A Genetic Screen for Modifiers of UFO Meristem Activity Identifies Three Novel FUSED FLORAL ORGANS Genes Required for Early Flower Development in Arabidopsis

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ABSTRACT

In a screen to identify novel genes required for early Arabidopsis flower development, we isolated four independent mutations that enhance the Ufo phenotype toward the production of filamentous structures in place of flowers. The mutants fall into three complementation groups, which we have termed FUSED FLORAL ORGANS (FFO) loci. ffo mutants have specific defects in floral organ separation and/or positioning; thus, the FFO genes identify components of a boundary formation mechanism(s) acting between developing floral organ primordia. FFO1 and FFO3 have specific functions in cauline leaf/stem separation and in first- and third-whorl floral organ separation, with FFO3 likely acting to establish and FFO1 to maintain floral organ boundaries. FFO2 acts at early floral stages to regulate floral organ number and positioning and to control organ separation within and between whorls. Plants doubly mutant for two ffo alleles display additive phenotypes, indicating that the FFO genes may act in separate pathways. Plants doubly mutant for an ffo gene and for ufo, ify, or clv3 reveal that the FFO genes play roles related to those of UFO and LFY in floral meristem initiation and that FFO2 and FFO3 may act to control cell proliferation late in inflorescence development.

Understanding the molecular basis of pattern formation is a fundamental goal of developmental biology. Elegant genetic and molecular studies have revealed that mechanisms involving morphogen gradients (Belvin and Anderson 1996; Rivera-Pomar and Jackle 1996) and cell-cell communication (Duffy and Perrimon 1996; Kornfeld 1997) are used during various developmental processes in animals. In plants, which rely primarily on coordinated cell division and cell expansion for morphogenesis (Meyerowitz 1997), the genetic mechanisms of pattern formation are not as well characterized. However, a number of studies over the past 10 years have dramatically illustrated the utility of analyzing flower development, such as that of Arabidopsis thaliana, as a means of gaining insight into these processes (Coen and Meyerowitz 1991; Ma 1994; Okada and Shimura 1994; Weigel 1995).

The mature Arabidopsis flower consists of four concentric whorls of organs arranged in a stereotypical pattern: four sepals in the outermost whorl, four petals in the second whorl, six stamens in the third whorl, and two carpels in the innermost whorl that fuse to form the gynoecium. The development of this complex structure from a small group of undifferentiated floral meristem cells set aside from the inflorescence meristem requires the activity of distinct sets of genes responsible for elaborating the various patterning elements. These genes combine into regulatory pathways required for specifying floral meristem identity and for controlling floral organ primordia number, identity, and positioning (Weigel 1995; Weigel and Clark 1996; Meyerowitz 1997). Yet, little is known about the mechanisms of correct allocation of cells to floral organ primordia or about the establishment and maintenance of discrete boundaries between floral organs, which are central to the formation of such patterns in the developing flower.

Genetic approaches have begun to address questions about boundaries and cell allocation through the identification of several genes with roles in these processes. In particular, mutations in the Arabidopsis UNUSUAL FLORAL ORGANS (UFO), Petunia no apical meristem (nam), and Arabidopsis CUP-SHAPED COTYLEDONS1 (CUC1), and CUC2 genes have been shown to cause defects that include inappropriately fused floral organs (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995; Souer et al. 1996; Aida et al. 1997). UFO flowers exhibit a wide variety of phenotypes, including the formation of mosaic organ types and fused adjacent organs in whorls 2 and 3, suggesting that UFO acts to define the boundaries between growing organs or to control cell proliferation within growing organs. nam flowers often produce an extra whorl of petals, many fused to neighboring stamens, while cuc1 and cuc2 flow-
ers show whorl-specific sepal-sepal and stamen-stamen fusions.

Molecular analyses of UFO and nam reveal nonuniform RNA expression patterns consistent with some of their roles in boundary specification in developing flowers. During early flower development, UFO RNA becomes detectable in the center of floral primordia, expands outward into the emerging sepal primordia while being downregulated in the center—forming a cup-shaped domain—and is then further restricted to the base of the petal primordia (Ingram et al. 1995; Lee et al. 1997). nam RNA is found early in stripes between the floral and bract primordia and later in rings around stamen primordia (Souer et al. 1996). Interestingly, UFO, nam, CUC1, and CUC2 are all either expressed in the embryonic shoot apical meristem (SAM) or affect its development, so some genetic mechanisms used in floral development seem to be closely related to those functioning in embryonic development (Souer et al. 1996; Aida et al. 1997; Lee et al. 1997). UFO and its ortholog, FIMBRIATA of Antirrhinum, encode novel proteins (Simon et al. 1994; Ingram et al. 1995) containing an F-box, a Skp1p-binding motif found in a number of cell-cycle regulatory proteins (Bai et al. 1996), and components of E3 ubiquitin ligase complexes active in ubiquitin proteolysis (Feldman et al. 1997; Skowrya et al. 1997). nam and CUC2 share sequence similarity with each other and are part of a large gene family, but their biochemical function remains unclear (Souer et al. 1996; Aida et al. 1997; Sablowski and Meyerowitz 1998).

We have attempted to isolate additional genes required for early floral patterning events by enhancer screening, using ufo mutant plants as a starting population. Such screens have been used successfully to identify genes functioning in related processes in both animals (Simon et al. 1991) and plants (Bowman et al. 1993; Liu and Meyerowitz 1995). Genetic interactions with UFO have identified a large, diverse class of genes required for flower formation in Arabidopsis (Levin and Meyerowitz 1995). Mutations in UFO cause the partial conversion of floral meristems into shoot meristems, and the inflorescence meristems of ufo mutant plants occasionally produce filamentous structures in place of flowers. Double-mutant combinations of ufo with filamentous flower (fil), clavata1 (clv1), clavata3 (clv3), launig (lug), and hanaba tanaru (han) prevent the formation of most flowers and produce filamentous structures in their place (Levin and Meyerowitz 1995). The floral meristem identity gene LEAFY (LFY; Weigel et al. 1992) is also a member of this class of genes, as the inflorescences of ify clv1 and ify lug double mutants also form predominantly filamentous structures (Clark et al. 1993; Liu and Meyerowitz 1995). If a double mutant forms filamentous structures, the two genes may act in different, though probably related, floral patterning processes. In the absence of both genes, insufficient patterning information may be supplied for flower development to proceed, and filamentous structures result.

