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FILAMENTOUS FLOWER Controls the Formation and Development of Arabidopsis Inflorescences and Floral Meristems

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Phenotypic analysis of single and multiple mutants as well as in situ localization analysis of the expression patterns of floral genes have revealed that the *FILAMENTOUS FLOWER* (*FIL*) gene plays important roles in establishing the inflorescence in Arabidopsis. As previously reported, the *fil* mutant generates clusters of both filamentous structures and flowers with floral organs of altered number and shape. The structural resemblance of the filamentous structures to peduncles and the expression pattern of the *APETALA1* (*AP1*) gene have shown that these filamentous structures are underdeveloped flowers that fail to form receptacles and floral organs, indicating that one of the roles of the *FIL* gene is to support the development of the floral meristem. That *FIL* also is involved in fate determination in the floral meristem is demonstrated by the homeotic conversion of flowers to inflorescences in *fil ap1* double mutants and in *fil ap1 cauliflower* triple mutants. In double mutants with flowering-time loci (i.e., *ft* or *fwa*), *leafy* (*lfy*), and *unusual floral organs (ufo)*, filamentous structures are formed, but very few or no flowers with floral organs develop. The enhanced phenotype in the *fil ap1* and the *fil lfy* double mutants suggests that the FIL protein may work together with AP1 and LFY proteins. The *FIL* gene also may be involved in the cell fate determination of floral organ primordia, possibly by controlling the spatial expression patterns of the class A and C floral organ identity genes.

INTRODUCTION

The development of molecular genetic analyses using Arabidopsis mutants has led to the discovery of many genes involved in the process of reproductive growth. This process has five basic stages: formation of the inflorescence meristem, determination of floral meristem identity, determination of floral organ number and arrangement, determination of organ identity, and maturation of floral organs (Okada and Shimura, 1994).

Formation of the inflorescence meristem is controlled by a set of genes in which mutations cause a shift in the timing of the transition from vegetative to reproductive growth (Coupland, 1995). The floral meristem is formed in a helical manner at the top of the inflorescence (Ottline-Leyser and Furner, 1992). Determination of floral meristem identity and development of the floral meristem are known to be controlled by several genes. These include *LEAFY* (*LFY*), *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), *UNUSUAL FLORAL ORGANS* (*UFO*), two flowering-time loci (*FT* and *FWA*), and *APETALA2* (*AP2*) (Komaki et al., 1988; Kunst et al., 1989; Irish and Sussex, 1990; Bowman et al., 1993; Shannon and Meeks-Wagner, 1993; Kempin et al., 1995; Levin and Meyerowitz, 1995; Weigel and Nilsson, 1995; Wilkinson and Haughn, 1995; Ruiz-Garcia et al., 1997).

Floral organ primordia are formed at the middle stage of floral organogenesis. The number and arrangement of the organs are controlled by the size of the floral meristem. The increased number of organs in the *clavata1* (*clv1*) mutant corresponds with the increased size of the meristem (Clark et al., 1993). The opposite is true for the *wuschel* (*wus*) mutant, which has a small meristem with a decreased number of floral organs (Laux et al., 1996). However, in the case of the *perianthia* (*pan*) mutant, the increase in organ number does not correlate with the size of the floral meristem (Running and Meyerowitz, 1996).

The cell fate of the organ primordia is determined by a set of homeotic genes. According to the proposed ABC model, floral organ primordia develop into one of four different kinds of floral organs—sepals, petals, stamens, or carpels—depending on which of the homeotic gene groups is being expressed at early stages in primordia development: namely, group A, a combination of groups A and B, a combination of groups B and C, and group C. Identified genes of group A are *AP1* and *AP2*, and *LEUNIG* (*LUG*) is known to support

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the functions of group A genes. Identified group B genes are *APETALA3* (*AP3*) and *PISTILLATA* (*PI*); group C includes *AGAMOUS* (*AG*) (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Liu and Meyerowitz, 1995).

FILAMENTOUS FLOWER (*FIL*) is known as the gene that controls inflorescence and flower architecture, because the *fil* mutant (the former name was FL54 mutant) forms two types of flower-related structures: type A flowers have an aberrant number and arrangement of floral organs and immature stamens, and type B structures have a filament with no floral structures or with only one sepal at the top (Komaki et al., 1988; Okada and Shimura, 1994). Further studies are required to determine the molecular function of the *FIL* gene in the developmental processes that form inflorescences and flowers.

In this study, we analyzed the role of the *FIL* gene by using morphological, developmental, genetic, and molecular approaches. The study consisted of three types of experiments. First, we analyzed the morphology of the *fil* mutant phenotypes by using two independently isolated mutants, *fil-1* and *fil-2*. Because the two alleles show similar structural abnormalities in the inflorescence meristem and floral meristem, we characterized the *fil-1* rather than the *fil-2* mutant. Second, we analyzed the phenotypes of a series of double and triple mutants constructed by crossing *fil* and other mutants carrying defects in their inflorescences or flowers. Third, we examined the in situ expression pattern of several genes that are known to play major roles in the process of inflorescence and flower development.

RESULTS

Characterization of the *fil* Inflorescence

The *fil* mutants initiated flowering at an earlier time than did the wild type. We counted the number of rosette leaves after the emergence of floral buds at the top of the inflorescence axis, because the number of rosette leaves is widely accepted as a standard parameter that determines flowering time (Koornneef et al., 1991, 1995). As shown in Table 1, flowering started earlier in the mutant than in the wild type under long- and short-day conditions.

The *fil* mutant often failed to produce tertiary lateral shoots, although formation of the secondary lateral shoots was not affected (Figure 1B). A lateral shoot develops from a meristem formed at the base of cauline leaves (Figures 1A and 2A). In the mutant, the lack of tertiary shoots was not caused by a defect in meristem growth but by a lack of meristem formation, because a meristem or a cell lump was not observed at the base of cauline leaves (Figure 2B). A meristem or a cell lump was not formed even when the secondary shoot was removed by cutting it just above the possible meristem formation site at the base of a cauline leaf (data not shown). Interestingly, tertiary lateral shoot formation was strongly repressed when the *fil* mutant was grown at the low temperature of 16°C (Figure 2C).

Because other phenotypes of the mutant were not enhanced at 16°C, it is difficult to postulate that the FIL protein is labile at 16°C. One possibility is that some FIL-associated protein(s) involved in the formation of a lateral shoot meristem might be temperature sensitive.

Characterization of the *fil* Floral Meristem

As previously reported, flowers of the *fil* mutant can be divided into two types (Komaki et al., 1988). One type, termed A, is a flower with floral organs of aberrant number, shape, and arrangement (Figures 2D and 2E). The other, termed B, has a filament with or without a sepal-like structure (Figure 2F). Also, the primary inflorescences of the mutant can be divided into three regions. As indicated in Figure 1B, region 1 has a cluster of ~15 type A flowers. Region 2 comprises a cluster of ~20 type B structures. Region 3 contains a mixture of five to 20 type A flowers and 10 to 20 type B flowers. The growth activity of the inflorescence meristem decreases after the formation of region 3 and stops after a few (one to five) carpelloid structures have formed at the top (Figure 2G). Similar partitioning of structure was observed in the lateral inflorescence as well as in the primary inflorescence. The effect of genetic background on this phenomenon is discussed later.

Type A flowers have an aberrant structure and number of floral organs. The number of sepals varies from three to five, and the number of petals and stamens is decreased. Petals and stamens are often replaced by filaments (Figure 2H). The number of carpels varies from two to five. Analysis using scanning electron microscopy revealed that the aberrant number of floral organs resulted from defects in the formation of the normal number of organ primordia (Figures 2I to 2K). The decrease in floral organ number was more prominent in type A flowers in region 3 than in those in region 1

Table 1. Transition to Reproductive Phase in *fil-1* Mutants

Strain	Number of Rosette Leaves at the Time of Flowering	
	Under Long-Day Conditions[a]	Under Short-Day Conditions[b]
Wild type (Landsberg *erecta*)	7.9 ± 0.2[c]	14.4 ± 0.6[d]
fil-1	6.5 ± 0.2[e]	10.5 ± 0.6[f]

[a] Continuous illumination.
[b] Eight hours of light and 16 hr of darkness.
[c] Eleven plants examined.
[d] Ten plants examined.
[e] Twenty plants examined.
[f] Eleven plants examined.

