescence meristem. (B) Stage 5 flower. The abaxial (ab) and adaxial (ad) sepals are larger than the two lateral (l) ones. (C) Stage 6 flower with all but one lateral sepal (s) removed. (D) Early stage 9 flower, four sepals removed. The petal primordia (p) are still small, whereas the stamens (st) are already well developed. (E) A young secondary inflorescence, with the subtending cauline leaf removed. Note that the first two organs produced, two cauline leaves (c), arise approximately opposite each other in lateral positions. im′, secondary inflorescence meristem.

(F–J) Weak *lfy-5* allele. (F) Inflorescence apex with flower buds at stages equivalent to stages 1 through 5 of wild type. The flower buds are surrounded by extra tissue that corresponds to rudimentary bracts (arrowheads). (G) Stage 5 flower. The pattern of the sepals is abnormal, in that the adaxial sepal (ad) is smaller than the two lateral sepals (l). Compare with (B). (H–I) Stage 6 flowers, four sepals removed. The flower in (H) is slightly younger than the wild-type flower in (C). Five or six primordia (st′), which are similar in size to wild-type stamen primordia, surround the prospective gynoecium (g). (J) Stage 8/9 flower, four sepals removed. All primordia surrounding the central gynoecium (g) have grown at a rate that is similar to wild-type stamens, although some are more stamen-like (st′) and others more petal-like (p′). The top of the gynoecium (g) is also abnormal. Compare with (D).



(K–T) Strong *lfy-6* allele. (K) Inflorescence apex with flower buds at stages equivalent to stages 1 through 5 of wild type. The buttresses at the flanks of the inflorescence meristem (im) are triangular in shape; they are developing bracts (b). (L) Close-up of a bract primordium with a flower bud (f') arising on the base of the bract. The bud is clearly separated from the main apex. (M–N) Flowers at a stage equivalent to stage 5 of wild type (B). The outer four sepals arise in an approximately cruciform pattern, but the pattern is often distorted. The adaxial sepal (ad) arises often at a higher position than the abaxial one; an extreme case is shown in (N). The primordia interior to the outer four sepals are not arranged in a whorled pattern. (O–P) Flowers at a stage equivalent to stage 6 of wild type (C). (O) The abaxial sepal (ab) has been removed. Of the lateral sepals (l), the left one is reduced and barely visible. A tentative spiral pattern can be superimposed on the primordia starting with the adaxial sepal (ad). The primordia succeeding the adaxial sepal are numbered from 2 to 5 according to their assumed position in the phyllotactic spiral. (P) Both medial sepals and the right lateral sepal have been removed (l, left lateral sepal). No clear whorled or spiral pattern can be recognized for the primordia interior to the outer four sepals. (Q) A flower at a later stage with six sepal primordia removed. A tentative spiral pattern is indicated. (R) A flower in which the interior-most primordia appear to be sepal/carpel mosaics (sc) as deduced from their shape and their partial congenital fusion. There do not appear to be additional, younger primordia in the center (arrowhead), indicating that the flower is determinate. (S–T) Secondary inflorescences developing in the axils of cauline leaves. im', secondary inflorescence meristem. (S) As in wild type (E), the first two cauline leaves (c) arise laterally, giving a developing secondary inflorescence a distinct appearance from wild-type (B) or mutant (M, N) flowers. (T) A later inflorescence with the two oldest leaves removed. It develops in a fashion similar to that of the primary apex (K); leaves/bracts (b) arise in a spiral pattern.

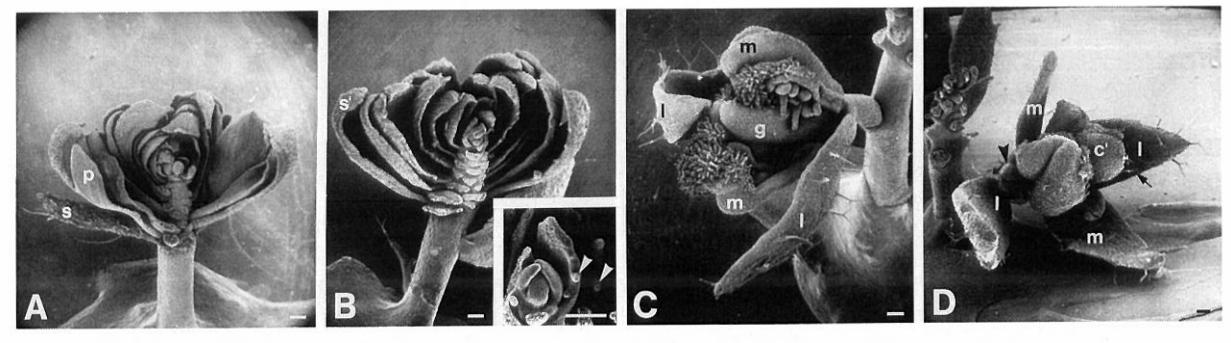


Figure 4. Mature Phenotype of Double Mutants between *lfy-6* and Floral Homeotic Mutations

Bars represent 100 μm.

(A) Amorphic *ag-1* mutant in the *er* background. The flower is indeterminate, and only sepals (s) and petals (p) develop.

(B) *ag-2; lfy-6* in the *er* background. The flower is indeterminate, as is the flower of the *ag* single mutant. The outer organs are sepal-like (s'). The inset is an enlarged view of the floral apex and shows that the more interior organs exhibit carpelloid characteristics, as evident from developing ovules (arrowheads). Compare with Figures 2F, 2G.

(C) *ap2-2* flower. The lateral first-whorl organs (l) are leaves; the medial first-whorl organs (m) are carpels. The interior carpels forming the gynoecium (g) are not fused.

(D) *ap2-2; lfy-6* flower. The lateral (l) and the medial (m) first-whorl organs are leaves. The interior organs are carpels (c') with some leaf character such as stellate trichomes (arrow). Note secondary flower buds (arrowhead). Compare with Figures 2F, 2G.

female and male fertile, although fertility is reduced compared with wild type.

Whereas all alleles produce sepal-like and carpel-like organs, well-developed petals and stamens are only observed in weak and, less frequently, in intermediate alleles. Thus, the formation of petals and stamens is more sensitive to loss of *LFY* activity than is the formation of sepals and carpels.

Typical for all classes of *lfy* alleles is that solitary carpelloid bracts appear on the inflorescence later in development (Figure 2K). These often fuse, and the apex terminates with a mass of carpelloid tissue (Figure 2P).

Developing *lfy* Flowers Exhibit Inflorescence Traits

To determine the origin of the defects seen in *lfy* mutants, we investigated the early development of secondary inflorescences and flowers in *lfy* mutants. The development of the supernumerary secondary inflorescences in *lfy* mutants closely resembles the development of wild-type secondary inflorescences (Figures 3E, 3S, 3T; Schultz and Haughn, 1991). This observation confirms the notion that the early-arising flowers are completely transformed into inflorescence shoots. The following description will focus on the development of the later-arising abnormal flowers in two mutants representing opposite ends of the phenotypic series of *lfy* alleles. The development of wild-type Arabidopsis flowers has been described in detail (Müller, 1961; Bowman et al., 1989; Hill and Lord, 1989; Smyth et al., 1990).

The inflorescence apex of the strong *lfy-6* allele produces bracts where flowers emerge in wild type (compare Figures 3A and 3K). Mutant flowers arise on the base of these bracts, but the emergence of flower buds is delayed relative to wild type (Figures 3K, 3L). The first four floral organs, which develop into sepals in wild type and into mostly sepal-like organs in *lfy* mutants, arise in a strong *lfy* allele in a fashion similar to wild type, although their pattern is often not perfectly cruciform, but somewhat twisted (Figures 3B, 3M, 3N). In addition, the adaxial first-whorl organ often arises higher on the receptacle than the abaxial one, indicating that the phyllotaxis of the outer organs is not strictly whorled as in wild-type flowers, but intermediate between the whorled mode of wild-type flowers and the spiral mode of inflorescence shoots. This becomes more obvious as the succeeding organs develop (Figures 3M–3Q), when often a spiral pattern can be superimposed on the emerging organs. This pattern, however, is normally not perfectly spiral as is the case for a bona fide inflorescence meristem. One difference between flowers and shoots is that the latter are indeterminate. Close inspection of the region enclosed by the interior-most carpel-like primordia did not reveal any aborted organs, proving that *lfy* flowers are truly determinate (Figure 3R). The analysis of early development of *lfy-6* flowers substantiates the view that these flowers have features of both wild-type flowers and inflorescence shoots.