Not every double-mutant combination of genes in this class displays this enhanced phenotype; e.g., clv1 clv3 and ufo ify double mutants reveal epistatic relationships (Clark et al. 1995; Levin and Meyerowitz 1995). In these cases, the two genes are likely to act in the same process. Functionally, the members of this class of genes seem to have little in common: UFO, LFY, and FIL (Komaki et al. 1988) play roles in establishing floral meristem identity, while LUG has a role in regulating organ width and a cadastral function in regulating the extent of floral organ identity gene expression (Liu and Meyerowitz 1995). CLV1, CLV3, and HAN appear to control meristem size, as clv1 and clv3 mutants form larger meristems and more floral organs (Clark et al. 1993; Clark et al. 1995; Liu and Meyerowitz 1995), while han flowers have reduced organ numbers (H. Saikai, personal communication). However, each of these genes functions in very early floral patterning events, suggesting that the production of filamentous structures in one of the above mutant backgrounds might be a useful phenotypic marker with which to identify novel genes required for the initial stages of flower development.

In this article we describe the results of our genetic screen to identify enhancers of the UFO phenotype toward the production of filamentous structures. We have identified three FUSED FLORAL ORGANS genes, mutations in any one of which cause the fusion of adjacent floral organs within and/or between whorls. ffo1 and ffo3 mutants display very similar vegetative and floral organ-fusion phenotypes, but the two genes appear to perform their floral organ boundary-restricting roles at different stages of development. The ffo2 mutant phenotype is restricted to the inflorescence and has a different type of defect in floral organ separation. The phenotypes of double mutants between alleles from each complementation group are additive, indicating that though the three genes function in the same process, they may act in separate pathways to control floral organ separation. Additional double-mutant analysis with other members of the filamentous structures class indicates that the FFO genes act in related processes with UFO and LFY in the initial stages of flower development and may also play a role in controlling meristematic cell proliferation.

MATERIALS AND METHODS

Arabidopsis growth conditions: Seeds were sown and plants grown as described previously (Levin and Meyerowitz 1995).

Mutagenesis: ufo homozygous seeds were washed with 0.1% Tween-20 for 15 min, subsequently mutagenized with 0.2% EMS (ethyl methanesulfonate) for 12 hr, washed with sterile water for 15 min, washed several times with 0.1% Tween-20 for a total of 5.5 hr, and then sown on soil mix. 1700 M1 plants
germinated and produced M2 seeds, which were collected from each individual M1 plant. Approximately 40 M2 plants were screened from each of 975 M1’s.

**Genetic and phenotypic analysis:** The isolated enhancer mutants were crossed to wild-type Landsberg erecta (Ler) plants. F2 plants from this cross were screened to identify the mutant phenotype in the absence of the ufo-6 allele. The single-mutant F2 plants were backcrossed two additional times to Ler before all analysis except the ffo complementation tests, which were performed with singly backcrossed lines. The ffo1-3 allele was identified in an EMS mutagenesis of 35S::PI plants in the Nossen ecotype and was backcrossed three times to Ler before analysis. The ffo2-2 allele was identified in an EMS mutagenesis of ag-4 mutants in the Ler ecotype and was backcrossed three times to Ler before analysis.

Complementation tests between the ffo mutants were performed by crossing plants homozygous for one ffo mutation to plants homozygous for another ffo mutation in all possible pairwise combinations. The F1 plants from each cross were scored for the presence of the mutant phenotype and F2 seeds collected in bulk. To confirm the F1 results, approximately 60 F2 plants per cross were scored for the mutant phenotype or for the segregation of wild-type and doubly mutant plants as appropriate.

Additional complementation tests were conducted by crossing plants homozygous for either the ffo1-1, ffo2-1, or ffo3-1 mutation to plants homozygous for either the cuc1, cucumber (cuc2), pinoid-8, or pin-formed-5 mutation (Okada et al. 1991; Bennett et al. 1995; Aida et al. 1997). The F1 plants from each cross were wild type, suggesting that the mutations were not allelic. To confirm these results, approximately 60 F2 seeds per cross were scored for the segregation of wild-type and doubly mutant plants. Due to the similarity between ffo1 and cuc2 mutant phenotypes, F2 plants from putative double mutants and from 25 ffo1 and cuc2 single mutants were sown and the plants scored to verify the presence of the double mutant. Genetic mapping experiments (see next paragraph) indicated linkage between FFO2 and aitogoma (Elliot et al. 1996; Kuchler et al. 1996), which lies near aptal2 (ap2) on chromosome 4. To test for complementation, pollen from homozygous ant plants was crossed onto homozygous ffo2-2 carpels. The presence of wild-type plants in the F1 and F2 generations confirmed that ffo2 and ant are not allelic.

The map locations of the FFO loci were determined by two-factor genetic mapping. Plants homozygous for each ffo mutation were crossed to plants homozygous for a linked visible marker, and at least 150 F2 plants scored to identify the double mutant. F2 seeds from 28 single-mutant F1 plants were collected and sown, and the F3 families analyzed for segregation of doubly mutant plants. According to the frequency of recombination in the F3 generation, FFO1 is located on chromosome 2, approximately 25 cm from CLV3; FFO2 is located on chromosome 4, approximately 19 cm from AP2; and FFO1 is located on chromosome 5, approximately 21 cm from HAN (H. Sakai, personal communication).

In experiments to determine the frequency of cauline leaf fusion, to identify the organ subtending the first flower, and to determine the number and identity of floral organs present (Tables 2 through 5), all plants were grown at the same time, in the same location, and at a similar density of plants per pot. For analysis of cotyledon development, 200 seeds per genotype were sown on Murashige and Skoog basal salt mixture (MS) plates at a density of 30 seeds/plate, incubated at 24°C for 5 days, placed under lights (600 f.c. of constant cool-white fluorescent) at 23°C and scored after 5 days’ growth.