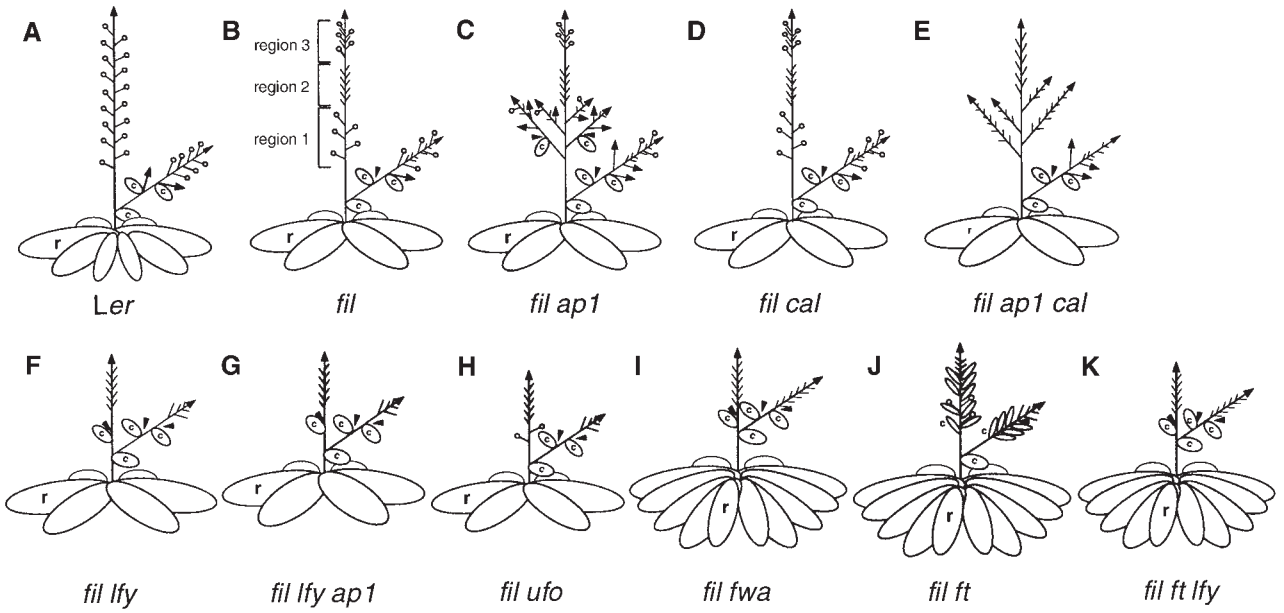

Figure 1. Morphology of the Wild Type and Mutants.

(A) Wild-type Landsberg *erecta* (L*er*).
(B) *fil* mutant.
(C) *fil ap1* double mutant.
(D) *fil cal* double mutant.
(E) *fil ap1 cal* triple mutant.
(F) *fil lfy* double mutant.
(G) *fil lfy ap1* triple mutant.
(H) *fil ufo* double mutant.
(I) *fil fwa* double mutant.
(J) *fil ft* double mutant.
(K) *fil ft lfy* triple mutant.
Arrowheads indicate a lack of the tertiary inflorescence meristem at the base of a cauline leaf. An open circle with a stalk and a short bar drawn on the inflorescence indicate a flower (including a type A flower) and a type B filament, respectively. A black triangle at the top of an inflorescence indicates a meristem. c, cauline leaf; r, rosette leaf.

(Table 2). A decrease in organ number implies the possibility that the size of the floral meristem is reduced. To test this model, we measured the size of the domed inner meristem at stage 3. The meristem of wild-type flowers was 44.4 ± 2.5 μm in diameter, whereas that of mutant flowers in region 1 was 40.2 ± 1.2 μm and that of flowers in region 3 was 35.5 ± 2.2 μm. The decrease in meristem size paralleled the decrease in floral organ number. This result supports the hypothesis regarding the reduced size of the meristem. A similar observation was reported in the case of *clv1* (Clark et al., 1993) and *wus* (Laux et al., 1996).

Type A flowers show a variety of homeotic changes between floral organs (Komaki et al., 1988). As listed in Table 2, the most frequently observed change is that petals are converted to sepals (Figure 2L). Other changes are carpelloid sepals (Figure 2M), staminoid petals (Figure 2N), carpelloid stamens (Figure 2O), sepaloid stamens (Figure 2P), and petaloid stamens (Figure 2Q). This homeotic conversion of floral organs suggests that the *fil* mutation alters the expression pattern of homeotic genes in the floral meristem; however, we did not observe a drastic change in the expression pattern in the mutant, as is discussed later. In addition to homeotic conversion, stamens often lack anthers, indicating that *FIL* is required for full growth of stamen primordia.

Peduncles of type A flowers are markedly longer than those of the wild type. The length of peduncles of wild-type mature flowers was 3.5 ± 0.1 mm, whereas that of type A flowers of the mutant was 5.3 ± 0.3 mm. Scanning electron microscopy revealed that the pattern and size of the epidermal cells of the mutant peduncles are the same as those of the wild type (Figures 3A and 3B). Therefore, the long peduncle of the mutant may have resulted from additional cell divisions, suggesting that the *FIL* gene controls the division of peduncle cells but not their elongation.

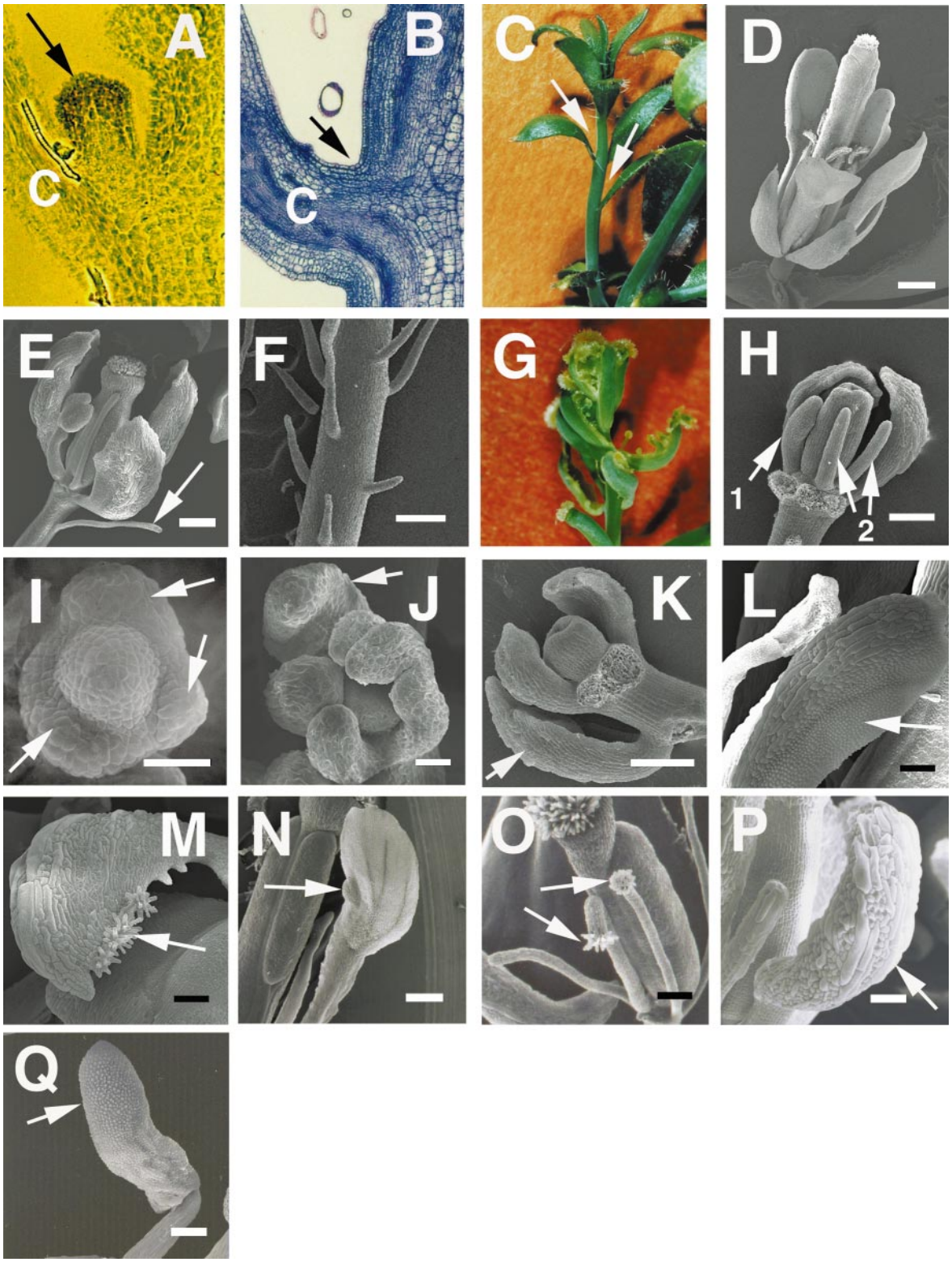

Figure 2. Inflorescence Meristems and Flowers of the Wild Type and the *fil* Mutant.

An additional structural abnormality of type A flowers in region 3 is a filament or a sepal-like structure that is formed at the middle or at the base of peduncles at the abaxial side of flowers (Figures 2E, 2K, and 3D). Although wild-type Arabidopsis flowers do not have bracts, several plant species have a bract on the peduncle at the abaxial side. Therefore, the additional structure in type A flowers could be interpreted as being a bract. If so, it would be interesting to know how the *fil* mutation activated the bract-forming mechanism, which appears to be suppressed in wild-type plants.

Close examination of type B structures revealed that the pattern and size of the epidermal cells are similar to those of peduncles of young buds of wild-type flowers and of the type A flowers of the *fil* mutant (Figure 3C). The diameter of the type B structure is 70 to 100 μm, which is similar to that of peduncles at developmental stage 5 of wild-type flowers. Thus, it is conceivable that type B structures are immature flowers that stopped their development without forming floral organs, possibly having failed to form receptacles. This interpretation also is supported by the rare cases in which a small floral bud is formed at the top of the filament (data not shown). In some cases, a sepal-like organ is formed at the abaxial side, and two or three short, thin filaments form at the adaxial side on top of type B structures (Figures 3E and 3F). The pattern of epidermal cells of the sepal-like organ resembles that of sepals (Figure 3E). In the development of wild-type flowers, there is a spatial order in sepal formation. A sepal at the abaxial side is formed first, then a sepal forms at the adaxial side, and finally two sepals form simultaneously at both lateral sides. Formation of a sepal-like organ at the abaxial side of type B structures could be interpreted as a result of floral meristem development stopping after a sepal had formed at the abaxial side. The short filaments that often form at the adaxial side could be immature sepals at the adaxial and lateral sides. Alternatively, the sepal-like organ could be a bract. Considering that some of the type A flowers carry a bractlike organ, the type B structures could be explained as type A flowers whose development was halted after forming the bractlike organ.