In a weak allele such as *lfy-5*, the flower buds are surrounded by extra tissue resembling rudimentary bracts (Figure 3F). The presence of rudimentary bracts indicates that the later-arising flowers in *lfy-5* also have some characteristics of a secondary inflorescence. The outer four sepals and the central gynoecium arise largely normally (Figures 3G, 3J). The pattern in which the petaloid and stamenoid organs arise between the outer sepals and the central gynoecium appears to be whorled rather than spiral (Figures 3H, 3I), although it does not resemble the wild-type pattern.

Are Floral Homeotic Genes Active in *lfy* Mutants?

Since *lfy* mutants exhibit only a partial transformation of later-arising flowers into inflorescence shoots, we were curious what effect the floral homeotic mutations have on the *lfy* mutant phenotype. These mutations fall into three classes (Bowman et al., 1989, 1991). The first class,

represented by *apetala2* (*ap2*), affects the outer two whorls of floral organs and transforms sepals into carpels and petals into stamens. The second class, represented by *apetala3* (*ap3*) and *pistillata* (*pi*), affects the second and third whorl and transforms petals into sepals and stamens into carpels. The third class, represented by *agamous* (*ag*), affects the third and fourth whorls and transforms stamens into petals and carpels into sepals. In addition, *ag* mutants are indeterminate, such that the fourth-whorl sepals constitute the first whorl of another *ag* flower.

We constructed double mutants between a strong *lfy* allele, either *lfy-6* or *lfy-7*, and a representative of each of the three classes of homeotic genes (see Experimental Procedures for identification of double mutants). None of the homeotic mutants affects the early-arising structures in *lfy* mutants, confirming that the early-arising flowers are completely transformed into secondary inflorescence shoots. We monitored three aspects of the later-arising flowers: phyllotaxis, number, and identity of floral organs. With respect to the pattern of organ emergence (i.e., spiral or whorled phyllotaxis), *lfy-6* is epistatic to the two homeotic mutations *ap2-2* and *ag-2* (data not shown). With respect to the number of organs in a flower, both *ap2* and *ag* are epistatic to *lfy*. A major effect on the specification of organ identity is only observed in the *ap2-2; lfy-6* double mutant. In the following, we will briefly discuss the phenotypes of the different double mutants.

AP3

Flowers of double mutants between the strong *lfy-7* allele and the *ap3-1* mutant (Bowman et al., 1989) are not very different from the flowers of a strong *lfy* single mutant, except that mosaic organs with stamen or petal characteristics are absent (data not shown). This is consistent with the fact that the organs affected by *ap3-1*, petals and stamens, are almost completely missing in strong *lfy* alleles.

AG

In flowers of a double mutant between *lfy-6* and the strong *ag-2* allele (Bowman et al., 1991), many more organs develop than in *lfy-6* single mutants, reflecting the indeterminate nature of *ag* mutants (Figures 4A, 4B). In addition, the transition from sepals to sepal/carpel mosaic organs, while proceeding from outer to more interior organs, is delayed.

AP2

A novel phenotype is also observed in a double mutant between the strong *lfy-6* allele and the strong *ap2-2* allele (Figures 4C, 4D). The double mutant flowers have fewer organs than *lfy-6* single mutant flowers have (5.7 vs. 14.5 organs), similar to the effect of *ap2-2* in a *LFY* wild-type background (5.6 vs. 15.8 organs; Bowman et al., 1991). The outer organs are more leaf-like than in *lfy-6* single mutants, similar to lateral first-whorl organs in *ap2-2* (Bowman et al., 1991). The remaining organs are most often organs intermediate between carpels and leaves. In contrast to *lfy* single mutants, no sepal-like organs are observed.

In summary, the double mutant experiments indicate that the products of the homeotic genes *AG* and *AP2* are still active in a *lfy* mutant background, while the activity of *AP3* appears to be very much reduced.

The *ap1-1* Mutation Enhances the *lfy* Mutant Phenotype

Our molecular analysis (see below) indicated that the strong *lfy* alleles represent a complete loss of function. If residual *LFY* activity can be ruled out as the cause for the remaining floral characteristics in strong *lfy* mutants, there must be other factors that interact with *LFY* in determining floral meristem identity. Therefore, we expected that a complete transformation of flowers into inflorescence shoots would only be achieved when these other factors are eliminated in addition to *LFY*. One candidate for a mutation affecting floral meristem identity is the *ap1-1* mutation, which causes the development of ectopic secondary flowers in the axils of first-whorl floral organs (Irish and Sussex, 1990; Figure 5D). The development of secondary flowers can be interpreted as a partial conversion of a floral meristem into an inflorescence meristem. The first-whorl organs, when fully developed, are leaf-like, and they often arise at different levels on the receptacle (Irish and Sussex, 1990; Figure 5B). The pattern in which they emerge is often not strictly cruciform as in wild-type, but slightly twisted, similar to the first-whorl organs in a strong *lfy* mutant. This implies a phyllotaxis of the first-whorl organs that is intermediate between a spiral and a whorled mode, further corroborating the notion of a partial transformation of the floral meristem. The second and third whorls are also affected, in that the second-whorl petals are most often absent (Irish and Sussex, 1990), and the pattern in which the third-whorl stamens arise is often irregular (Figure 5C).

We constructed double mutants of *ap1-1* and the strong *lfy-6* allele as well as of *ap1-1* and the weak *lfy-5* allele. Both combinations show a dramatic enhancement of the *lfy* single mutant phenotype. No clear transition between secondary inflorescences and flower-like structures is evident. The first two organs of the later-arising, transformed flowers arise laterally, approximately opposite each other (Figures 5F, 5J), similar to what is observed in wild-type secondary inflorescences (Figure 3E). The succeeding organs emerge in a spiral fashion (Figures 5F, 5J, 5G, 5K). Most organs are very leaf-like, similar to the first-whorl organs of *ap1-1* single mutants (Figures 5H, 5L). In the axils of the leaf-like organs, secondary buds arise (Figures 5G, 5H, 5K, 5L). The synergistic effect is most obvious in the *ap1-1; lfy-5* double mutant. Whereas *lfy-5* single mutant flowers are much more normal than *lfy-6* flowers (Figures 2F, 2O), this difference largely disappears in the *ap1-1* background. As with *lfy* and *ap1-1* (Irish and Sussex, 1990; Bowman, 1991) single mutants, the severity of the phenotype of the double mutant flowers decreases acropetally.

Molecular Cloning and Identification of the *LFY* Gene

To understand how *LFY* exerts its function at the molecular level, we cloned the *LFY* gene. Using visible as well as restriction fragment length polymorphism (RFLP) markers, *LFY* was mapped to the lower half of chromosome 5 (Schultz and Haughn, 1991; see Experimental Procedures). Starting with the nearest RFLP marker, about 600 kb of contiguous genomic DNA was isolated by chromo-