**Strain construction:** Double mutants were constructed by crossing a plant homozygous for an ffo mutation or a ufo mutation with a plant homozygous for the other mutation. All double-mutant phenotypes were confirmed in the F3 generation by analyzing the segregation of the double mutant among the progeny of an F2 parent homozygous for only one of the mutations.

**Scanning electron microscopy:** Samples were collected, fixed, mounted, coated, and photographed as described previously (Levin and Meyerowitz 1995).

**Transmission electron microscopy:** Tissues were fixed, stained, postfixed, embedded, and stained as described previously (Liu et al. 1997) and visualized using a Phillips 201 electron microscope.

**Image processing:** Slides and negatives were scanned and digitized with a Polaroid SprintScan 35/LE scanner. Images were adjusted for brightness, contrast, and color and assembled for figures with Adobe Photoshop (version 3.0; Mountain View, CA). Figures were printed with a Kodak XLS 8300 Digital Printer.

**RESULTS**

**Isolation of mutants enhancing the ufo phenotype:** To identify genes necessary for the initial stages of floral development, we performed an EMS mutagenesis in which we screened for plants with an enhancement of the ufo weak filamentous structure defect (see Levin and Meyerowitz 1995). In order to collect sufficient M2 seeds from each M1 plant, we selected the ufo-6 mutant for mutagenesis because it was the weakest available ufo allele and had fertility close to wild-type levels (Levin and Meyerowitz 1995). Because the double-mutant plants that we sought to identify in this screen were expected to lack flowers and thus be sterile, we collected M2 seeds from individual M1 plants. This approach allowed us to recover the enhancer mutation of interest from the heterozygous M2 sibling plants. By screening the progeny of approximately 975 ufo-6 M2 plants, we recovered 12 mutations that enhanced the ufo filamentous structure defect (J.Z.L., J.C.F., and E.M.M., unpublished results).

In this article, we focus on four of these enhancer mutations that share a similar single mutant phenotype. We have named these genes FUSED FLORAL ORGANS (FFO) because a mutation in any one of them results in inappropriately fused floral organs. Complementation tests among the four mutations indicated that we had identified mutations in three genes: FFO1, FFO2, and FFO3 (Table 1). Two independent FFO1 alleles, ffo1-1 and ffo1-2, were isolated in this screen. In unrelated mutageneses, we isolated two other mutants with similar phenotypes and assigned them by complementation tests to FFO1 (ffo1-3) and FFO2 (ffo2-2), respectively (Table 1). The ffo mutations all complemented the cup-shaped cotyledons1 (cuc1), cuc2, pinoid (pid), and pin-formed (pin) mutations, all of which have fused floral organ phenotypes (Goto et al. 1987; Okada et al. 1991; Bennett et al. 1995; Aida et al. 1997). ffo1 and ffo3 alleles are recessive, while the ffo2-1 allele is semidominant (see below). Preliminary map-position data for each of the ffo mutations indicated that they behaved in a Mendelian
TABLE 1
Fused floral organ (ffo) alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Isolation No.</th>
<th>Inheritance</th>
<th>Allele strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>ffo1-1</td>
<td>971</td>
<td>Recessive</td>
<td>Stronger</td>
</tr>
<tr>
<td>ffo1-2</td>
<td>607</td>
<td>Recessive</td>
<td>Weaker</td>
</tr>
<tr>
<td>ffo1-3</td>
<td>6.1</td>
<td>Recessive</td>
<td>Weaker</td>
</tr>
<tr>
<td>ffo2-1</td>
<td>3-4</td>
<td>Semidominant</td>
<td>Stronger</td>
</tr>
<tr>
<td>ffo2-2</td>
<td>870</td>
<td>Recessive</td>
<td>Weaker</td>
</tr>
<tr>
<td>ffo3-1</td>
<td>833</td>
<td>Recessive</td>
<td>—</td>
</tr>
</tbody>
</table>

All mutants were isolated in a Landsberg erecta (Ler) genetic background with the exception of 6.1, which was isolated in Nossen and backcrossed three times to Ler before analysis.

fashion and were likely to be new genetic loci (see materials and methods). In addition, we isolated one additional mutation that enhanced ufo-6 and caused floral organ fusion; we showed that this mutation was a weak pin-formed allele.

Phenotypes of ufo ffo double mutants: To determine whether the degree of enhancement varied with ufo allele strength, we compared the double-mutant phenotypes of ffo1-1, ffo2-1, and ffo3-1 in combination with both ufo-6 and ufo-2. The ufo-2 phenotype is generally more severe than that of ufo-6 and is representative of the strong ufo alleles (Figure 1A; Levin and Meyero-witz 1995). In double-mutant combinations with the weak ufo-6 allele, ffo1-1, ffo2-1, and ffo3-1 formed a few Ufo-like flowers in basal positions on the primary inflorescence and filamentous structures in the apical positions. Filamentous structures were also observed to terminate most axillary inflorescences. Although each ufo-2 ffo double-mutant phenotype had a slightly higher frequency of filamentous structures compared with that of its respective ufo-6 ffo double mutant, no qualitative differences were observed between the double-mutant combinations with ufo-2 and ufo-6 (Figures 1 and 2). These minor differences are likely to be caused by the