It is known that floral meristems of Arabidopsis are generated in a helical manner interspersed by an angle of ~135° (Figure 3G; Ottline-Leyser and Furner, 1992). We confirmed that type A flowers of the *fil* mutant follow the same phyllotactic regulation (Figures 3H and 3I). It is worth noting that primordia of type B structures also were generated with an angle of ~135° (Figure 3J), strongly indicating that type B structures are immature floral buds.

Analysis of Double Mutant Inflorescences

To examine the role of the *FIL* gene in the formation and maintenance of the inflorescence meristem, we generated a series of double mutants with a number of well-characterized floral meristem identity genes, *AP1*, *FIL*, *CAL*, *UFO*, *FT*, and *FWA*, and analyzed structural abnormalities in their inflorescences. In the case of the *fil ap1* double mutant, nearly 15 flowers formed at the base of the inflorescence, corresponding to region 1 of the *fil* single mutant, and these showed a complete homeotic change from a flower to an inflorescence with flowers (Figures 1C, 4B, and 4C). Because flowers of the *ap1* mutant have bractlike sepals with additional small flowers at their base, the *ap1* mutation shows a partial conversion of a flower to an inflorescence (Figure 4A).

Figure 2. (continued).

(A) A transverse section of a wild-type plant showing lateral shoot meristems formed at the base of cauline leaves (arrow).
(B) A transverse section of the *fil-1* mutant. No lateral shoot meristem is formed at the base of the cauline leaf (arrow).
(C) The *fil-1* mutant grown at 16°C. The lateral shoot meristems at the base of the cauline leaves are not formed (arrows).
(D) A wild-type flower.
(E) A type A flower of the *fil* mutant with abnormal floral organs and with a thin filament (arrow) on the abaxial side of the peduncle.
(F) A cluster of the type B structures of the *fil* mutant.
(G) Carpelloid bracts produced on the top of the inflorescence of the *fil* mutant.
(H) A flower at stage 9 with five sepals, no petals, an abnormal stamen (arrow 1), and two filaments (arrows 2). Three of the abaxial sepals were removed.
(I) A type A flower at stage 3 with three sepal primordia (arrows).
(J) A type A flower at stage 5 with five sepals and a bractlike structure at the abaxial side (arrow).
(K) A type A flower at stage 7 with no primordia in whorls 2 and 3. Note this flower has a bractlike structure at the abaxial side (arrow). Two lateral sepals were removed.
(L) A sepaloid petal of a *fil* mutant flower showing epidermal cells specific to petals (arrow) and to sepals.
(M) A carpelloid sepal with stigmatic papillae (arrow).
(N) A staminoid petal of a *fil* mutant flower with antherlike cells (arrow).
(O) Carpelloid stamens of a *fil* mutant flower with stigmatic papillae at the top (arrows).
(P) An anther of a sepaloid stamen of a *fil* mutant flower showing epidermal cells specific to sepals (arrow).
(Q) An anther of a petaloid stamen of a *fil* mutant flower showing epidermal cells specific to petals (arrow).
C, cauline leaf. Bars = 500 μm for **(D)** to **(F)**; 100 μm for **(H)** and **(K)** to **(Q)**; and 25 μm for **(I)** and **(J)**.

Table 2. Floral Organ Number in Flower Mutants and Double Mutants

	Whorl 1			Whorl 2			Whorl 3					Whorl 4
Strain	Sepal[a]	Carpelloid Sepal	Extra Flower	Petal	Stamenoid Petal	Sepaloid Petal	Stamen	Carpelloid Stamen	Filament	Sepaloid Stamen	Petaloid Stamen	Carpel
L*er*[b]	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
fil-1[c] (region 1)	3.7 ± 0.2	0.0 ± 0.0 (0.5%)[d]	0.0 ± 0.0	1.3 ± 0.2	0.0 ± 0.0 (1.3%)[d]	0.1 ± 0.1 (5.5%)[d]	0.8 ± 0.2	0.0 ± 0.0 (0.5%)[d]	3.7 ± 0.5	0.0 ± 0.0 (1.0%)[d]	0.0 ± 0.0 (1.3%)[d]	2.7 ± 0.6
fil-1[e] (region 3)	3.0 ± 0.2	0.0 ± 0.0 (1.6%)[f]	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0 (1.0%)[f]	0.0 ± 0.0 (3.4%)[f]	0.0 ± 0.0	0.0 ± 0.0 (0.6%)[f]	3.7 ± 0.4	0.0 ± 0.0 (1.0%)[f]	0.0 ± 0.0 (1.6%)[f]	2.4 ± 0.5
fil-2[g] (region 1)	3.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	0.0 ± 0.0	4.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.5
fil-2[h] (region 3)	3.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	3.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.4
ap2-1[i]	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.2	1.5 ± 0.2	0.0 ± 0.0	5.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
fil-1 ap2-1[j] (region 1)	3.1 ± 0.4	1.0 ± 0.4	1.3 ± 0.4	0.0 ± 0.0	0.8 ± 0.3	0.0 ± 0.0	1.1 ± 0.6	0.6 ± 0.3	0.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	2.8 ± 0.6
fil-1 ap2-1[k] (region 3)	0.0 ± 0.0	2.3 ± 0.4	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.5
ap1-17[l]	3.4 ± 0.1	0.0 ± 0.0	0.8 ± 0.2	3.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
fil-1 ap1-17[m] (region 1)	2.3 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.4
fil-1 ap1-17[n] (region 3)	1.4 ± 0.2	0.7 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.2
ap1-1[o]	1.8 ± 0.5	0.0 ± 0.0	2.9 ± 0.3	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	5.1 ± 0.3	0.0 ± 0.0	1.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
fil-1 ap1-1[p]	1.0 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.2

[a] Filamentous organs were included.
[b] Twenty samples of Landsberg *erecta* plants were tested.
[c] Thirty-five samples of *fil-1* flowers of region 1 were counted.
[d] Four hundred flowers were examined to identify the frequency of homeotic changes.
[e] Thirty-seven samples of *fil-1* flowers of region 3 were counted.
[f] Five hundred flowers were examined to identify the frequency of homeotic changes.
[g] Thirty samples of *fil-2* flowers of region 1 were counted.
[h] Twenty samples of *fil-2* flowers of region 3 were counted.
[i] Twenty-six samples of *ap2-1* flowers were counted.
[j] Eight samples of *fil-1 ap2-1* flowers of region 1 were counted.
[k] Nineteen samples of *fil-1 ap2-1* flowers of region 3 were counted.
[l] Twenty-five samples of *ap1-17* flowers were counted.
[m] Eighty-two samples of *fil-1 ap1-17* flowers of region 1 were counted.
[n] Sixty-three samples of *fil-1 ap1-17* flowers of region 3 were counted.
[o] Eighteen samples of *ap1-1* flowers were counted.
[p] Thirty samples of *fil-1 ap1-1* flowers were counted.

The phenotype of the *fil ap1* double mutant can be interpreted as the *fil* mutation enhancing the phenotype of the *ap1* mutation, suggesting that the FIL protein works coordinately with the AP1 protein in the process of floral meristem formation. After a cluster of converted flowers formed, a cluster of type B structures formed on the inflorescence of the double mutant (Figures 4B and 4D). The number and shape of type B structures were the same as those of the *fil* mutant. In region 3, however, type A flowers were formed. The number of floral organs of type A flowers was decreased, and most of these organs changed to filamentous structures. It is interesting that the type B structure of the *fil ap1* double mutant did not show any sign of being converted to an inflorescence, whereas the type A flower did. This result may indicate that AP1 is not involved in the formation of the type B structure.

The *fil cal* double mutant had no additional abnormalities when compared with the *fil* mutant (Figure 1D). This result is not surprising, however, because the *cal* single mutant does not show any structural abnormalities, possibly due to the redundancy between *CAL* and *AP1* (Kempin et al., 1995). However, *fil ap1-1 cal* triple mutants (Figures 1E and 4F) showed a severe phenotype compared with *fil ap1-1* or *ap1-1 cal* double mutants (Figures 4B and 4E). Flowers in region 1

were converted to inflorescences. In region 2, a cluster of type B structures was formed, but the growth of the inflorescence stopped without forming region 3. As a result, the triple mutant had a multibranched inflorescence with type B structures but no flowers. These results indicate that *AP1* and *CAL* are not required to form type B structures but are indispensable in the formation of type A flowers, possibly because the genes are required in the process of forming receptacles and floral organs.

The *fil lfy* double mutant also showed a drastic structural change in its inflorescence when compared with the *fil* or *lfy* mutants (Figures 1B and 4G). The double mutant failed to produce type A flowers but formed a cluster of filaments as well as a few bractlike or carpel-like organs at the top of the inflorescence (Figures 1F, 4H, and 4I). The inflorescence appeared to lack regions 1 and 3. In addition, the *fil lfy* double mutant failed to generate tertiary lateral inflorescences, although cauline leaves were formed normally. The secondary inflorescences were often lacking (Figure 4H). These results indicate that the *lfy* mutation enhances the phenotype of the *fil* mutant and that both *FIL* and *LFY* genes are required for formation and maintenance of the floral meristem and inflorescence meristem.