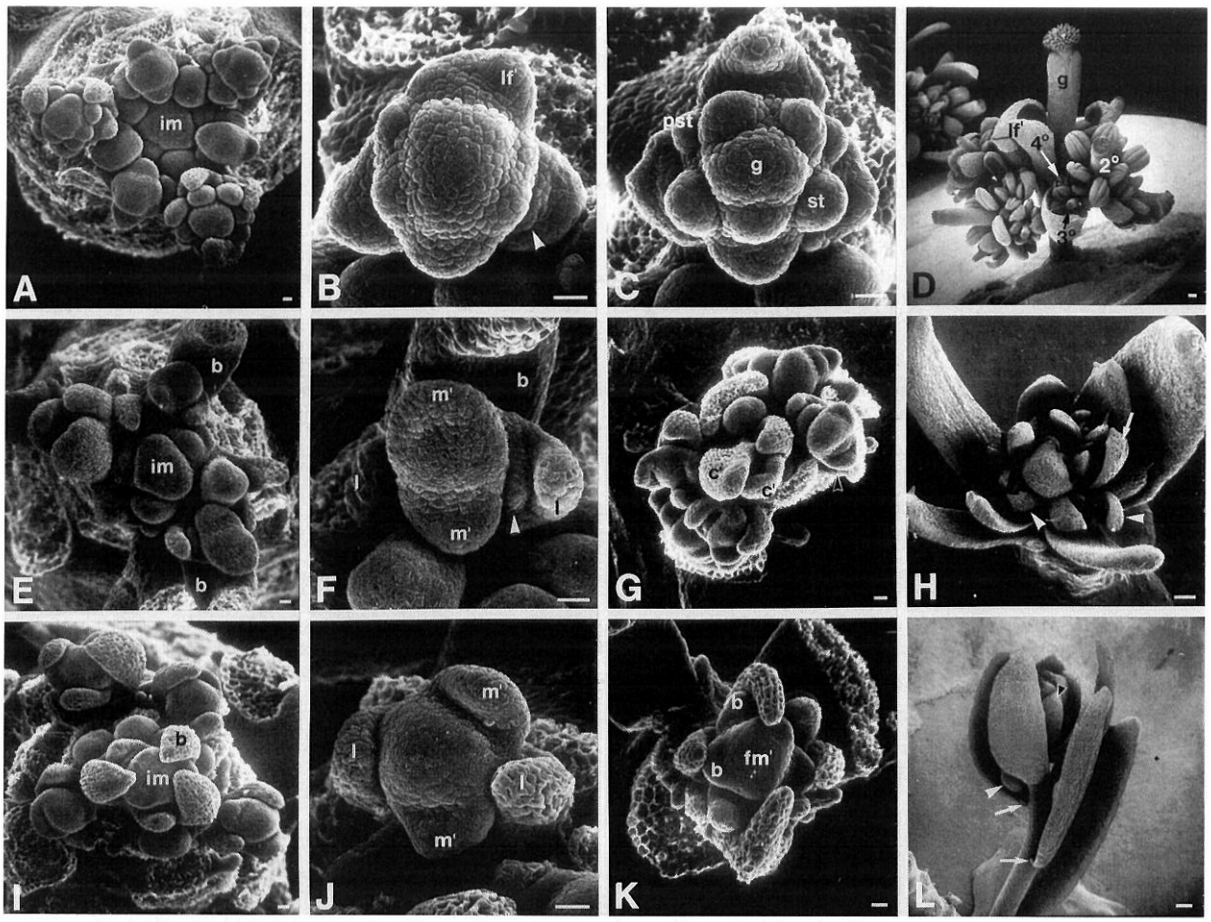


Figure 5. *ap1; lfy* Double Mutants

Bars represent 10 μm (A–C, E–G, I–K) or 100 μm (D, H, L).

(A–D) *ap1-1*. (A) Inflorescence apex with flowers at stages equivalent to stages 1 through 6 of wild type. im, inflorescence meristem. (B) Stage 5 flower. The first-whorl organs, which will develop into leaf-like organs (lf'), are abnormal in shape. Their pattern may be distorted, as in the extreme case shown here. An axillary flower bud (arrowhead) starts to arise in the axil of the right lateral first-whorl organ. (C) Stage 6 flower. The pattern in which the stamens (st) arise is abnormal (compare with Figure 3C). The two smaller primordia will probably develop into petal/stamen mosaic organs (pst). g, gynoecium. (D) Mature flower, the first one produced by an inflorescence. Secondary (2°), tertiary (3°), and quaternary (4°) flowers are visible. The first-whorl organs (lf') are very leaf-like, but have fewer trichomes, when compared with cauline leaves. The central gynoecium (g) is normal.

(E–H) *ap1-1; lfy-5*. (E) Inflorescence apex with transformed flowers at stages equivalent to stages 1 through 5 of wild type. Bracts (b) are more prominent than in the *lfy-5* single mutant (compare with Figure 3F). (F) Flower at a stage comparable to stage 5 of wild type. The two lateral organs (l), which arise first, are larger than the "medial" ones (m'), as in a developing secondary inflorescence (compare with Figure 3E). A secondary bud (arrowhead) is visible in the axil of the right lateral organ. (G) Flower at a later stage with six leaf-like organs removed. There are numerous secondary buds, which repeat the pattern of the primary buds (compare the bud indicated by an arrowhead with [F]). The later-developing primordia appear to be carpelloid (c'), but they arise also in a spiral pattern and rarely form a gynoecial cylinder. (H) A nearly mature flower consisting of numerous leaf-like organs with secondary buds in their axils (arrowheads). Some of the epidermal cells of the leaf-like organs have a morphology characteristic of carpels (arrow).

(I–L) *ap1-1; lfy-6*. (I) Inflorescence apex with transformed flowers at stages equivalent to stages 1 through 5 of wild type. (J) Flower at a stage comparable to stage 5 of wild type. The two lateral organs (l) are larger than the "medial" ones (m'), as in a developing secondary inflorescence (compare with Figure 3E). The organs are not arranged in a cruciform pattern (compare with Figures 3M, 3N). (K) Flower at a later stage with three leaves removed. The transformed flower repeats the pattern of the main apex (compare with [I]). (L) A mature flower consisting of numerous leaf-like organs with stipules at their bases (arrows) and secondary buds in their axils (arrowhead). Some of the epidermal cells have morphological characteristics of carpels (triangle).

some walking. By RFLP analysis, we could define a distal, but not yet a proximal, limit for *LFY* within the walk (Figure 6A). While this work was in progress, the *FLO* gene from the distantly related species Antirrhinum majus was cloned (Coen et al., 1990). *flo* mutants exhibit a phenotype reminiscent of our strong *ap1; lfy* double mutants, suggesting that *FLO* and *LFY*, or *AP1*, might be homologous genes. We isolated a genomic Arabidopsis clone cross-hybridizing to a *FLO* cDNA and found that this clone mapped to our walk in a position that is compatible with it being *LFY* (Figure 6A). To determine whether this gene was indeed *LFY*, we sequenced the genomic DNA encoding the *FLO* homolog from wild-type Arabidopsis and from 8 independently isolated *lfy* mutants. All 8 mutant alleles

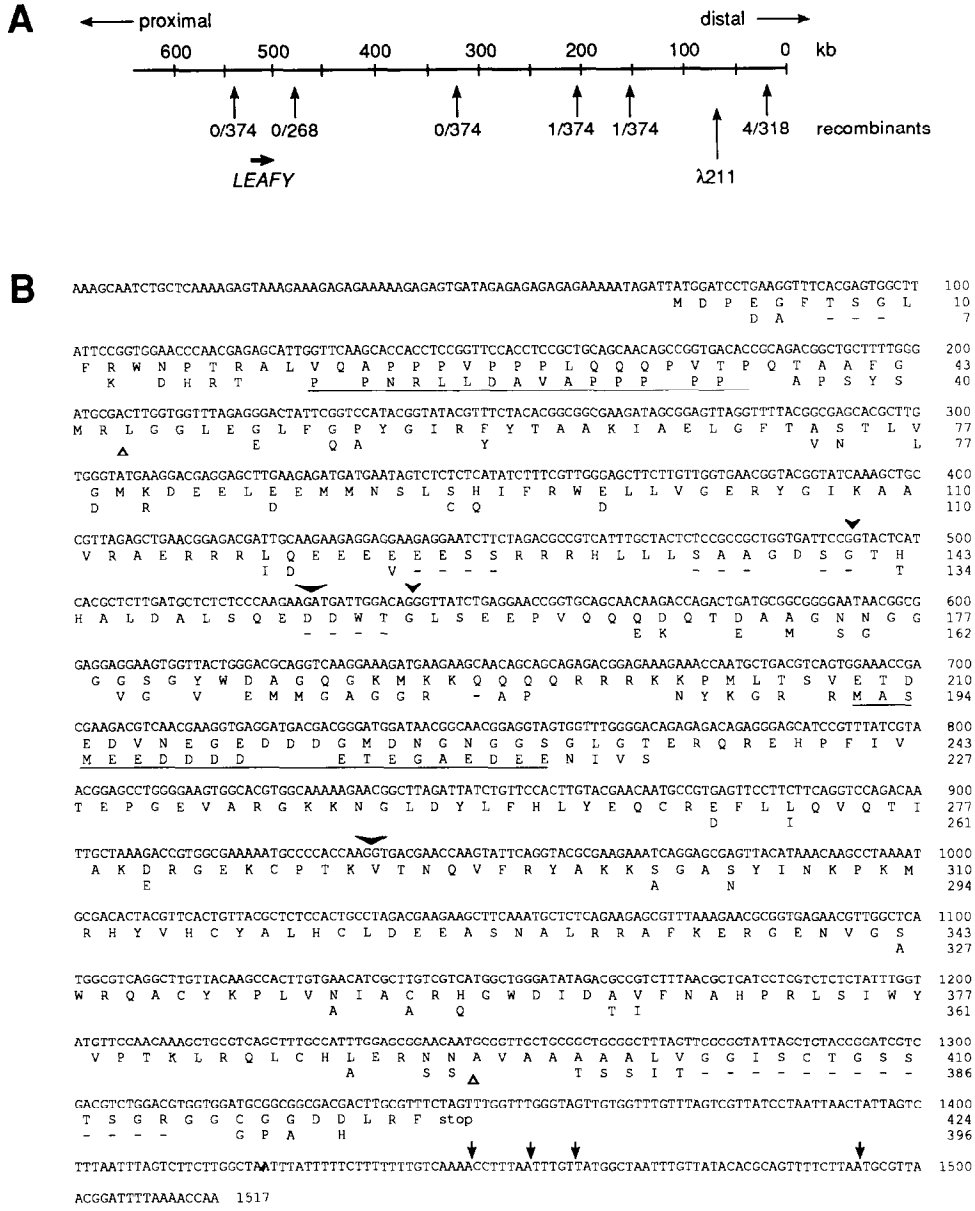


Figure 6. Cloning and Sequence of *LFY*

(A) Chromosomal walk in the *LFY* region. The entry point was the genomic clone 211 of Chang et al. (1988). Proximal and distal refer to the orientation on the chromosome. The numbers indicate the fraction of cross-overs detected between a Wassilewskija-0 chromosome carrying the *lfy-7* mutant allele and a Niederzenz-0 chromosome carrying a *LFY* wild-type allele. Recombinants were detected by RFLP analysis. See text and Experimental Procedures for details.