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Figure 1.—Phenotypes of ufo-2 ffo double-mutant and ffo single-mutant plants. (A) A ufo-2 inflorescence with a reduced flower and several tiny filamentous structures. The flowers contain mosaic organs and consist predominantly of sepals and carpels, reflecting a reduction in class B organ identity gene activity. (B) A ufo-2 ffo1-1 inflorescence. Some of the more basal filamentous structures are topped with stigmatic tissue (arrowheads). (C) A ufo-2 ffo2-1 inflorescence. (D) A ufo-2 ffo3-1 inflorescence terminating in filamentous structures (arrowheads) and leaflike structures (arrows). (E) A wild-type Ler flower. (F) An ffo1-1 flower with two partially fused sepals (arrow). (G) An ffo2-1 flower composed of two sepals and a petal/sepal mosaic organ (arrow), two narrow petals, five stamens, a filament-like organ (arrowhead), and the central gynoecium. (H) An ffo3-1 flower with two completely fused sepals (arrow) and a bulge in the apical part of the valves just below the stigma (arrowhead).
Figure 2.—Phenotypes of wild-type, ffo mutant, and ufo-2 ffo double-mutant inflorescences and flowers visualized by SEM. Numbers refer to stages of floral development, which are according to Smyth et al. (1990). (A) A ufo-2 ffo-1 inflorescence. (B) A ufo-2 ffo-1 inflorescence. (C) A ufo-2 ffo-1 inflorescence with basal filamentous structure topped by stigmatic tissue (arrowhead). (D) Higher magnification of a ufo-2 ffo-1 filamentous structure from C. The apical adaxial surface is made up of stigmatic cells. (E) A wild-type Ler inflorescence. A total of eight stage 1-5 floral primordia have been initiated. The stage reached by each bud older than stage 1 is shown. (F) An Ler stage 3 flower. (G) An Ler stage 5 flower viewed from the side. The boundary between the abaxial and the lateral sepal is marked by a deep groove (arrow). (H) An ffo-1 inflorescence. The floral primordia at these early stages of development appear indistinguishable from the wild type. (I) An ffo-1 stage 5 flower viewed from the side. The abaxial and the lateral sepal are almost completely fused to one another (arrow). (J) Higher magnification of the stage 5 flower shown in I. The epidermal cells of the abaxial and the lateral sepal form a continuum across the presumptive boundary site. (K) An ffo-3-1 inflorescence. (L) An ffo-3-1 stage 3 flower with two adjacent first-whorl organ primordia fused to one another (arrow). (M) Another ffo-3-1 stage 3 flower in which the first-whorl sepal primordia have arisen fused together into a ring. (N) An ffo-2-2 inflorescence. A total of 11 stage 1-5 floral primordia have been initiated, more than are formed on wild-type inflorescences, and multiple buds at approximately the same developmental stage are visible. The stage reached by each bud older than stage 1 is shown. (O) An ffo-2-2 stage 5 flower viewed from the side. The first-whorl sepal vary greatly in size and are spaced with three on one side of the meristem and one on the other rather than equidistantly from one another. (P) An ffo-2-2 stage 4 flower. Congenital fusion between two adjacent sepal is shown (arrow). In A-C, bars = 100 μm; in D-P, bars = 10 μm.
fact that the ufo-2 mutation causes a stronger reduction in UFO function than ufo-6 (Levin and Meyerowitz 1995; Lee et al. 1997). Thus, these enhancers do not seem to exhibit allele specificity in their enhancement of the ufo filamentous structure defect. In light of these results, we present the phenotypes of only the ufo-2 ffo double mutants in more detail.

The filamentous structure defects of ufo-2 ffo2 and ufo-2 ffo3-1 double mutants were examined using both light microscopy (Figure 1) and scanning electron microscopy (SEM) (Figure 2). Most ufo-2 ffo2 and ufo-2 ffo3-1 inflorescences produced some basal flowers, followed by filamentous structures at the apical positions. The filamentous structures were usually thin green cylindrical protrusions from the stem (Figures 1C and 2A), with those in the more basal positions sometimes bearing stellate (branched) trichomes (Figures 1D and 2B). ufo-2 ffo2 inflorescences eventually terminated in tiny filamentous structures (Figure 1C), while ufo-2 ffo3-1 inflorescences eventually terminated in a mass of leaves enclosing carpelloid tissue (Figure 1D). The filamentous structures produced by the ufo ffo2 and ufo ffo3-1 double mutants appear similar to structures previously described for many other single- and double-mutant genotypes (Komaki et al. 1988; Clark et al. 1993; Levin and Meyerowitz 1995; Liu and Meyerowitz 1995; Talbert et al. 1995).

Compared to ufo-2 ffo2 and ufo-2 ffo3-1 double mutants, the ufo-2 ffo1-1 double mutant had a slightly stronger enhancement of the ufo filamentous structure defect, in that they formed fewer basal flowers. One striking feature of these plants was that many of the filamentous structures near the base of the inflorescence had carpelloid characteristics (Figures 1B and 2, C and D). The filamentous structures found in other ufo double mutants are not carpelloid (Clark et al. 1993; Levin and Meyerowitz 1995), except for those in the most apical positions on the inflorescence (Levin and Meyerowitz 1995). The apical carpelloid filamentous structures of these other ufo double mutants are likely to be related to the premature termination of ufo single-mutant inflorescences in carpelloid structures (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995).

Transmission electron microscopy of filamentous structures of ufo-2 double mutants: Previous studies of ufo double mutants that produce filamentous structures rather than flowers have raised the issue of whether these structures are more closely related to leaves or to flowers (Levin and Meyerowitz 1995). To address this issue, we used transmission electron microscopy (TEM) to analyze filamentous structures from three different ufo-2 double mutants. Transverse sections were taken at the base of ufo-2 cv3-1, ufo-2 fil, and ufo-2 ffo1-1 filamentous structures and compared to those taken at the base of wild-type flower pedicels and cauline leaves (Figure 3). Transverse sections were also taken at the tips of ufo-2 cv3-1 filamentous structures, and the cells present were found to be very similar to those at the base. We found that the cells that make up the filamentous structures were often irregular in shape, particularly in the epidermal and underlying cortical layers, and had cell walls of uneven thickness (Figure 3, A–F). The ground tissue appeared to be composed mainly of highly vacuolated, mature cells of a few cell types, and intercellular air spaces were abundant. Vascular bundles were notably absent from the filamentous structures of all genotypes examined (compare Figure 3, A–F with Figure 3, H and I). The most basal section of one ufo-2 ffo1-1 filamentous structure showed a single vascular tracheid. This vascular cell was absent in subsequent sections of the same filamentous structure.