Because both *fil lfy* and the *fil ap1* double mutants showed enhanced abnormality in their inflorescences, and because the homeotic conversion from flowers to shoots in the *ap1 lfy* double mutant is known to occur (Figure 4J; Huala and Sussex, 1992; Weigel et al., 1992), we constructed the *fil lfy ap1* triple mutant and examined its phenotype. Interestingly, the triple mutant showed the same inflorescence structure as did the *fil lfy* double mutant (Figure 1G). This finding indicates that the *AP1* gene acts downstream of the *FIL* and *LFY* genes.

The *UFO* gene is reported to function in floral meristem formation and maintenance (Figure 4K; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). The structure of the *fil ufo* double mutant is similar to that of the *fil lfy* double mutant. After only one or two type A flowers appeared, a cluster of type B structures formed (Figure 1H). Homeotic conversion of a flower to an inflorescence was not observed. Tertiary inflorescences were not produced, although cauline leaves were formed. Therefore, the *ufo* mutation had an effect similar to that of the *lfy* mutation in the absence of *FIL* gene function. The result is consistent with the model that *UFO* works with *LFY* in the process of floral meristem development (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995).

The study of some flowering-time mutants, such as *fwa* and *ft* (Figures 4L and 4N), suggests that the corresponding wild-type genes play roles in controlling the structure of the inflorescence as well as in determining the timing of flowering, because these mutants show an enhanced phenotype of their floral structure when combined with the *ap1* or *lfy* mutation (Madueño et al., 1996; Ruiz-García et al., 1997). The bolting time of the *fil fwa* double mutant is delayed but is the same as that of the *fwa* single mutant (data not shown). However, the structure of the inflorescence is changed drastically from that of parental lines. As shown in Figures 1I and 4M, the *fil fwa* double mutant failed to produce type A flowers. Similar to the *fil lfy* double mutants, type B structures were formed immediately after bolting. Secondary and tertiary inflorescences were often missed.

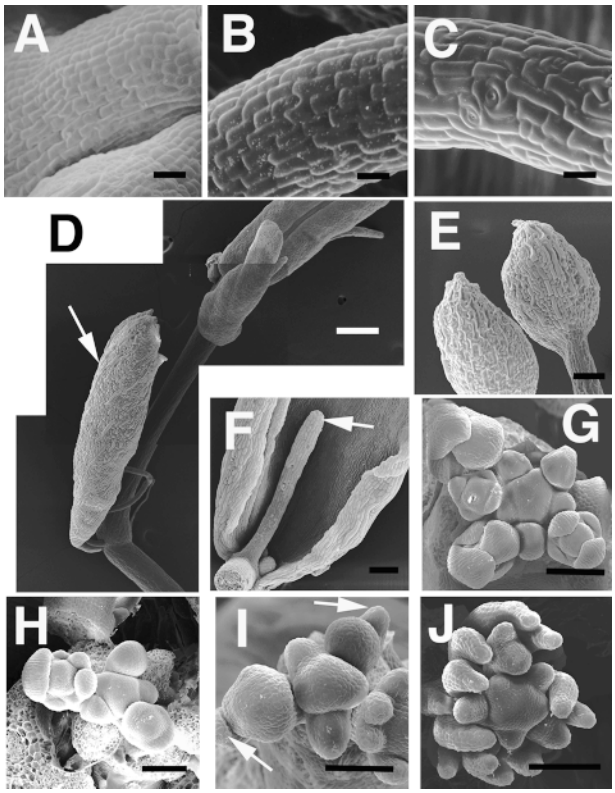

Figure 3. Inflorescence Meristems and Flowers of the Wild Type and the *fil* Mutant.

(A) Enlarged view of a peduncle of a wild-type flower at stage 5.
(B) Enlarged view of a peduncle of a type A flower of the *fil* mutant at stage 5.
(C) Enlarged view of a type B structure of the *fil* mutant.
(D) Type A flower with a bractlike organ (arrow) at the middle part of the peduncle.
(E) Abaxial side of sepal-like organs formed at the top of type B structures of the *fil* mutant.
(F) Enlarged view showing the inside of sepal-like organs formed at the top of the type B structure. A filament formed at the adaxial side (arrow).
(G) Top view of a wild-type inflorescence.
(H) Top view of an inflorescence of the *fil* mutant forming type A flowers.
(I) Top view of an inflorescence of the *fil* mutant in region 3. Bractlike organs are shown in a floral bud (arrows).
(J) Top view of an inflorescence of the *fil* mutant forming type B structures in region 2.
Bars = 25 μm for **(A)** to **(C)**; 500 μm for **(D)**; and 100 μm for **(E)** to **(J)**.

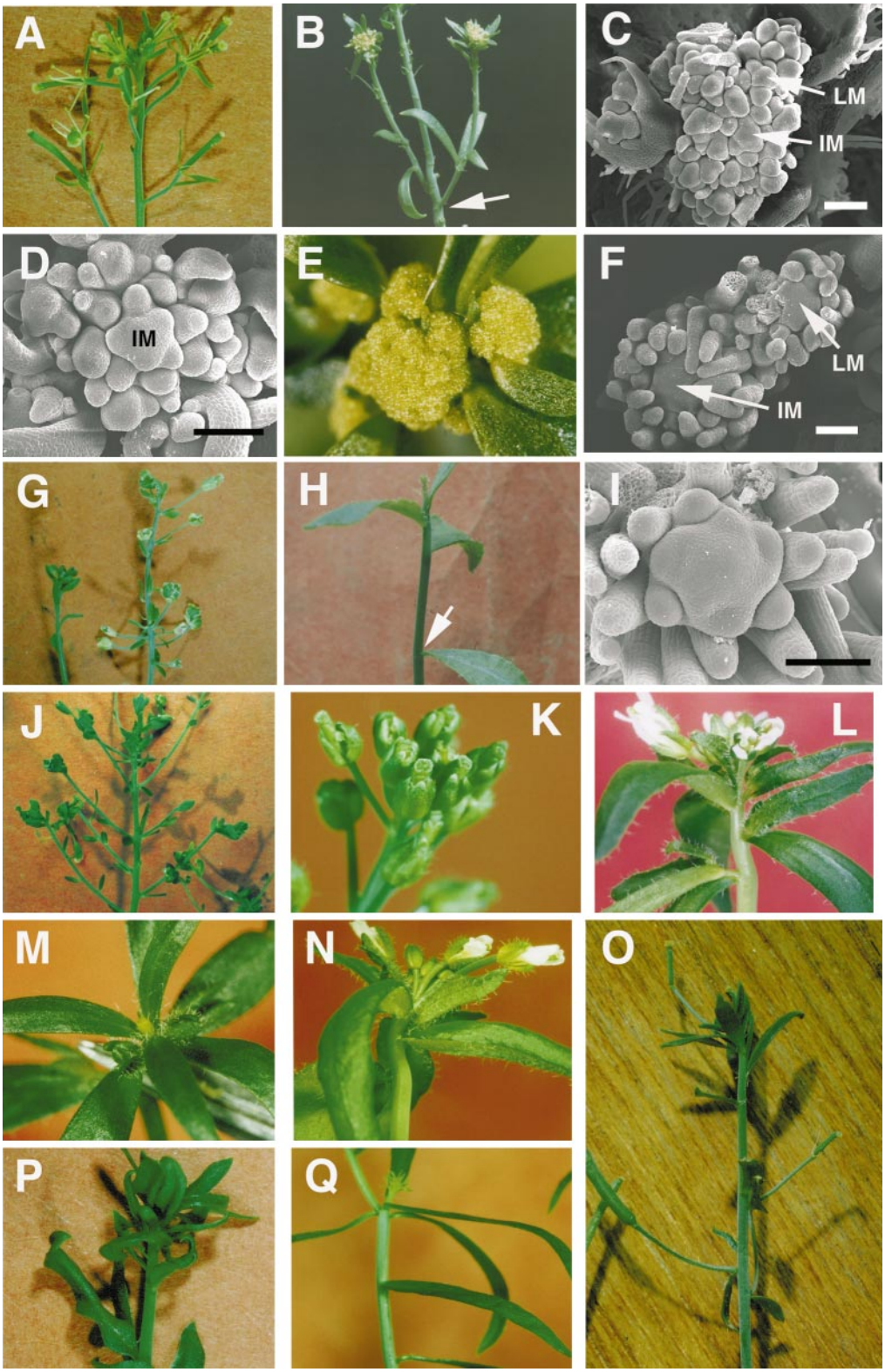

Figure 4. Inflorescences of Double Mutants.

The mutant phenotype indicates that the *fwa* mutation has an enhancing effect on the *fil* mutation. The similarity of the phenotypes of the *fil lfy* and *fil fwa* double mutants suggests that *FWA* and *LFY* genes may have a redundant function in the formation of the lateral inflorescence and development of the floral meristem.

Although the flowering time of the *fil ft* double mutant was the same as that of the *ft* mutant, the inflorescence of the double mutant showed a unique structure. Approximately 10 floral buds were formed in the lower part of region 1; however, unlike the wild type or the *ft* or *fil* mutant, most of the buds were accompanied by a cauline leaflike structure (Figures 1J and 4O). The flowers were self-fertile and set seed, but the number of floral organs was decreased. In the upper part of region 1, however, there was a cluster of >20 cauline leaves with no floral buds (Figure 4P). The appearance of a cauline leaflike structure at the base of floral buds may indicate that the *ft* mutation caused a partial conversion of the floral meristem to the inflorescence meristem.