(B) cDNA sequence of *LFY*. The two large arrowheads above the sequence indicate the two introns of 458 and 910 bp, respectively. The two smaller arrowheads point to an additional splice donor site and an additional splice acceptor site at the first intron. Five out of ten sequenced cDNAs have the complete sequence shown here, four cDNAs are the result of splicing from the second splice donor site to the second splice acceptor site and are missing the four codons coding for DDWT, and one cDNA is the result of splicing from the first splice donor site to the first splice acceptor site and is missing the 12 codons coding for GTHHALDALSQE. The predicted amino acid sequence is shown below the nucleotide sequence. Below the *LFY* amino acid sequence, amino acids that are different in the predicted protein product of *FLO* (Coen et al., 1990) are shown. Gaps in the *FLO* sequence are indicated by dashes. The two open triangles below the *FLO* sequence indicate the positions of 3 and 1 additional amino acids, respectively, in the *FLO* sequence. A proline-rich domain near the amino terminus and an acidic domain in the middle of the proteins are underlined. The arrows above the 3′-untranslated sequence indicate alternative polyadenylation sites.

are associated with single codon changes in this gene (Table 1), demonstrating that *LFY* is the Arabidopsis homolog of *FLO*.

The molecular lesions in the different *lfy* alleles correlate well with the severity of the mutant phenotypes. Four strong alleles all have new stop codons, while 4 intermediate and weak alleles have missense mutations. Surprisingly, 3 of the strong alleles, *lfy-1*, *lfy-6*, and *lfy-8*, have

stop codons in the same position. In *lfy-1* (Haughn and Somerville, 1988), which was isolated from a different genomic background than the other 2 strong alleles, we also found silent changes within the *lfy* coding region, confirming its independent origin. Although the fourth strong allele, *lfy-7*, arose in a T-DNA insertion mutagenesis (Feldmann, 1991), it also has only a single base pair change, creating a premature stop codon.

The *LFY* gene encodes a message of about 1.6 kb as determined by Northern blot hybridization to poly(A)$^+$ RNA extracted from young flowers (data not shown). Compared with other floral control genes, *LFY* transcripts are rare. The abundance of *LFY* clones in two different cDNA libraries prepared from young flowers up to stage 10 is about two orders of magnitude lower than that of cDNAs for the homeotic genes *AG*, *AP3*, and *PI* (Yanofsky et al., 1990; Jack et al., 1992; K. Goto and E. M. M., unpublished data). We isolated overlapping clones representing a full-length cDNA by conventional and anchored polymerase chain reaction (PCR) (Saiki et al., 1988; Frohman et al., 1988). The genomic structure of the *LFY* transcribed region is similar to that of the Antirrhinum gene *FLO*, with two introns in homologous positions (Figure 6B). We found some heterogeneity at the first intron, with two different splice donor sites and two different splice acceptor sites being used in at least three different combinations. Of eight sequenced 5′ clones, seven start at the same position 71 bp upstream of the initiation ATG. There is considerable heterogeneity at the 3′ end, with at least five different polyadenylation sites being detected (Figure 6B). The three different *LFY* splice variants have a coding potential for proteins of 412, 420, and 424 aa. A search comparing the deduced *LFY* protein sequence with the GenBank data base detected significant homology only with the *FLO* protein. Alignment of the 420 aa isoform with the 396 aa *FLO* protein (Coen et al., 1990) reveals 70% (277/396) identical amino acids and 82% (326/396) identical and conserved residues (Figure 6B). The protein sequences are most highly conserved in the C-terminal parts. In addition, two domains, a proline-rich domain near the N-terminus and an acidic domain in the middle of the proteins (both underlined in Figure 6B), are conserved, although not at the level of primary sequence. Coen et al. (1990) have suggested that these domains might indicate that *FLO*—and by extension, *LFY*—is a transcriptional activator.

LFY RNA Is Strongly Expressed in Very Early Floral Primordia

Phenotypic analysis of *lfy* mutant alleles indicates that the *LFY* wild-type product is required very early in flower development. To learn how *LFY* expression correlates with the mutant phenotype, we studied the expression of *LFY* RNA in wild-type and mutant plants by in situ hybridization to tissue sections.

Wild Type

The most striking result of these experiments is the finding of strong *LFY* expression in young flower primordia surrounding the inflorescence apex. In contrast, we detected no expression in the inflorescence meristem proper, which is best documented in transverse sections (Figures 7A–7C; see Figure 3A for a scanning electron microscopic [SEM] view of a wild-type apex). The earliest expression of *LFY* is in cell groups that apparently have not begun to separate from the inflorescence meristem as buttresses ("1" in Figure 7C). Thus, the earliest expression appears to be in the anlagen of the floral primordia. During stage 1 of flower development (Smyth et al., 1990), expression of *LFY* RNA in the floral primordia increases ("4" in Figure 7C). Strong, uniform *LFY* expression is maintained in floral primordia of stage 1 through early stage 3 (Figures 7, 8A). During stage 3, shortly after the sepals have started to arise, *LFY* expression abates in the center of the incipient flower (Figure 8B). Strong expression is maintained in the sepal primordia until the end of stage 4 (Figures 7D, 8A). Although low levels of *LFY* expression can be detected in the central region between the sepals in stage 4 and stage 5, when the petal and stamen primordia arise, no clearly localized pattern can be recognized as is the case for the homeotic genes *AG* and *AP3* at these stages (Drews et al., 1991; Jack et al., 1992). During stage 6, when the petal and stamen primordia grow and the gynoecium emerges, the pattern resolves and *LFY* RNA can be detected in petal and stamen primordia and in the prospective gynoecium (Figures 8C, 8D). The expression in the stamens becomes restricted to the basal part of the primordia, from which the filaments will develop (Figure 8E). The expression in petals, filaments, and gynoecium is maintained until the end of stage 9 (Figures 8G, 8H), after which no *LFY* expression is detected (data not shown). The early expression is roughly 5-fold stronger than the late expression after stage 6, as judged by the density of hybridization grains in the same inflorescence.

Flowers and leaves arise in homologous positions at the flanks of an Arabidopsis shoot meristem. To address the question of whether *LFY* is expressed in all lateral primordia produced by the shoot meristem, we studied *LFY* expression in young plants before the shoot meristem starts to produce flowers. Only a weak signal was detected over young cauline-leaf primordia (Figure 8I), demonstrating that strong *LFY* expression is specific to floral primordia.

AP1

If *LFY* expression is indeed indispensable for the formation of normal flowers, we expect to detect *LFY* RNA in ectopic flower primordia formed in certain mutant backgrounds. In *ap1-1* mutant plants, ectopic secondary flowers arise in the axils of first-whorl organs of the primary flowers (see Figure 5; Irish and Sussex, 1990). *LFY* is expressed normally in the apex of *ap1-1* inflorescences (Figure 9A). In addition, strong *LFY* expression characteristic of young floral primordia is detected in ectopic floral buds that appear in the axils of first-whorl primordia at later stages (Figure 9B).