When we compared the ground tissue from the filamentous structures of the three double mutants, we observed a range of cell types—from very distinctive ufo-2 cv3-1 cells to ufo-2 ffo1-1 cells, which resembled those in wild-type leaves. The cells of ufo-2 cv3-1 filamentous structures were nearly devoid of cytoplasm, and prominent starch grains were the only cytoplasmic structures present in nearly every cell (Figure 3, A and B). ufo-2 fil filamentous structures also consisted mainly of highly vacuolated storage cells containing multiple starch grains. However, cells containing large amounts of cytoplasm and diverse organelles, including chloroplasts, were also present (Figure 3, C and D). ufo-2 ffo1-1 filamentous structures contained a relatively higher proportion of densely cytoplasmic cells as well as some polyhedral cells composed of a large vacuole and surrounding cytoplasm, the latter containing numerous chloroplasts and starch grains (Figure 3, E and F). These polyhedral cells closely resemble the spongy parenchymal mesophyll cells found at the margins of wild-type cauline leaves (compare Figure 3, F and G) and are less similar to the densely packed, more irregular and starchless cells present in wild-type pedicels (Figure 3, H and I). Thus, at the cellular level, the filamentous structures of some genotypes may be more closely related to leaves than to flowers, while those of other genotypes, such as ufo-2 cv3-1, have neither leaflike nor floral character.

Analysis of ffo single mutants: ffo1: From each ufo-6 ffo double mutant, we isolated and characterized the ffo single mutant. The ffo1 mutants displayed floral defects in the first and third whorls. We present data for the ffo1-1 mutant because the other two ffo1 mutants displayed similar, though slightly weaker, phenotypes. In the first whorl of wild-type flowers, the sepals attach to the base of the receptacle but do not fuse to each other (Smyth et al. 1990). By contrast, in ffo1-1 mutants, the sepals fuse partially or completely along their margins (Table 2 and Figure 1F). The frequency and extent of the fusion defect became more extreme in apical flowers. To determine whether the sepals from ffo1-1 mutants fuse congenitally or postgenitally, we used SEM to examine the earliest stages of flower development in ffo1-1 mutant flowers. We found that the sepals of ffo1-1
Figure 3.—Transmission electron micrographs of ufo-2 double-mutant filamentous structures compared with wild-type pedicels and cauline leaves. (A) A ufo-2 clv3-1 filamentous structure. (B) Higher magnification of the ground tissue cells shown in A. (C) A ufo-2 fil filamentous structure. (D) Higher magnification of the ground tissue cells shown in C. (E) A ufo-2 flo1-1 filamentous structure. (F) Higher magnification of the ground tissue cells shown in E. (G) A wild-type cauline leaf from the third node. Shown are mesophyll parenchyma cells at the margin of the leaf blade. (H) A different region of the wild-type cauline leaf from the third node shown in G, showing the vascular bundles, including a prominent tracheid (T), which forms one of the lateral veins in the leaf blade. (I) Cells and vascular bundles, including tracheids (T), from a wild-type flower pedicel. (S) starch granule. In A, C, E, G, and I, bars = 10 μm; in B, D, F, and H bars = 1 μm.
TABLE 2

<table>
<thead>
<tr>
<th>Positions of flowers</th>
<th>ffo1 organ counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ffo1-1 flower No.</td>
</tr>
<tr>
<td></td>
<td>1-5</td>
</tr>
<tr>
<td>Whorl 1 (sepal-sepal junction)</td>
<td></td>
</tr>
<tr>
<td>Sepals unfused</td>
<td>3.00</td>
</tr>
<tr>
<td>Sepals fused &lt;50%</td>
<td>1.00</td>
</tr>
<tr>
<td>Sepals fused &gt;50%</td>
<td>0.04</td>
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<tr>
<td>Total</td>
<td>4.04</td>
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<tr>
<td>Whorl 2</td>
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<td>Total</td>
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</tr>
<tr>
<td>Whorl 3</td>
<td></td>
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<tr>
<td>Stamens</td>
<td>4.96</td>
</tr>
<tr>
<td>Stamens fused</td>
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<tr>
<td>Other (rSt, FSt)</td>
<td>0.04</td>
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<td>Total</td>
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<td>Whorl 4</td>
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</tr>
<tr>
<td>Carpels</td>
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<tr>
<td>Whorl 1 fused organs</td>
<td>0.60</td>
</tr>
<tr>
<td>Whorl 3 fused organs</td>
<td>0.56</td>
</tr>
</tbody>
</table>

The average number of organs per whorl is given. Lea, Landsberg erecta; rSt, rudimentary stamen; FSt, filament/stamen organ.

* The first 25 flowers were examined on each of five plants.

* The first 25 flowers were examined on each of six plants.

* The number of sepals-sepal junctions completely unfused, fused along <50% of the margin, or fused along >50% of the margin.

* Number of flowers with defect/total number of flowers.

mutant flowers appeared indistinguishable from wild-type through stage 4 (compare Figure 2, E and H). At stage 5, when the petal and stamen primordia appear, developing wild-type sepals had deep grooves between them (Figure 2G), while some adjoining ffo1-1 mutant sepals were observed to be fused in basal positions or along the entire length of the margin (Figure 2, I and J). Thus, sepal fusion in ffo1-1 mutant flowers appears to occur postgenitally.

In the third whorl of wild-type flowers, five or six stamens attach to the base of the receptacle but do not fuse to each other (Smyth et al. 1990). By contrast, in ffo1-1 mutants, the number of stamens in the basal flowers was increased, and two stamens often displayed partial fusion (Table 2). In more apical flowers, the frequency of extra stamens and of stamen-stamen fusion events decreased compared with that of the basal flowers (Table 2). In the second whorl, petal-petal fusion was observed only once in 150 flowers; no defects were observed in the fourth whorl (Table 2). The size of individual floral organs in ffo1-1 mutants was not altered (Figure 1F). As is seen for mutants in floral meristem identity genes such as LFY and UFO (Weigel et al. 1992; Levin and Meyerowitz 1995), the first flower formed by ffo1-1 mutants was subtended by a leaf or filamentous structure on about 15% of primary inflorescences.