We then examined the phenotype of the *fil ft lfy* triple mutant. In this case, bolting time was the same as that of the *ft* mutant. Interestingly, the triple mutant showed an inflorescence structure nearly identical to that of the *fil lfy* or *fil fwa* double mutants (Figure 1K). The triple mutant produced type B structures but no type A flowers (Figure 4Q), and formation of lateral inflorescences was aborted. More than 80% of the cauline leaves on the main inflorescence lacked secondary inflorescences. The similar inflorescence structure indicates that the *FT* gene may work downstream of the *FIL* and *LFY* genes in the formation and maintenance of the inflorescence meristem. It should be noted that the structural defects in the inflorescence were not always observed in double mutants of *fil* with late-flowering mutants. For example, the inflorescence structure of the *fil fca* double mutant was the same as that of the *fil* mutant (data not shown).

Analysis of Double Mutant Floral Structures

We examined the role of the *FIL* gene in the development of floral organs by constructing a set of double mutants. The flower of a plant having strong alleles of the *ap2* mutant has two sepals in the abaxial and adaxial positions; however, two sepals at medial position are converted homeotically to carpels (Figure 5B). The structure of the pistil is normal, but petals and stamens are absent (Komaki et al., 1988; Kunst et al., 1989). The flower of a plant with a weak allele, *ap2-1*, had four leaflike sepals, four staminoid petals, six stamens, and a pistil (Figure 5A and Table 2). Although the inflorescence of the *fil ap2-1* double mutant resembled that of the *fil* mutant, the structure of the floral organs of type A flowers formed in region 3 was the same as that of plants with strong alleles of *ap2.* The *fil ap2-1* double mutant had a homeotic change of two sepals at the medial position to carpels and lacked petals and stamens (Figures 5C and 5D). The enhanced floral structure indicates that the *FIL* gene

Figure 4. (continued).

(A) An inflorescence of the *ap1* mutant.
(B) The *fil-1 ap1-1* double mutant showing a homeotic change from flowers to shoots. The shoots converted from a flower are not accompanied by cauline leaves (arrow).
(C) Top view of an inflorescence of the *fil-1 ap1-1* double mutant forming lateral shoot meristems.
(D) Top view of an inflorescence of the *fil-1 ap1-1* double mutant forming type B structures.
(E) An inflorescence of the *ap1 cal* double mutant.
(F) Top view of an inflorescence of the *fil-1 ap1-1 cal* triple mutant forming a lateral shoot meristem.
(G) An inflorescence of the *lfy* mutant.
(H) The primary inflorescence of the *fil-1 lfy-6* double mutant. Type B structures are formed at the meristem. Secondary shoot meristems are not formed at the base of the cauline leaves (arrow).
(I) Enlarged view of an inflorescence meristem of the *fil-1 lfy-6* double mutant (shown in **[H]**) forming type B structures.
(J) An inflorescence of the *ap1 lfy* double mutant.
(K) An inflorescence of the *ufo* mutant.
(L) An inflorescence of the *fwa* mutant.
(M) The primary inflorescence of the *fil-1 fwa-1* double mutant. Similar to the *fil lfy* double mutant (shown in **[H]** and **[I]**), the type B structures are formed at the meristem.
(N) An inflorescence of the *ft* mutant.
(O) Region 1 of the *fil-1 ft-1* double mutant forming type A flowers.
(P) Upper part of region 1 of the *fil-1 ft-1* double mutant showing a cluster of cauline leaves.
(Q) The primary inflorescence of the *fil-1 ft-1 lfy-6* triple mutant. Similar to the *fil lfy* double mutant (shown in **[H]** and **[I]**), the type B structures are formed at the meristem.
IM, inflorescence meristem; LM, lateral shoot meristem. Scale bars in **(C)**, **(D)**, **(F)**, and **(I)** = 100 μm.

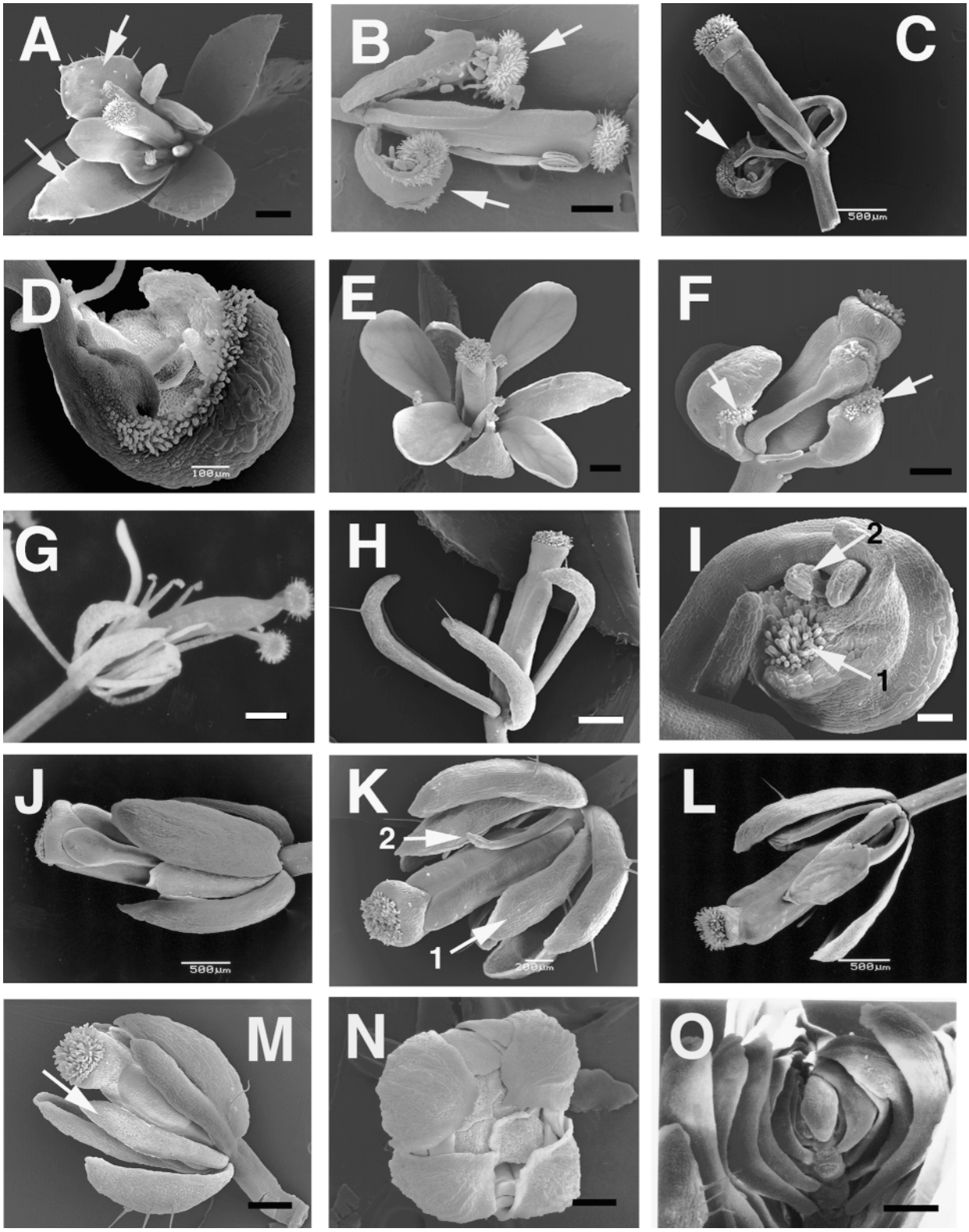

Figure 5. Floral Structure of the Mutants.

may work coordinately with *AP2* in the formation, fate determination, and growth of floral organ primordia formed in whorls 1, 2, and 3.

Similar enhancement of floral organ structure was found in type A flowers of a double mutant carrying *fil* and a weak allele of the *ap1* mutation, *ap1-17* (the former name was FL1; described by Okada and Shimura [1994]). A flower of this mutant had four leaflike sepals with an extra flower at the base of each sepal (Figure 5E and Table 2). The structure and number of other floral organs were normal. Strong alleles of the *ap1* mutant result in plants with flowers having a reduced number of petals, stamens, and sepals (Bowman et al., 1993). The phenotype of the *fil ap1-17* double mutant showed an enhanced phenotype, namely, petals and stamens were lacking, and several sepals showed a homeotic change to carpels (Figure 5F and Table 2). The enhanced phenotype of the double mutant suggests that FIL and AP1 proteins may work together in floral organogenesis as well as in the formation and maintenance of the inflorescence meristem.

Another class A–related gene, *LUG*, is known to affect the growth of floral organs and determination of floral organ identities in whorls 1 and 2 (Liu and Meyerowitz, 1995). The flower of the *lug* mutant has narrow floral organs that sometimes show homeotic transformation in whorls 1 and 2, it has an aberrant number of floral organs in whorls 2 and 3, and the carpels fail to fuse properly (Liu and Meyerowitz, 1995; Figure 5G).

In the *fil lug* double mutant, the phenotype of type A flowers was additive, namely, there was a decreased number of narrow sepals (Figure 5H). Although sepals of type A flowers did not show any homeotic changes, the sepal-like organ found at the top of type B structures was sometimes converted to carpels (Figure 5I). These results indicate that *FIL* and *LUG* work independently in the process of flower development.

An additive phenotype also was observed in type A flowers of double mutants carrying *fil* and a mutation in a class B gene, either *PI* or *AP3*, namely, petals were converted to sepals, and stamens were partially converted to carpels or filaments (Figures 5J and 5K). A similar conversion of floral organs was observed in *fil ufo* double mutants (Figures 5L and 5M). Flowers of *fil ag* double mutants also showed an additive phenotype. Type A flowers showed reiterated sepals and petals or sepaloid petals, like a flower of the *ag* mutant; however, the number of sepals and petals was decreased, and these structures became narrower than those of the *ag* mutant (Figures 5N and 5O). The additive phenotype of flowers in the series of double mutants suggests that *FIL* does not work together with *PI*, *AP3*, *UFO*, or *AG* in the process of flower development.