TERMINAL FLOWER

In *terminal flower-2* (*tfl-2*) mutant plants, the primary inflorescence produces only two to five normal flowers and then terminates with a flower that has more than the normal complement of floral organs (Alvarez et al., 1992). This phenotype has been interpreted as a transformation of the normally indeterminate inflorescence meristem into a determinate floral meristem (Shannon and Meeks-

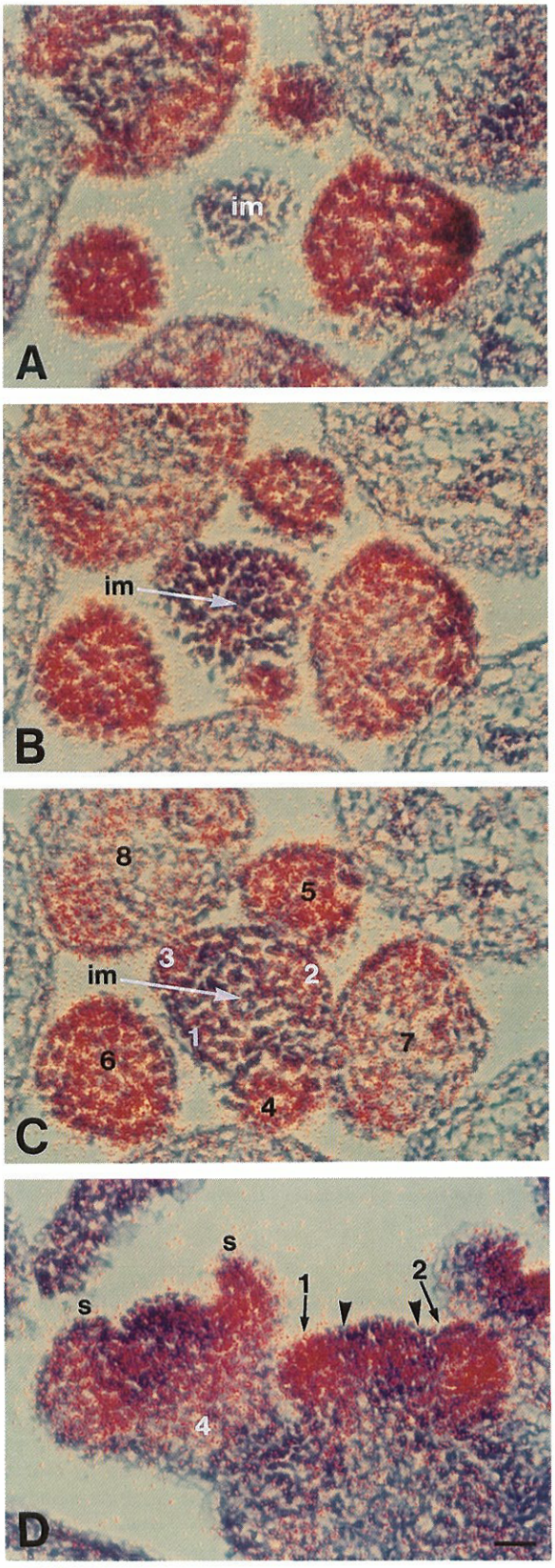


Figure 7. Expression of *LFY* RNA during Early Stages of Wild-Type Flower Development

Bar represents 20 μm. For an SEM view of a similar apex, see Figure 3A. Expression of *LFY* RNA was determined by in situ hybridization to 8 μm thick tissue sections. The tissue was stained with toluidine blue. All photos are bright-field/dark-field double exposures. For the dark-field exposures, a red filter was used, causing the silver grains of the emulsion to appear red.

(A–C) Three consecutive transverse sections of an inflorescence apex with (A) being uppermost. The region in the center of the apex, the inflorescence meristem proper (im), does not accumulate *LFY* RNA. The developing floral primordia, which surround the inflorescence meristem in a spiral pattern, are numbered 1 through 8, with 1 corresponding to the youngest primordium. A weak signal is detected at the flanks of the apex, corresponding to the anlage of a floral primordium (1) and floral buds of early stage 1 (2, 3). Expression increases during stage 1 (4), and is maintained throughout stage 2 (5, 6).

(D) Longitudinal section of an inflorescence apex. Strong expression is detected in stage 1 (1) and 2 (2) flowers on both sides of the inflorescence apex. The region between these two flower buds, as indicated by arrowheads, probably corresponds to an early stage 1 bud and shows a lower level of expression. In a stage 4 flower (4), strong expression is detected in the sepals (s), but not in the central region between the sepals.

Wagner, 1991; Alvarez et al., 1992). The transformation of inflorescence meristems into floral meristems is most dramatic in the lateral secondary inflorescence shoots, which are often replaced by solitary flowers (Alvarez et al., 1992). In young wild-type plants shortly after floral induction, the secondary inflorescence shoot in the axil of a cauline leaf is very small and does not express *LFY* (Figure 9C). In contrast, in a *tfl-2* plant of similar age, the secondary meristem has developed precociously into a large bud and expresses *LFY* RNA at high levels (Figure 9D), indicating that the secondary inflorescence meristem has been transformed into a floral meristem.

LFY

Finally, we studied *LFY* RNA expression in a strong *lfy* mutant. Our phenotypic analysis had suggested that the later-arising *lfy* flower primordia have a mixed identity of inflorescence and floral meristems—organs arise in a spiral phyllotaxis typical for inflorescences, but they consist largely of cell types typical for floral organs. The early expression of *LFY* RNA in *lfy-6* is normal, in that the lateral organs produced by the primary inflorescence meristem strongly express *LFY* (Figures 9E, 9F). These organs are floral bracts, which do not develop in wild type. When the floral primordia arise in the axils of the bracts, they also express *LFY* RNA at a high level, while the expression in the bracts diminishes (Figure 9E). Shortly thereafter, *LFY* expression in the floral bud begins to deviate from the wild-type pattern. The mutant floral meristems express *LFY* RNA strongly in the lateral organs they produce, thereby repeating the pattern of the primary inflorescence meristem (Figure 9F). The level of *LFY* expression is often lower than in the primary inflorescence, indicating again that the transformation of floral into inflorescence meristems is incomplete.

Discussion

In the developmental pathway leading to the formation of flowers in Arabidopsis thaliana, inflorescence meristems give rise to floral meristems, which then produce floral organ primordia. We have demonstrated that two factors,

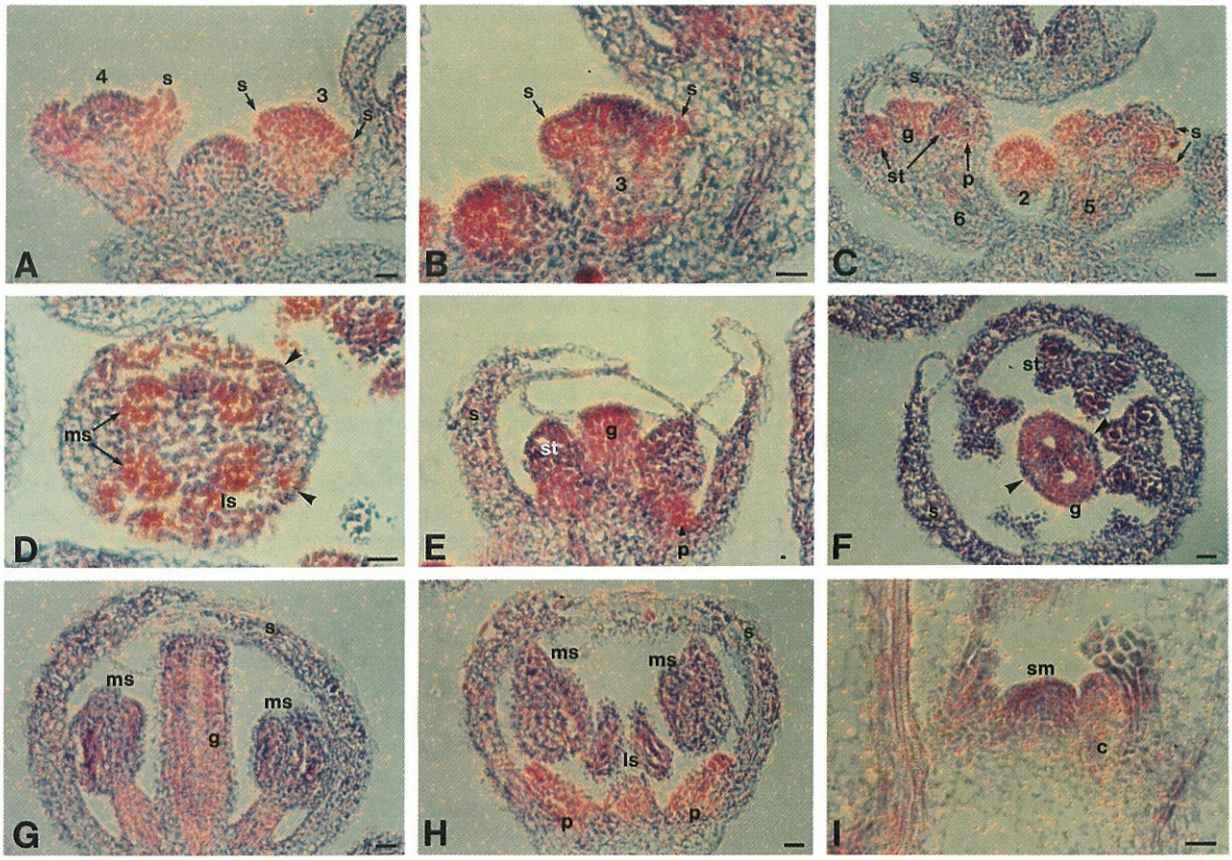


Figure 8. Expression of *LFY* RNA during Later Stages of Wild-Type Flower Development

Bars represent 20 μm. Longitudinal sections, except (D) and (F). For an SEM view of similar stages, see Figures 3A–3D. Numbers refer to stages of floral development according to Smyth et al. (1990).