In addition to the floral defects exhibited by ffo1 mutants, the growth of the margins of the cauline leaves and the cotyledons showed similar types of abnormalities. In all ffo1-1 mutants examined, one or both margins of one or more cauline leaves were attached inappropriately at their base to the stem of the primary inflorescence (Table 3 and Figure 4B). In contrast, cauline leaf-stem fusion occurs in only 1/4 of wild-type plants, and only at

TABLE 3

<table>
<thead>
<tr>
<th>Cauline leaf defect</th>
<th>Allele</th>
<th>Plants affected (%)</th>
<th>Nodes affected(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>Lea</td>
<td>25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>ffo1-1</td>
<td>100</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>ffo1-2</td>
<td>100</td>
<td>100</td>
<td>67</td>
</tr>
<tr>
<td>ffo1-3</td>
<td>63</td>
<td>63</td>
<td>52</td>
</tr>
<tr>
<td>ffo2-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ffo2-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ffo3-1</td>
<td>53</td>
<td>53</td>
<td>7</td>
</tr>
</tbody>
</table>

All plants were grown at the same time under the same conditions. Lea, Landsberg erecta; n, number of plants scored.

* Nodes counted beginning with the most basal on the inflorescence.
the lowermost node (Table 3). On elongated stems, the leaf tissue often appeared to have been torn, with sections of the base of the cauline leaf tissue attached to the stem, but not to the rest of the cauline leaf (Figure 4B). This defect was observed in only primary inflorescences, and its severity decreased acropetally (Table 3). At a frequency of about 2%, we observed inappropriate fusion of the margins of both cotyledons in fo1-1 mutants. Plants with such defects usually recovered to form normal rosettes as the true leaves developed and emerged in a normal pattern. No other changes in the growth and development of fo1 mutants were observed.

ff03: Like fo1 mutants, fo3-1 mutant plants showed floral defects primarily in the first and third whorls. fo3-1 flowers had a variable number of partially or completely fused sepals in the first whorl and fused stamens in the third whorl (Table 4 and Figure 1H). We also observed a small increase in stamen number in basal fo3-1 flowers compared to wild type. Only minor defects were observed in the second whorl of fo3-1 flowers (Table 4). The gynoecia of fo3-1 flowers displayed a bulge in the apical part of the valves just below the stigma and exhibited slightly decreased fertility, with a corresponding decrease in silique elongation (Figure 1H). As observed in fo1-1 mutants, the first flower formed by fo3-1 mutants was subtended by a leaf or filamentous structure on about 20% of primary inflorescences. Overall, the fo3-1 floral phenotype was similar to that of fo1, although fo3-1 seemed to be slightly weaker.

However, using SEM we found that fo3-1 flowers differed from fo1 flowers at the earliest stages of flower development. Some fo3-1 flowers showed congenital fusion of the first-whorl sepals detectable as early as stage 3 (Figure 2, K and L), when wild-type sepal primordia are distinguishable as discrete mounds of cells (Figure 2F). In stage 3 fo3-1 mutant flowers, we could ob-
TABLE 4

<table>
<thead>
<tr>
<th>Positions of flowers</th>
<th>ffo3-1 flower No.</th>
<th>Lea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-5</td>
<td>6-10</td>
</tr>
<tr>
<td>Whorl 1 (sepal-sepal junction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepals unfused</td>
<td>2.76</td>
<td>1.12</td>
</tr>
<tr>
<td>Sepals fused &lt;50%</td>
<td>1.20</td>
<td>2.08</td>
</tr>
<tr>
<td>Sepals fused &gt;50%</td>
<td>0.12</td>
<td>0.88</td>
</tr>
<tr>
<td>Total</td>
<td>4.08</td>
<td>4.08</td>
</tr>
<tr>
<td>Whorl 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petals</td>
<td>4.04</td>
<td>3.96</td>
</tr>
<tr>
<td>Petals/stamens</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Filaments</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>4.12</td>
<td>4.00</td>
</tr>
<tr>
<td>Whorl 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamens</td>
<td>5.80</td>
<td>5.60</td>
</tr>
<tr>
<td>Stamens fused</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>Other (rSt, FSt)</td>
<td>0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Whorl 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpels</td>
<td>2.08</td>
<td>2.04</td>
</tr>
<tr>
<td>Whorl 1 fused organs</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>Whorl 3 fused organs</td>
<td>0.08</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The average number of organs per whorl is given. Le, Landsberg erecta; rSt, rudimentary stamen; FSt, filament/stamen organ.

- The first 25 flowers were examined on each of five plants.
- The first 25 flowers were examined on each of six plants.
- The number of sepal-sepal junctions completely unfused, fused along <50% of the margin, or fused along >50% of the margin.
- Number of flowers with defect/total number of flowers.

The ffo3 plants also exhibited several nonfloral defects that altered cotyledon growth, cauline leaf development, and inflorescence internode elongation (data not shown). At a frequency of about 5%, ffo3 seedlings had one of the following defects: one cotyledon shriveled up, cotyledons fused together at one of the two margins, or the first true leaf was much larger than the second true leaf. In over 50% of the ffo3 mutants, a cauline leaf defect similar to that of ffo1 was observed (Table 3); in other cases, there appeared to be a bulge of tissue on the abaxial side of the leaf at the junction of the leaf with the stem. All ffo3 plants examined were shorter than wild-type plants of the same age and displayed decreased internode elongation.

ffo2: Although the ffo2 mutants had fused floral organs, their phenotypes suggest that the underlying defect involved a different aspect of floral development than that affected in ffo3-1 and ffo1 mutants (see discussion). ffo2 mutants exhibited variable defects in all four whorls of the flower, with ffo2-1 flowers displaying slightly stronger phenotypes than ffo2-2 flowers. In ffo2 mutants, we observed an increase in the number of floral organs in whorl 2 (Table 5). Organs in both whorls were often narrower—and, at a low frequency, were reduced to filament-like cylindrical organs (Figure 1G and Table 5). Some ffo2 first-whorl organs had petal tissue on the margin of the sepals (Figure 1G); this defect was usually accompanied by the absence of the adjacent petal in the second whorl.