Expression of Floral Genes in *fil* and the Double Mutants

Because the analysis of the double mutants indicated that the functional interaction of *FIL* and *LFY* genes was required for the formation and maintenance of the floral meristem and the inflorescence meristem, we examined the expression pattern of *LFY* in the *fil* mutants by using *LFY* mRNA as a probe. In the floral meristem of the wild type, the expression of *LFY* is maintained in floral primordia at stage 1 to early stage 3. After stage 3, *LFY* expression abates in the center dome. Strong *LFY* expression is maintained in the sepal primordia until the end of stage 4, and *LFY* expression is maintained in petals, filaments, and gynoecia until the end of stage 9 (Weigel et al., 1992). The expression pattern of the *LFY* gene in type A flowers of the *fil* mutant was similar to

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Figure 5. (continued).

(A) An *ap2-1* flower. The four sepals are converted to leaves (arrows).
(B) A flower from a plant with a strong allele of *ap2*, *ap2-3.* Two sepals at abaxial and adaxial positions are converted to carpels (arrows).
(C) A flower of the *fil-1 ap2-1* double mutant. Two sepals at abaxial and adaxial positions are converted to carpelloid sepals (arrow).
(D) Enlarged view of a carpelloid sepal of the *fil ap2-1* double mutant (shown in **[C]**).
(E) A flower from a plant with a weak allele of *ap1*, *ap1-17.*
(F) A flower of the *fil ap1-17* double mutant with carpelloid sepals (arrows).
(G) A *lug* flower.
(H) A type A flower of the *fil-1 lug-10* double mutant.
(I) Enlarged view at the tip of a type B structure of the *fil-1 lug-10* double mutant (shown in **[H]**) that has a carpelloid sepal at the top. Stigmatic papillae (arrow 1) and ovules (arrow 2) are shown.
(J) An *ap3* flower.
(K) A type A flower of the *fil-1 ap3-3* double mutant showing sepaloid petals (arrow 1) and stamens lacking an anther (arrow 2).
(L) A *ufo* flower. A sepal has been removed.
(M) A type A flower of the *fil-1 ufo-2* double mutant showing sepaloid petals (arrow).
(N) An *ag* flower.
(O) A type A flower of the *fil-1 ag-1* double mutant. Sepals and petals were removed to show the inside.
Bars = 500 μm for **(A)** and **(E)** to **(H)**; and 100 μm for **(B)**, **(I)**, and **(M)** to **(O)**.

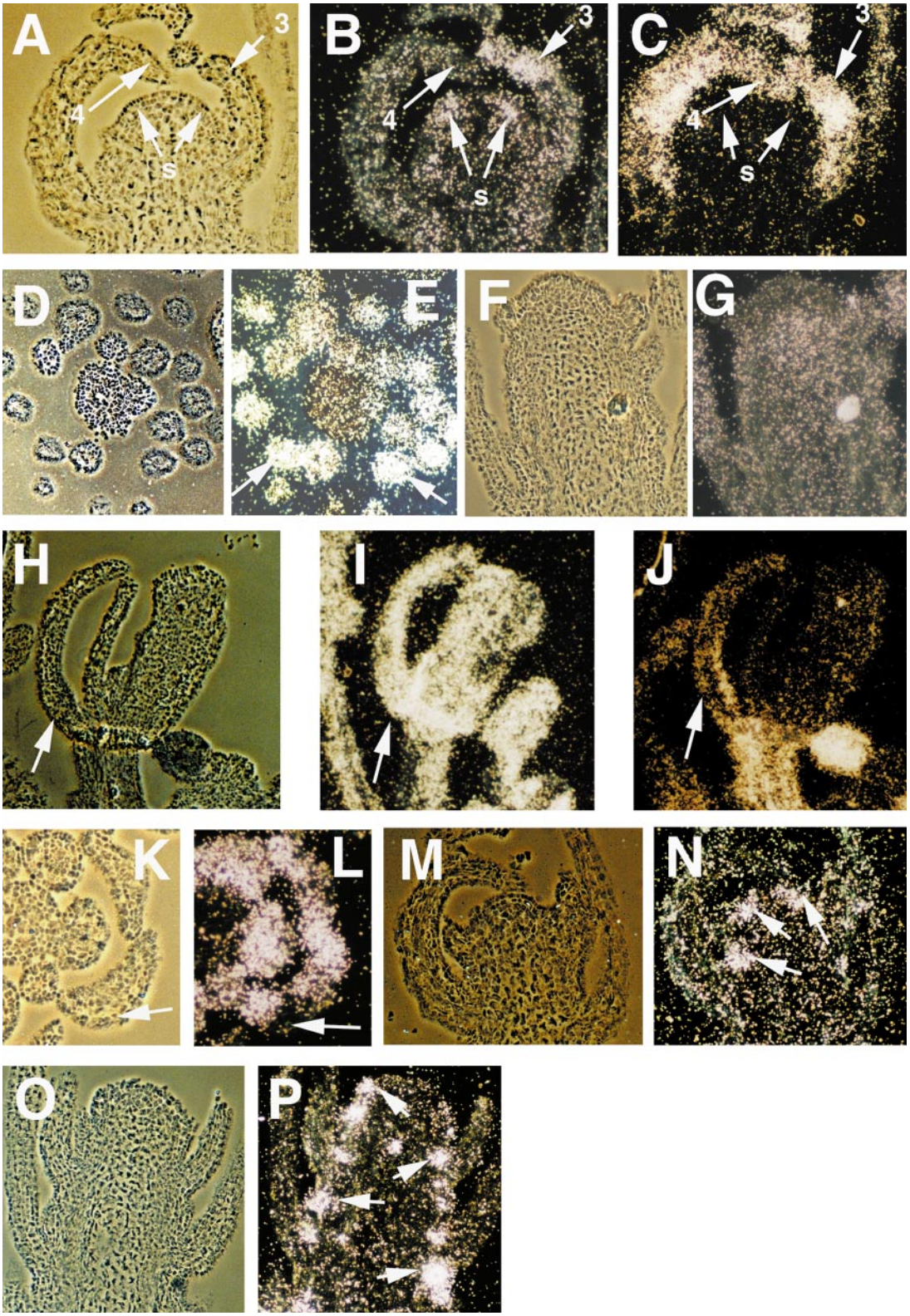

Figure 6. Expression Patterns of Flower-Related Genes in *fil-1* and Double Mutants.

that of the wild type (data not shown). However, in type B structures, the pattern of *LFY* gene expression changed as the structures grew. Expression was detected in the meristem corresponding to stage 1 of wild-type floral buds, but it was localized at the top of the meristem at stage 3, and it gradually weakened and disappeared at stage 4 (Figures 6A and 6B). It could be argued that *FIL* supports the maintenance of *LFY* gene expression in the floral meristem and that the failure of flower development in the type B structure is a result of the disappearance of *LFY* gene expression. The latter result is consistent with the previous observation that *LFY* gene expression in early stages, stages 1 to 9, is necessary for normal development of the floral meristem (Weigel et al., 1992).

Another floral meristem identity gene, *AP1*, is reported to be expressed uniformly in the floral meristem of the wild type from stage 1. As the meristem develops, expression of *AP1* has been observed in the outer whorls and in peduncles but ceases in the central region of the meristem (Mandel et al., 1992). In type A flowers of the *fil* mutant, the pattern of *AP1* gene expression was similar to that of the wild-type flowers. Strong expression of this gene also was observed in type B structures (Figures 6A and 6C to 6E), which is consistent with our hypothesis that the type B structure is an aborted flower forming a peduncle only. The expression level of the *AP1* gene was reduced in type B structures of *fil lfy* double mutants (Figures 6F and 6G), indicating that *AP1* expression is positively controlled by *FIL* and *LFY* and that expression of *AP1* may not be required for the growth of the type B structure.

As we described earlier, *fil ap2-1* and *fil ap1-17* double mutants showed a homeotic change of a carpelloid sepal. Therefore, we tested the *AP1* expression pattern in the outer whorls of the double mutants. Consistent with the identity of the organ, the expression of *AP1* was not observed in the floral buds, except for the peduncles (Figures 6H and 6J). The *fil* mutation may be responsible for the repression of *AP1* gene expression in outer whorls of floral buds of the double mutants, because the expression of *AP1* was not suppressed in these regions of *ap2-1* and *ap1-17* flowers (data not shown). These results suggest that the *FIL* gene may work as a positive regulator of *AP1* expression coordinately with *AP2* or with *AP1* itself.

We examined the expression pattern of class B and C genes. As determined from the in situ pattern, *AP3* gene expression basically was not affected in the inflorescence and floral meristems of the *fil* mutant. In a mutant flower with sepaloid petals, *AP3* was expressed in petals in the region showing the features of petal tissue but not in the region converted to sepal tissue (Figures 2L, 6K, and 6L). The correlation of the formation of sepal tissue and the lack of *AP3* gene expression in petals are consistent with the widely accepted ABC model of cell fate determination in flowers (Weigel and Meyerowitz, 1994).

Expression of another class B gene, *PI*, also appeared to be normal in the floral buds. However, ectopic expression of *PI* was observed in the inflorescence meristem. As shown in Figures 6M and 6N, several patches of cells at the apex were shown to express the *PI* gene. The role of the unusual expression of *PI* is not clear, because the phenotype of the inflorescence of the *fil pi* double mutant was the same as that of the *fil* mutant, although the flower structure of the double mutant resembled that of the *pi* mutant.