(A–B) In early stage 3, shown in (A), *LFY* is expressed uniformly throughout the floral bud. In a slightly later stage 3 flower, shown in (B), expression is weaker in the center than in the arising medial sepals (s). Expression in the center is further reduced during stage 4, shown in (A), but does not disappear completely.

(C) Expression in the sepals (s) is reduced during stage 5. A patchy pattern is apparent in the center. At stage 6, the sepals (s) are largely devoid of *LFY* RNA, whereas expression is detected in the primordia of the petals (p), the stamens (st), and the central gynoecium (g).

(D) In this transverse section of a stage 6 flower of similar age as the one in (C), a signal is visible in the medial (ms) and lateral stamens (ls) as well as in the margins of the sepals (arrowheads). The small petal primordia are out of the plane of section.

(E) In a stage 7 flower, expression is detected in petals (p), at the base of the stamens (st), and in the gynoecium (g).

(F) In this transverse section of a stage 9 flower, a nonuniform signal in the two-carpelled gynoecium (g) is apparent. Arrowheads point to regions with lower levels of *LFY* expression.

(G–H) Two parallel sections of the same stage 9 flower. Weak expression is detected in the petals (p), in the filaments of medial (ms) and lateral stamens (ls), and in the gynoecium (g).

(I) Section of a young shoot apex shortly before the first floral primordia appear. No expression is detected in the shoot apical meristem (sm), but a weak signal is detected over a young cauline leaf primordium (c).

the products of the *LFY* and *AP1* genes, act synergistically in promoting floral over inflorescence development. Our molecular analysis of the *LFY* gene has revealed that *LFY* is expressed very early in floral anlagen and floral primordia, consistent with it having a direct role in establishing floral meristem identity.

LFY Controls Floral Meristem Identity

In wild-type Arabidopsis, floral meristems derive from inflorescence meristems, yet they execute a developmental program very different from inflorescence meristems. Thus, there must be factors that promote the determination of floral meristems as opposed to inflorescence meristems. *LFY* is one of these factors, and inactivation of *LFY* causes inflorescence shoots to develop in place of flowers. Although only the early-arising flowers in *lfy* mutants are completely transformed into inflorescence shoots, several features suggest that the later-arising flowers have partial inflorescence characteristics. First, many of the floral organs arise in a spiral phyllotaxis, which is typical of inflorescences. Second, the flowers are subtended by bracts, which resemble the cauline leaves that subtend secondary inflorescence shoots. Third, secondary flowers occasionally develop within the mutant flowers. Fourth, the outermost floral organs can be leaf-like, and even if predominantly sepal-like, they can have some morphological characteristics of cauline leaves such as stellate trichomes. Fifth, the lateral organs arising on the flanks of

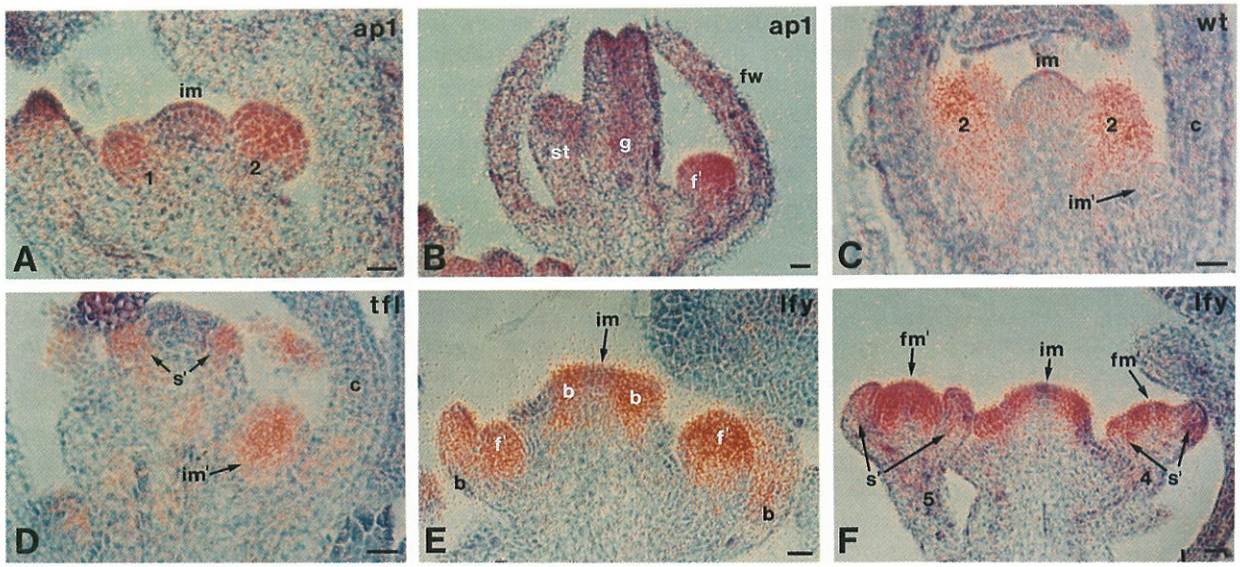


Figure 9. Expression of *LFY* RNA in Mutant Plants

Bars represent 20 μm. All panels are longitudinal sections. Numbers indicate stages of floral development.

(A–B) *ap1-1*. (A) Early expression in stage 1 and 2 buds is normal (compare with Figure 7D). (B) At later stages, ectopic buds (f') appear in the axil of first-whorl organs (fw). These buds show strong *LFY* expression at a level comparable to the early floral primordia arising on the inflorescence apex. im, inflorescence meristem; st, stamen; g, gynoecium.

(C) A young wild-type plant after a few floral buds have been initiated. In the axil of a cauline leaf (c), a small secondary inflorescence meristem (im'), which is devoid of *LFY* expression.

(D) A young *tfl-2* plant at the same age as the wild-type plant shown in (C) (12 to 13 days). In the axil of a cauline leaf (c), a well-developed bud, which shows strong *LFY* expression, is visible. This bud corresponds to a secondary inflorescence meristem (im') that has been transformed into a floral meristem. The primary inflorescence meristem expresses *LFY* RNA at its flank. The intensity of the signal indicates that this is probably expression in the ectopic sepals (s') developing on the inflorescence meristem, which has been transformed into a floral meristem and which is farther along in development than the secondary inflorescence meristem (Alvarez et al., 1992).

(E–F) Strong *lfy-6* mutant. (E) Strong expression is detected in the primordia flanking the inflorescence meristem (im). These primordia correspond to bract (b) primordia (for an SEM view, see Figure 3K). Floral buds (f') develop only later in the axils of these bracts. Strong expression is detected in the developing floral buds, while the signal in the growing bracts decreases. (F) At stages equivalent to stage 4 to 5 of wild type (compare with Figures 8A, 8C), strong expression is detected in the center of the abnormal flowers, whereas expression in the first-whorl sepal-like organs (s') is reduced. In the center, strong signal is detected in regions flanking the floral meristem (fm'), repeating the pattern of the primary inflorescence meristem.

the transformed floral meristem strongly express (nonfunctional) *LFY* RNA, as is typical for inflorescence meristems.

The residual floral character of the transformed flowers is revealed by the presence of organs that have epidermal cells with the characteristics of wild-type sepals and carpels. The floral character has been further confirmed by the interaction of strong *lfy* alleles with homeotic mutants that specifically affect the identity of floral organs. In double mutants homozygous for *lfy* as well as one of the floral homeotic mutations *ap2* and *ag*, a novel phenotype is observed, implying that both *AP2* and *AG* are at least partially active in a *lfy* mutant background. In contrast, an *ap3* mutation, which represents a third class of floral homeotic genes, has very little effect in a *lfy* mutant background, suggesting that *LFY* is an activator of *AP3*. The effect of *LFY* on organ identity, however, cannot be mediated only by *AP3*, since different types of organs develop in *ap3* and *lfy* single mutants as well as in double mutants such as *ap3; ag* and *ag; lfy* (Bowman et al., 1989, 1991).