With SEM, we observed several defects in ffo2 sepal primordia initiation and development. Wild-type sepals arise equidistantly from one another relative to the position of the adaxial sepal, which invariably arises nearest the apical meristem (Figure 2E; Smyth et al. 1990). In over 50% the ffo3-1 mutants, a cauline leaf defect similar to that of ffo1 was observed (Table 3); in other cases, there appeared to be a bulge of tissue on the abaxial side of the leaf at the junction of the leaf with the stem. All ffo3 plants examined were shorter than wild-type plants of the same age and displayed decreased internode elongation.

With SEM, we observed several defects in ffo2 sepal primordia initiation and development. The sepal primordia continue to develop in a regular fashion, with the two medial and the two lateral sepals other cases, there appeared to be a bulge of tissue on the abaxial side of the leaf at the junction of the leaf similar to one another in size (Figure 2F). In wild-type sepals also varied widely in size and shape, even in the same flower (Figure 2O). In addition, congenital fusion between two neighboring sepal primordia was detected (Figure 2P). Unlike the other ffo mutants, ffo2 mutant flowers did not have more than two fused sepals per flower, and the fusion of the two sepals was complete: that is, it extended along the entire margin of the sepals (Figure 1G).

In the third whorl of ffo2 mutant flowers, we observed

serve two adjoining sepal primordia arising, connected by a continuous ridge of cells (Figure 2L). In rare cases, all four sepal primordia arose fused together into a cone (Figure 2M).
fused stamens, stamen mosaic organs, and reduced stamen-like organs (Figure 1G and Table 5). The severity of all these defects increased acropetally (Table 5). The surface of the gynoecia appeared normal, but there was some reduction in fertility. ffo2 mutants also had a disruption in the phyllotaxy of the flowers on their inflorescences and seemed to initiate more flowers than wild-type inflorescences at a similar stage of development (compare Figure 2, N and E). While double mutants, we found fusion of the mosaic first-whorl organs similar to that observed in ffo1 mutants (Figure 4D). In addition, these double mutants seemed to have a reduction in the number of second-whorl petals, possibly due to their fusion with first-whorl organs. In ap2-1 flowers, the first-whorl organs are the ffo2-1 allele is semidominant. In about 30% of heterozygotes, at least one of the following defects was observed: an increased number of floral organs in whorl 1 and more rarely in whorl 2; fused floral organs in whorls 1 and 3; and narrow or reduced floral organs in whorls 1, 2, and 3. Plants heterozygous for the ffo2-2 allele rarely displayed decreased petal number in the second whorl but otherwise appeared wild type.

Role of organ identity in ffo1 mutant floral organ fusion: Because the floral defects of the ffo1 mutants were essentially limited to the first and third whorls, we examined the consequences of altering floral organ identity in the ffo1 mutant background. 35S::PI plants, which express PISTILLATA (PI) under the control of the constitutive CaMV 35S promoter, produce petal tissue at the margins and base of the first-whorl organs and sepal tissue in the upper-central region of these organs (Figure 4C; Krizek and Meyerowitz 1996). Because ffo1-1 second-whorl petals did not fuse, we tested whether ffo1-1 35S::PI double mutants with petal tissue along the margins of the first-whorl organs would fuse. In these double mutants, we found fusion of the mosaic first-whorl organs similar to that observed in ffo1 single mutants (Figure 4D). In addition, these double mutants seemed to have a reduction in the number of second-whorl petals, possibly due to their fusion with first-whorl organs. In ap2-1 flowers, the first-whorl organs are leaves, and the second-whorl organs are mostly petal/stamen mosaic organs (Bowman et al. 1991). We found that 18% of ap2-1 ffo1 double-mutant flowers contained fused first-whorl organs (Figure 4F), compared with all of the ffo1 single-mutant flowers and none of the ap2-1 single-mutant flowers (Figure 4E; flowers #10-20 counted in each case). Thus, the fusion defect observed in ffo1 mutant flowers is likely to be a consequence of the position of the organs in the floral meristem and not of their identity. Surprisingly, flowers from these double mutants also had an enhancement of the ap2-1 phenotype toward that of a stronger ap2 phenotype, e.g., carpelloby of first-whorl organs and absence of second-whorl organs (Figure 4F).

### TABLE 5

<table>
<thead>
<tr>
<th>Positions of flowers</th>
<th>ffo2-1 flower No.</th>
<th>Ler^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-5</td>
<td>6-10</td>
</tr>
<tr>
<td>Whorl 1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepals</td>
<td>4.20</td>
<td>3.52</td>
</tr>
<tr>
<td>Sepals fused</td>
<td>0.04</td>
<td>0.72</td>
</tr>
<tr>
<td>Total^f</td>
<td>4.24</td>
<td>4.24</td>
</tr>
<tr>
<td>Whorl 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petals</td>
<td>3.88</td>
<td>3.32</td>
</tr>
<tr>
<td>Petals/ filaments</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Filaments</td>
<td>0.00</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>3.88</td>
<td>3.40</td>
</tr>
<tr>
<td>Whorl 3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamens</td>
<td>5.32</td>
<td>5.68</td>
</tr>
<tr>
<td>Stamens fused</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>Other (PSt, rSt,F, StC)</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Total</td>
<td>5.60</td>
<td>5.96</td>
</tr>
<tr>
<td>Whorl 4:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpels</td>
<td>2.04</td>
<td>2.00</td>
</tr>
<tr>
<td>Whorl 1 fused organs^a</td>
<td>0.00</td>
<td>0.32</td>
</tr>
<tr>
<td>Whorl 2 fewer than 4 petals^a</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>Whorl 3 fused organs^a</td>
<td>0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The average number of organs per whorl is given. Ler, Landsberg erecta; PSt, petal/stamen; rSt, rudimentary stamen; F, filament; StC, stamen/carpel.