The *AG* gene also was expressed ectopically in the inflorescence of the *fil* mutant. *AG* expression was high in several sectors of cells (Figures 6O and 6P). The role of the ectopic expression of *AG* in the inflorescence is not clear, because there are no additional structural abnormalities in

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Figure 6. (continued).

(A) to **(C)** Longitudinal sections of a meristem of the *fil-1* mutant generating type B structures that correspond to flowers at stages 3 and 4. **(A)** provides a bright-field view. In **(B)**, expression of the *LFY* gene at the apical region of the type B structure at stage 3 (arrow 3) is shown, but expression at stage 4 is not apparent (arrow 4). Also shown is ectopic expression of the *LFY* gene in the stem (arrows S). In **(C)**, expression of the *AP1* gene in the type B structures is shown (arrows 3 and 4), but expression in the meristem is not apparent (arrows S).

(D) and **(E)** Transverse sections of an inflorescence of the *fil-1* mutant in region 2. **(D)** provides a bright-field view. **(E)** shows *AP1* expression in type B structures (arrows) but not in the inflorescence meristem (center).

(F) and **(G)** Longitudinal sections of the *fil lfy* double mutant. **(F)** provides a bright-field view. Reduced expression of the *AP1* gene in the type B structures is shown in **(G)**.

(H) to **(J)** Type A flower of the *fil ap2-1* double mutant. Arrows show the carpelloid sepal. **(H)** provides a bright-field view. **(I)** shows *AG* expression in the carpelloid sepal. **(J)** shows *AP1* expression in the peduncle but not in the carpelloid sepal.

(K) and **(L)** Transverse sections of a flower of the *fil* mutant. **(K)** provides a bright-field view. **(L)** shows the absence of *AP3* expression in the sepaloid tissue of the sepaloid petal (arrows in **[K]** and **[L]**).

(M) and **(N)** Longitudinal sections of an inflorescence of the *fil* mutant. **(M)** provides a bright-field view. **(N)** shows ectopic *PI* expression in cell clusters in the inflorescence (arrows).

(O) and **(P)** Longitudinal sections of the inflorescence of the *fil* mutant. **(O)** provides a bright-field view. **(P)** shows ectopic *AG* expression as patches at the base of type B structures. (Some of the patches are marked with arrows.)

the inflorescence of the *fil ag* double mutant in comparison with that of the *fil* mutant. *AG* expression also was detected in the carpelloid sepals of *fil ap2-1* and *fil ap1-17* double mutants (Figures 6H and 6I). This result is consistent with previous studies showing that *ap2* mutations cause a conversion of sepals to carpels by permitting the expression of *AG* in sepal primordia (Drews et al., 1991).

As shown in Table 2, a variety of homeotic conversions was observed in *fil* flowers. There may be two possible explanations for this. One is that the *fil* mutation caused spatial or temporal disturbance in the expression pattern of the homeotic genes. Alternatively, the floral organ primordia were formed at an incorrect position. For example, a primordium formed on the border of whorls 2 and 3 will develop a chimeric organ of petal and stamen. Further examination is required to determine the mechanism. The results of the in situ analysis demonstrated that *FIL* is required for supporting the normal expression pattern of several genes working in the formation and development of inflorescence and floral meristems.

DISCUSSION

Our analysis of the *fil* mutant clearly has shown that the major roles of the *FIL* gene are to control the formation of inflorescence meristems and the formation and development of floral meristems. The expression pattern of known floral genes is consistent with the suggested role of the *FIL* gene in floral organogenesis of Arabidopsis.

FIL Modifies the Formation of the Inflorescence Meristem

Among the pleiotropic phenotypes of the *fil* mutants, we noticed two features related to the formation of the inflorescence meristem. One was the timing of the phase change as the plants entered the reproductive growth stage. The mutants started to form an inflorescence meristem a few days earlier than did the wild type under long- or short-day conditions. In combination with late-flowering mutations, such as *ft* and *fwa*, the flowering time of the double mutants was delayed almost to the same extent as that of the single, delayed-flowering mutant, suggesting that the *FIL* gene plays a minor function in the determination of flowering time. Another alteration was the lack of tertiary lateral inflorescence meristem formation. Because the secondary inflorescence was formed normally, the reason for the defect of the tertiary inflorescence remains unknown. In addition, it is not known whether the two phenotypes, early flowering and the defect in lateral inflorescence formation, are the result of the same or related genetic regulatory systems. The observation that the latter phenotype is enhanced at low temperature but that the former phenotype is not suggests that the two phenotypes are controlled by separate systems.

FIL Has a Role in the Formation of Floral Meristems

The role of the *FIL* gene in floral meristem formation was suggested in light of the phenotype of double mutants. Flowers in region 1 of the *fil ap1* double mutant were transformed into inflorescences (Figure 1C). Neither of the parental mutants showed such a clear homeotic conversion. This drastic change of the inflorescence indicates that the FIL and AP1 proteins interact and contribute to the formation of floral meristems.

Interestingly, a similar phenotype showing a homeotic change from a flower to an inflorescence was observed in *lfy ap1* double mutants (Huala and Sussex, 1992). These results suggest that *FIL* and *LFY* genes at least partially share a common role in the formation and fate determination of floral meristems.

In the case of the *fil lfy* double mutant, the inflorescence produced filaments but failed to form flowers (Figure 1F). By comparing the inflorescence of the *fil* mutant with that of the double mutant, we found that the double mutant had region 2 but lacked regions 1 and 3. The lack of region 1 might be interpreted to result from the decreased potential to form floral meristems in the double mutant. An alternative interpretation is that type A flowers are replaced by type B structures. The number of type B structures that formed on the inflorescence of the double mutant was lower than was the total number of type A flowers and type B structures formed on an inflorescence of the *fil* mutant, suggesting that the activity of the inflorescence meristem to generate type A flowers or type B structures is decreased in the *fil lfy* double mutant. Therefore, in either case, we can postulate that *FIL* and *LFY* genes control a key step in the process of floral meristem formation.

The *fil fwa* double mutant and *fil ft lfy* triple mutant showed a phenotype similar to that of the *fil lfy* double mutant, whereas the *fil ft* double mutant had a unique phenotype. It is known that the *ft* and *fwa* mutations show similar effects on the background of the *ap1* or *lfy* mutation (Madueño et al., 1996; Ruiz-García et al., 1997), but in the *fil* mutant, *ft* and *fwa* mutations contribute in a different manner. Our results indicate that the *FWA* gene has a redundant function with respect to the *LFY* gene and that the *FT* gene works downstream of *FIL* and *LFY* genes in the process of the floral meristem formation.

The Type B Structure Is an Underdeveloped Flower

The nature of the type B structures has been puzzling. Analysis using scanning electron microscopy showed that the pattern of the epidermal cells of the structure resembles that of peduncles. The transverse section of the structure

showed a cellular organization similar to that of peduncles (data not shown). These observations strongly indicate that a type B structure is an immature flower that forms a peduncle but lacks a receptacle and floral organs. The floral meristem of a wild-type plant develops to form a peduncle at the base and a receptacle at the top. Primordia of the floral organs are formed on the receptacle. In region 2 of the *fil* mutant inflorescence, the basal part of the floral meristem might develop to form a peduncle, but the apical part of the meristem might fail to form a receptacle and therefore cannot form floral organs. If the formation of the receptacle was permitted, some floral organs would be formed at the top of the peduncle-like filament. Thin filaments or a sepal-like structure often found at the top of the type B structure might be the result of the incomplete formation of a receptacle. This interpretation also is supported by the expression pattern of the *AP1* gene.

The detailed analysis of the *AP1* gene in the floral meristem revealed that gene expression was detected at the very early stage, stage 2, of meristem development and continued to later stages in the outer whorls (Mandel et al., 1992). The *AP1* gene is expressed uniformly in cells of floral meristems, including peduncles. Consistent with the reported results of *AP1* expression in the peduncles, we detected expression of the *AP1* gene in type B structures. In addition, meristems of type B structures emerged in a helical manner separated by ~135°, which is the same radial arrangement as seen in type A flowers and wild-type flowers. These results strongly indicate that the type B structure is an underdeveloped floral meristem. Our data also indicate that development of peduncles does not require the function of the *FIL* gene, which may be consistent with the observation that type A flowers have long peduncles.

FIL Supports the Development of the Floral Meristem

We have shown that the inflorescence meristem of the *fil* mutant forms two types of floral buds, type A flowers and type B structures. Type A flowers have four kinds of floral organs, although the number and shape of the organs are often aberrant. However, type B structures are considered to be immature flowers lacking the receptacle and floral organs. The structural abnormality of the flowers indicates that the *FIL* gene is responsible for the development and maturation of the floral meristem. In combination with the *FIL* gene, other floral genes, for example, *AP1*, *LFY*, *CAL*, *UFO*, *FWA*, and *FT*, have been shown to support the growth of the floral meristem. As summarized in Figure 1, the formation of type A flowers was strongly repressed in the double or triple mutants, although formation of type B structures was not affected severely. Considering that type B structures are underdeveloped flowers without a receptacle and floral organs, the genes listed above may not be required for the formation of peduncles. By using a genetic approach, we show that development of the floral meristem can be separated into two pathways—formation of the peduncle and formation of the receptacle. The floral organs are formed on the receptacle.