It is interesting that organs with floral characteristics develop in *lfy* mutants, although these organs emerge in a pattern more typical for inflorescences. This validates the view that specification of organ identity and organ position are largely independent (Battey and Lyndon, 1990).

Schultz and Haughn (1991) reported that the *ap2-6* allele (Kunst et al., 1989), which is slightly weaker than the *ap2-2* allele (Bowman et al., 1991), has no effect in a *lfy-1* mutant background. We have shown that with respect to organ identity *ap2-2* is largely epistatic to *lfy-6*, which has the same molecular lesion as *lfy-1*. A similar effect has been found with the weak *ap2-1* allele (D. W. and E. M. M., unpublished data; see Bowman et al. [1989] for a description of *ap2-1*). We currently do not understand why Schultz and Haughn (1991) did not observe an effect of *ap2-6* in a *lfy-1* background, but it might be due to different genetic backgrounds or to the idiosyncratic properties of the *ap2-6* allele.

Three strong *lfy* alleles are predicted to produce only a 31 aa peptide compared with the wild-type protein of about 420 aa. Since it is unlikely that this short peptide retains wild-type activity, we propose that partial floral development in strong *lfy* mutants is due to factors acting redundantly with *LFY*. One of these factors appears to be the product of the *AP1* gene, since the transformation of flowers into inflorescence shoots is more complete in *ap1-1;*

lfy double mutants than in *lfy* single mutants. The novel double mutant phenotype also demonstrates that the *ap1-1* mutant phenotype, a partial conversion of floral into inflorescence meristems, is not mediated by *LFY*. This conclusion is supported by the finding that the early expression pattern of *LFY* is not noticeably altered in *ap1* single mutants. Further molecular analysis is required to determine how the synergism between *LFY* and *AP1* action is achieved.

Early-arising flowers require more *LFY* activity than later-arising ones, since even weak *lfy* alleles cause a complete transformation of the early-arising flower into inflorescence shoots. This difference in requirement of *LFY* activity suggests that other factors promoting flower development accumulate during the life cycle of the plant and that they will eventually allow partial flower development in all *lfy* mutants. Although it is possible that the product of the *AP1* gene becomes more active during inflorescence development, this does not appear to be very likely, since the severity of the *ap1; lfy* double mutant phenotype decreases acropetally as well. Notably, the severity of the *ap1-1* single mutant phenotype also decreases acropetally (Irish and Sussex, 1990; Bowman, 1991).

LFY Acts Locally in Floral Primordia

LFY RNA is expressed strongly in early floral primordia, indicating that *LFY* acts locally within the floral primordium to determine its developmental fate. In the developing floral primordium, *LFY* expression is detected much earlier than expression of the homeotic genes *AG* and *AP3* (Drews et al., 1991; Jack et al., 1992), suggesting that *LFY* has a role in controlling the expression of floral homeotic genes. The expression pattern of *LFY*, however, makes it unlikely that *LFY* provides direct positional cues for defining the spatial limits of homeotic gene expression. In early stage 3, when region-specific expression of the homeotic genes *AG* and *AP3* is first detected (Drews et al., 1991; Jack et al., 1992), *LFY* still is expressed uniformly throughout the whole flower. Only during stage 3, after the *AG* and *AP3* expression patterns are established, do regional differences in *LFY* expression become apparent. Although *LFY* is expressed at later stages in all floral organs except sepals, this later expression pattern does not correlate well with the specific effects of *lfy* mutants on the different types of floral organs.

Inflorescence development in Arabidopsis can be broken down into two phases: First, the primary inflorescence meristem produces cauline leaves associated with secondary lateral inflorescences. Then, the inflorescence meristem switches to the formation of flowers. We interpret the *lfy* mutant phenotype as a transformation of flowers into secondary inflorescences. An alternative view is that *lfy* mutations have a heterochronic effect on the inflorescence meristem and simply delay the switch from the first phase to the second phase of inflorescence development, i.e., from the production of secondary inflorescences to the production of flowers, similar to the prolongation of juvenile development in the *Teopod* mutants of maize (Poethig, 1988). We prefer the former interpretation, since our molecular results indicate that *LFY* acts in the floral primordia rather than in the inflorescence meristem.

LFY and *FLO* Are Homologous Genes

The predicted protein products of the *LFY* gene from Arabidopsis and of the *FLO* gene from Antirrhinum are 70% identical. By Southern blot hybridization, we have not detected any other gene with close homology to *FLO* in the Arabidopsis genome (D. W. and E. M. M., unpublished data), suggesting that *LFY* is the true Arabidopsis *FLO* homolog. The phenotypes of *flo* mutations in Antirrhinum are much more severe than the *lfy* mutant phenotype, in that the transformation of flowers into inflorescence shoots is essentially complete (Carpenter and Coen, 1990; Coen et al., 1990). The only indication that the determination of the floral meristem is regulated by partially redundant factors in Antirrhinum comes from the very infrequent observation of carpelloid organs in the *flo-640* allele (R. Carpenter and E. Coen, personal communication; Coen et al., 1990). The sequence of this allele, however, has not yet been determined, and it is thus unknown whether it represents a complete loss-of-function allele. If, as in Arabidopsis, determination of the floral meristem is regulated by partially redundant factors in Antirrhinum, the factor(s) not encoded by the *FLO* gene play a much more minor role in Antirrhinum than the additional factor(s) in Arabidopsis. It will be interesting to learn whether an *AP1* homolog exists in Antirrhinum, and what its function is.

Both *LFY* and *FLO* are expressed in floral primordia before any overt sign of differentiation, reflecting the role of *LFY* and *FLO* in determining floral meristem identity (this work; Coen et al., 1990). It has been suggested that the transient expression of *FLO* in sepal, petal, and carpel primordia reflects the role of *FLO* in establishing floral organ identity in Antirrhinum (Coen et al., 1990). One way to execute such a function would be to control the spatial boundaries of homeotic gene expression. Our comparison of the expression pattern of *LFY* with that of the homeotic genes *AP3* and *AG* (see above) makes this scenario unlikely for Arabidopsis. Preliminary experimental data indicate that *lfy* mutations indeed have a more pronounced effect on the level of transcription than on the spatial pattern of expression of at least one of the homeotic genes, *AP3* (D. W., T. Jack, and E. M. M., unpublished data). We would like to point out, though, that the situation in Antirrhinum might be different, since the Antirrhinum homeotic gene *DEFA* appears to be activated slightly later than its Arabidopsis homolog *AP3* (Schwarz-Sommer et al., 1992; Jack et al., 1992). It is noteworthy that in contrast to the early expression of *LFY* and *FLO* in floral primordia, the later expression pattern is not conserved, since only *LFY*, but not *FLO*, is expressed in stamens. This finding provides further support for the notion that the later expression of *LFY*, and probably also of *FLO*, plays only a minor role in exerting the known functions of *LFY/FLO*.

Dicotyledonous plants are characterized by an astonishing variety of inflorescence structures (e.g., Weberling, 1981). Although Arabidopsis and Antirrhinum inflorescences are both of the raceme type, differences in inflores-

cence structure exist. As with most flowers of dicotyledonous plants, wild-type Antirrhinum flowers are subtended by leaf-like bracts. In contrast, Arabidopsis flowers are not subtended by bracts, and one function of the *LFY* gene is to suppress bract formation. The earliest expression of *FLO* is detected in bracts, but the bracts are unaffected by *flo* mutations (Coen et al., 1990). Similarly, the earliest expression of nonfunctional *LFY* RNA in a *lfy* mutant is detected in the ectopic bracts, suggesting that the bracts in *lfy* mutants are homologous to the bracts subtending Antirrhinum wild-type flowers. This result sheds light on the unexpected finding that *FLO* is expressed in wild-type Antirrhinum bracts: The reason is apparently that flower and subtending bract derive from a common anlage and that *LFY* and *FLO* are expressed in all cells of the common anlage. This common anlage appears to be subdivided into floral primordium and bract primordium by factors that are activated independently of *FLO* and *LFY*. In Arabidopsis, the bract-inducing factor has come under negative control by *LFY*, and bracts are therefore suppressed in wild-type Arabidopsis.

Functional conservation of floral control genes has recently been demonstrated for the homeotic genes *AP3* of Arabidopsis and *DEFA* of Antirrhinum, which are 58% identical at the amino acid level (Jack et al., 1992). Both the mutant phenotypes and the expression patterns of *AP3* and *DEFA* are very similar (Klemm, 1927; Bowman et al., 1989; Sommer et al., 1990; Jack et al., 1992; Schwarz-Sommer et al. 1992). In contrast, with the comparison of *LFY* and *FLO*, functional differences between the two distantly related species Arabidopsis and Antirrhinum are beginning to emerge. By interspecific transformation experiments we will be able to determine whether these differences are encoded in the *LFY/FLO* DNAs or in transacting factors.

Experimental Procedures

Genetic and Phenotypic Analyses

All double mutants segregated at the expected ratio of about 1:15. The genotype of double mutants was confirmed by molecular or genetic tests. For *ap3* and *ag*, putative double mutants were analyzed by PCR (Saiki et al., 1988) for the presence of the known *ap3-1* (Jack et al., 1992) or *ag-2* (Yanofsky et al., 1990) mutations. For *ap1-1*, seeds were harvested from individual *ap1-1* F_2 plants derived from the *lfy* × *ap1-1* crosses. All F_3 plants had to be homozygous for *ap1-1*, and two-thirds of the families segregated a new phenotype 1:3, identical to the one seen in approximately 1/16 of the F_2 generation, confirming that the new phenotype was the *ap1-1; lfy* double mutant phenotype. For *ap2-2*, the same strategy was employed, except that seeds from F_2 plants were not kept separate because of the low number of seeds from individual plants (*ap2-2* plants are semisterile).

All phenotypic analyses described were performed at 25°C. The phenotypes of the strong *lfy-6* allele, of the intermediate *lfy-3* and *lfy-9* alleles, and of the weak *lfy-5* and *lfy-10* alleles were also tested at 16°C. At 16°C, the number of cauline leaves increases both in *lfy* mutants and in wild type (Table 2). In the strong *lfy-6* allele, the number of organs in the abnormal flowers is increased, but the general phenotype is largely unchanged. The phenotype of the weak and intermediate alleles becomes more severe, in that the early-arising flowers are subtended by bracts, develop fewer petals and stamens, and produce more secondary flowers (Table 2; Figure 2M for *lfy-5*). The later arising flowers, however, show a similar phenotype as the early-arising ones at 25°C. The more severe phenotype at lower temperature does not simply resemble the phenotype of stronger alleles, and we interpret it as a slower transition from true secondary inflorescences to more flower-like structures.

SEM was performed as described by Bowman et al. (1989). The samples were viewed at 10 or 20 kV accelerating voltage.

Meiotic Mapping and Chromosomal Walking

For meiotic mapping with visible markers, *lfy-1* was crossed to the tester strain *ttg yi* (Koornneef et al., 1989), and F_2 plants were scored for the three mutant phenotypes. Only 1 *yi lfy* recombinant, which was also *ttg*, was found among 957 F_2 individuals. Using the product ratio method (Stevens, 1939), *lfy* was determined to be about 10.2 ± 3.2 cM from *yi* on the lower half of chromosome 5, in good agreement with the data of Schultz and Haughn (1991).

For meiotic mapping with RFLP markers, *lfy-7*, isolated from the Ws-0 ecotype, was crossed to wild-type plants of the Nd-0 ecotype. DNA was prepared from F_2 plants with *lfy* mutant phenotype. Seeds were harvested individually from the phenotypically wild-type F_2 siblings. DNA was prepared from F_3 families after they had been scored for segregation of *lfy* mutants. DNA from individual F_2 plants or from F_3 families was analyzed by Southern blot hybridization for the segregation of RFLP markers, first derived from the published RFLP maps (Chang et al., 1988; Nam et al., 1989), and then from the walk.

Yeast artificial chromosome (YAC) (Ward and Jen, 1990; Grill and Somerville, 1991) and cosmid (Yanofsky et al., 1990) libraries were used for chromosome walking. End probes from positive YACs were generated by plasmid rescue and by inverse PCR (Ochman et al., 1990). The ends were tested for detection of RFLPs between Ws-0 and Nd-0. Since the end fragments were often short and did not detect any RFLPs, they were also used to isolate cosmids. Cosmids helped to align the YACs, since in several cases ends of nonoverlapping YACs mapped within a cosmid. Progress of the walk was monitored by RFLP analysis. Details of the walk are available on request.

Cloning of the *FLO* Homolog

Appropriate conditions for cross-hybridization were first determined on genomic Southern blots. The insert of the *FLO* cDNA clone pJAM101 (Coen et al., 1990) was labeled with ^{32}P and hybridized to a genomic Southern blot at 55°C without formamide. Two washes at 65°C with 2 × SSPE resulted in a single strongly cross-hybridizing band. A genomic cosmid library (Yanofsky et al., 1990) constructed from the L-*er* ecotype was screened under the same condition, and several overlapping cosmids were isolated.

Isolation of *LFY* cDNAs

We used a genomic *LFY* probe to screen 5 × 10^6 pfu representing 1 × 10^6 primary pfu of the cDNA library of Yanofsky et al. (1990). Since no *LFY* cDNA clone was identified, we used anchored and conventional PCR to isolate full-length *LFY* cDNAs (Saiki et al., 1988; Frohman et al., 1988). Oligonucleotide primers were designed for the putative *LFY* coding region as deduced from the homology to the coding sequence of *FLO* (Coen et al., 1990). Total RNA was extracted from young L-*er* flowers predominantly younger than stage 10 as described (Crawford et al., 1986), and poly(A)$^+$ RNA was isolated using the PolyAttract system (Promega). Poly(A)$^+$ RNA (1 μg) was used to synthesize first-strand cDNA with the cDNA Cycle kit (Invitrogen), using either an oligo(dT) primer or a gene-specific primer near the 5′ end of the predicted coding region. The specifically primed reaction was dA tailed. One-tenth of the first-strand cDNA reactions were used for PCR. The *LFY* cDNA was amplified in three parts. The central part was amplified from the oligo(dT)-primed cDNA with two specific primers. The 3′ end was amplified from the oligo(dT)-primed cDNA with a gene-specific primer and an oligo(dT) primer containing a NotI site. Since the first round of amplification yielded only a "smear" of products when size fractionated on an agarose gel, fractions of different sizes were cut out from an LMP agarose gel and reamplified with a nested gene-specific primer and the same oligo(dT)/NotI primer. The 5′ end was amplified from the specifically primed cDNA using a nested gene-specific primer and the oligo(dT)/NotI primer. A specific product of about 200 bp was obtained after one round of PCR, isolated from an LMP agarose gel, and reamplified using a second nested primer and the oligo(dT)/NotI primer. The PCR products were digested with NotI and with enzymes

recognizing internal sites, such that the 5′ fragment extended from the 5′ end to the internal BamHI site, the central part spanned the region between the BamHI site and the HindIII site, and the 3′ fragment extended from the HindIII site to the 3′ end. The digested fragments were subcloned into pBstKS+ (Stratagene) and sequenced. The same poly(A)$^+$ RNA used for the PCR analysis was also used to construct a cDNA library in the λZAPII vector (Stratagene), which yielded about 5×10^6 primary pfu. An amplified aliquot (5×10^5 pfu) was screened with a *LFY* cDNA probe derived from the PCR cloning, and one partial cDNA clone was isolated.

Sequencing of Wild-Type and Mutant DNA

Overlapping fragments of the genomic *LFY* region encompassing two adjacent EcoRI restriction fragments totaling 7.3 kb were sequenced from various plasmid subclones using universal and gene-specific oligonucleotide primers. Eight PCR-derived 5′ cDNA clones were sequenced, and all contained *LFY* sequences (see text). Eight PCR-derived 3′ cDNA clones were sequenced, of which six contained *LFY* sequences. Of the PCR-derived cDNA clones spanning the central portion of the *LFY* transcript, five were sequenced completely, and another five were sequenced across the heterogeneous first intron (see Figure 6B). In addition, the 5′ and 3′ ends of the partial cDNA clone isolated from the λZAPII library were sequenced.

To obtain mutant sequences, genomic DNA of the different alleles was digested to completion with BamHI and cloned into the EMBL4 vector (Frischauf et al., 1983). Positive clones were identified, and the 10 kb BamHI fragments starting at nucleotide 3 of the *LFY* coding region were subcloned. The exons were sequenced using gene-specific primers.

In Situ Hybridization

As template for the hybridization probe, we used a 1.4 kb chimeric clone, pDW122, in which a *LFY* cDNA fragment that starts at the BamHI site immediately downstream of the initiation ATG is combined with a 3′ genomic fragment that extends 20 bp past the downstream-most polyadenylation site. pDW122 was linearized with BamHI, and [^{35}S]UTP-labeled probes were generated by runoff transcription with T3 RNA polymerase. Probes were used at a final concentration of 1×10^8 to 2×10^8 dpm/ml. Fixation of tissue, preparation of sections, hybridization, and washes were carried out as described by Drews et al. (1991) with minor modifications. Slides were exposed for 1 to 3 weeks.

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