The Mechanism Underlying the Formation of Type A Flowers and Type B Structures Is Not Known

One of the striking features of the *fil* mutant is the change of "phase" of the inflorescence meristem—the formation of three different regions as elongation occurs (Figure 1B). Regions 1, 2, and 3 include a cluster of type A flowers, a cluster of type B structures, and a cluster of both types of structures, respectively. The change was abolished in several double mutants. The inflorescence of the *fil ufo* double mutant stopped its growth after forming regions 1 and 2. The *fil lfy* and *fil fwa* double mutants and *fil ft lfy* triple mutant showed only one region with a cluster of type B structures. These results may indicate the involvement of *UFO*, *LFY*, *FT*, and *FWA* genes in the shift of the inflorescence meristem forming either type of floral meristem. No other mutants are reported to show a clear phase change in the inflorescence. Although several possibilities are available to explain the phenomenon, the molecular mechanism remains unknown.

FIL Controls the Number of Floral Organs

Different from type B structures, type A flowers have floral organs, but their number is altered from that of wild-type flowers. The number of sepals, petals, and stamens is decreased, but that of carpels is increased. This feature was observed in flowers of double mutants (Table 2). Two types of Arabidopsis mutants with altered organ number have been reported. A mutant with an increased number of organs, *clv1*, and a mutant with a decreased number, *wus*, have floral meristems of larger and smaller meristems, respectively, than does the wild type (Clark et al., 1993; Laux et al., 1996). Whereas the *pan* mutant, having a meristem of normal size, has an increased number of sepals, petals, and stamens, it has a normal number of carpels (Running and Meyerowitz, 1996). In the *fil* mutant, the size of floral meristem is decreased, but our observations suggest that the size of the four whorls is not decreased proportionally; that is, the size of whorls 1, 2, and 3 was decreased but that of whorl 4 was increased. To examine the role of the *FIL* gene in determining the size of whorls, we crossed the *fil* mutant with a plant having a strong *clv1* allele, *clv1-4.* The *clv1-4* mutant has a flower with five sepals, five petals, nine stamens, and six carpels, on average (Clark et al., 1993; S. Sawa, unpublished results). When compared with that of a flower of the *fil* mutant, the number of petals, stamens, and carpels is increased to two, six, and five, respectively, in a flower of the *fil-1 clv1-4* double mutant, but the number of sepals was not changed (S. Sawa, unpublished results). These

results are in line with a model in which the size of inner whorls may be determined proportionally by the size of the floral meristem, but the size of whorl 1 may be determined independently of the size of the meristem in the *fil* mutant.

FIL Controls the Expression Pattern of Floral Genes

The floral structures of *fil ap1* and *fil ap2* double mutants showed an enhanced phenotype similar to that of plants with a strong allele of the *ap2* mutation. Namely, flowers of the *fil ap1-17* and *fil ap2-1* double mutants showed homeotic conversion of sepals to carpels and lacked petals and stamens (Figures 5C, 5D, and 5F). The effect of the *fil* mutation on the cell fate of floral organs formed in the outer whorls is consistent with the altered expression pattern of the organ identity genes. In situ hybridization analysis showed that expression of the *AP1* gene was not detected; instead, the *AG* gene was expressed in the outer whorls. Because mutual repression of the expression of *AP1* and *AG* genes has been proposed from genetic and molecular analyses of the wild type and several mutants (Gustafson-Brown et al., 1994), it is not obvious whether the major defect of the *fil* mutation is in repression of *AP1* gene expression or in the expression of the *AG* gene in the outer whorls.

In situ analyses of the expression pattern of homeotic genes showed that *PI* and *AG* are ectopically expressed in the inflorescence of the *fil* mutant. In addition, the incorrect expression pattern of homeotic genes in the developing floral meristems is suspected to be the cause of several types of homeotically converted floral organs found in *fil* flowers. It is strongly suggested that *FIL* is responsible for the spatial and temporal control of the expression of the genes. Future analyses of the molecular function of the *FIL* gene will clarify the mechanism.

We are now attempting to isolate the *FIL* gene by using a map-based cloning procedure. Genetic analysis has shown that the *fil* locus is linked to molecular markers on chromosome 2 between nga168 (16 recombinants per 178 chromosomes) and pAtT51 (three recombinants per 90 chromosomes). The *FIL* gene was confirmed to be covered by a bacterial artificial chromosome clone.

We also are examining enhancer and suppressor mutations of the *fil* mutant among the progeny of backcrosses of *fil* (Landsberg *erecta* ecotype) with wild-type lines of the Wassilewskija, Columbia, or Enkheim ecotypes. The enhanced lines show inflorescences similar to those of the *fil ufo* double mutant, whereas flowers of the suppressed lines recovered the number of petals and stamens: 4.5 sepals, 2.9 petals, 5.7 stamens, and 2 carpels. Interestingly, suppressor lines did not produce type B structures. Further analysis of the enhancing or suppressing loci will help us to understand the molecular function of the *FIL* gene in the formation and development of inflorescence and floral meristems.

There are many cases in which mutants with an identical or similar inflorescence phenotype are found in Arabidopsis, Antirrhinum, and other plant species. Mutants with phenotypes closely resembling each other often carry a mutation in a structurally homologous gene. Mutants with a phenotype similar to that of the *fil* mutant, however, have not been reported for Antirrhinum or other species, with one exception: the Arabidopsis mutant *revoluta* has some similarity in that it forms filaments on the inflorescence and lacks a secondary or tertiary inflorescence (Talbert et al., 1995). There might be some functional relation between the *FIL* and *REVOLUTA* genes; however, the two genes are different because the *REVOLUTA* gene is reported to reside on chromosome 5.

METHODS

Plant Material and Growth Conditions

The *fil-1* mutant (the former name was *FL54*) and *ap1-17* mutant (the former name was *FL1*) were isolated from *Arabidopsis thaliana* ecotype Landsberg *erecta* by ethyl methanesulfonate mutagenesis (Komaki et al., 1988; Okada and Shimura, 1994). The *fil-2* mutant (isolated from ethyl methanesulfonate–mutagenized Landsberg *erecta*) was provided by E.M. Meyerowitz (California Institute of Technology, Pasadena). Other mutants and wild ecotypes were obtained from the Ohio State University Arabidopsis Biological Resource Center (Columbus, OH).

Seeds were sown on the surface of vermiculite in small pots and incubated at 4°C for 3 days. Plants were grown in a laboratory room under continuous illumination of 50 to 100 μE m^{-2} sec^{-1} at 22°C (normal condition) or 16°C (low-temperature condition). Some plants were grown under short-day growth conditions (8 hr of light and 16 hr of darkness) at 22°C.

Strain Construction

Double mutants carrying *fil* and other mutations were constructed by crossing the *fil* homozygote with a plant homozygous for the other mutation, except in the case of the *ag* and *lfy* mutants. Genotypes of the double mutants were confirmed by backcrossing them with parental single mutants. In the case of the *ag* and *lfy* mutants, heterozygotes were used as parental lines.

The *fil lfy ap1-1* triple mutants were constructed by crossing *fil ap1-1* double mutants with plants that were homozygous for *fil* and heterozygous for *lfy.* Because the phenotype of the possible triple mutants was indistinguishable from the *fil lfy* double mutant, we confirmed the *ap1-1* mutation by using the polymerase chain reaction check method (Sawa et al., 1997). The homozygosity of the *lfy* gene in the triple mutant was confirmed by the phenotypic similarity between the triple mutant and the *fil lfy* double mutant.

To isolate the *fil cal* double mutants, the *fil* mutant was backcrossed to the wild-type Wassilewskija ecotype.

Scanning Electron Microscopy

For scanning electron microscopy, young, primary inflorescences were fixed in a carnoa liquid mixture (isoamyl acetate–ethanol [1:3]),

and then the samples were rinsed twice with ethanol. The samples were treated with 2% tannic acid in ethanol for 10 hr, rinsed twice with ethanol, and incubated in an ethanol–isoamyl acetate (1:3) mixture for ~15 min. The samples were then immersed in isoamyl acetate for 15 min and dried in liquid carbon dioxide. Individual flowers were removed from inflorescences and mounted on scanning electron microscopy stubs. The mounted specimens were coated with gold and observed with a scanning electron microscope (model JSM-T20; Nippon Denshi, Tokyo, Japan) at an accelerating voltage of 20 kV. The images were photographed on Neopan SS 120 film (Fuji, Tokyo, Japan).

In Situ Hybridization

In situ hybridization was conducted essentially by following the procedure of Ito et al. (1997). The antisense gene-specific probes were prepared as follows. The probe of the *AG* gene was obtained from a pIAGX plasmid containing a 532-bp fragment from base pair positions 328 to 859 of the *AG* gene, as reported by Yanofsky et al. (1990). The *AP3* probe was prepared from pODS4 carrying the SpeI-DraI fragment from the cDNA clone (Okamoto et al., 1994). The *AP1* probe was prepared from pOAP1-2 having a 457-bp fragment from positions 477 to 933, as reported by Mandel et al. (1992), and the *LFY* probe was prepared from pSLFY9 carrying a 510-bp fragment from positions 773 to 1282, as reported by Weigel et al. (1992). The *PI* probe, obtained from pcPINX (Goto and Meyerowitz, 1994), was a gift from K. Goto (Kyoto University).

Mapping

For mapping of the *FIL* locus, we used the simple sequence length polymorphism mapping marker nga168 (Bell and Ecker, 1994) and restriction fragment length marker pAtT51 (provided by E.J. Richards, Washington University, St. Louis, MO) on chromosome 2. We determined the polymorphic nature of the genome of the F_2 progeny obtained from a cross between the *fil* mutant and a wild-type ecotype Columbia plant. Map distances were scored by using the Kosambi mapping function (Koornneef and Stam, 1992).

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