^a The first 25 flowers were examined on each of five plants.
^b The first 25 flowers were examined on each of six plants.
^c The number of first-whorl organs with petal tissue along their margins (see Figure 1F) increased in more apical positions.
^d 5% of the total first-whorl organs had petal tissue along their margins.
^e Number of flowers with defect/total number of flowers.
Phenotypes of \( \text{ffo} \) double mutants: The similarities observed between the \( \text{ffo} \) mutant floral phenotypes, particularly with respect to sepal fusion, suggested that the products of the three \( \text{FFO} \) genes might act in a common developmental pathway(s). We tested this hypothesis genetically by creating all double-mutant combinations of the \( \text{ffo1-1} \), \( \text{ffo2-1} \), and \( \text{ffo3-1} \) alleles. The double-mutant phenotype observed in each case was consistent with an additive interaction between the mutations (data not shown). Both \( \text{ffo1-1} \) and \( \text{ffo2-1} \) and \( \text{ffo3-1} \) \( \text{ffo2-1} \) flowers displayed fused sepal and stamen—characteristic of \( \text{ffo1-1} \) and \( \text{ffo3-1} \) flowers— and also narrow, reduced, and/or mosaic floral organs—characteristic of \( \text{ffo2-1} \) flowers. Fusions between sepal, which occurred at frequencies intermediate between those of the single mutants, were nearly complete along the length of the organs. \( \text{ffo1-1} \) \( \text{ffo3-1} \) flowers had an increased frequency of fused sepal and stamen per flower compared to either single mutant, and also an increased severity of the sepal fusion phenotype, such that each sepal was almost completely fused to its neighbors.

Interactions with \( \text{LEAFY} \) and \( \text{CLAVATA3} \): To determine whether the \( \text{FFO} \) genes interact with other members of the filamentous structures class of genes in addition to \( \text{UFO} \), we constructed plants doubly mutant for an \( \text{ffo} \) mutation and for either a \( \text{clv3-1} \) (Figure 5) or a \( \text{lfy} \) (Figure 6) allele. Plants homozygous for the \( \text{clv3-1} \) allele have enlarged apical and floral meristems, and mutant flowers have additional organs of each type, particularly stamens and carpels (Figure 5A; Clark et al. 1993). The apical meristems of \( \text{clv3-1} \) mutant plants are often fasciated, and the floral meristems generate additional whorls of carpels interior to the fourth whorl. The inflorescences of \( \text{ufo-2} \) \( \text{clv3-1} \) plants form up to 20 flowers, and then generate filamentous structures in place of later-arising floral meristems (Levin and Meyerowitz 1995).

In general, \( \text{ffo clv3-1} \) double-mutant plants had a less severe filamentous structures defect than \( \text{ffo ufo} \) double mutants. The phenotype of \( \text{ffo1-1 clv3-1} \) plants was additive early in inflorescence development, with basal flowers displaying fused sepal in whorl one and additional organs in all whorls (Figure 5B). After producing many flowers, some double-mutant inflorescences formed reduced flowers (flowers with severely reduced organ number in each whorl) and occasional filamentous structures, but this phenotype was not observed in every plant. The \( \text{ffo3-1 clv3-1} \) mutant phenotype was also additive early in inflorescence development, with basal flowers displaying fused sepal in whorl one and additional organs in all whorls. Later in development, the double-mutant phenotype became more severe: the inflorescence meristem enlarged and produced flowers reduced in size and organ number, before eventually terminating in apparently undifferentiated, overproliferating meristem tissue (Figure 5C). This phenotype appears to be more severe than that observed in \( \text{clv3-1} \) single-mutant plants at a similar stage of development (Figure 5A).

The \( \text{ffo2-1 clv3-1} \) mutant phenotype was additive early in inflorescence development, with basal flowers displaying (1) an organ number phenotype intermediate between those of the two single mutants, (2) reduced and mosaic organs, and (3) fused sepal in whorl 1. Later in development, \( \text{ffo2-1 clv3-1} \) inflorescences underwent fasciation, producing reduced flowers and tiny filamentous structures at the ends of the apparently overproliferating tissue (Figure 5D). This phenotype also appears to be more severe than that observed in \( \text{clv3-1} \) single-mutant plants at a similar stage of development (Figure 5A). Thus, the moderate synergism of the \( \text{ffo2-1 clv3-1} \) and \( \text{ffo3-1 clv3-1} \) double-mutant phenotypes indicates that both \( \text{FFO2} \) and \( \text{FFO3} \) interact with \( \text{CLV3} \) during the late stages of inflorescence meristem growth and suggests that these genes have a role in the control of cell proliferation in meristematic tissue.

Mutations in the floral meristem identity gene \( \text{LFY} \) cause a number of inflorescence and flower defects (Schultz and Haughn 1991; Sussex 1991; Weigel et al. 1992), some of which are quite similar to those caused by mutations in \( \text{UFO} \) (Levin and Meyerowitz 1995). Ify mutants are characterized by defects
including an increased number of secondary inflorescences, occasional filamentous structures forming in place of flowers, and premature termination of flowering, with leaflike and carpeloid organs at the apex of the inflorescence meristem (Weigel et al. 1992). The phenotype of double mutants constructed with ufo-2 and weak lfy alleles resembles that of strong lfy mutants, while the phenotype of double mutants constructed with ufo-2 and a strong lfy allele is indistinguishable from that of the strong lfy mutant (Levin and Meyerowitz 1995). Unlike ufo cv3 plants, ufo lfy double mutants do not show any enhancement in the formation of filamentous structures.

To test for interactions between LFY and FFO genes, we constructed double mutants with an allele of ffo1, ffo2, or ffo3 and a strong lfy allele, lfy-26 (Figure 6A). In contrast to its behavior with ufo, lfy strongly enhanced the phenotypes of all three ffo mutants toward the production of filamentous structures (Figure 6, B–D). ffo1 lfy-26 double-mutant inflorescence meristems did not form any flowers, generating only filamentous structures on the flanks of the inflorescence meristems (Figure 6B) before terminating in a few leaflike and carpeloid organs characteristic of lfy-26. ffo3-1 lfy-26 double mutants had a similar phenotype to that of the ffo1-1 lfy-26 double mutants, but generally formed more leaflike organs in a spiral phyllotaxy before terminating (Figure 6C). ffo2-1 lfy-26 plants formed at most one flower, with reduced or absent second- and third-whorl organs and unfused carpels—subtended by a long bract—then generated filamentous structures and/or leaflike organs along the flanks of the inflorescence meristem (Figure 6D). Like the ffo1-1 lfy-26 and the ffo3-1 lfy-26 double-mutant plants, ffo2-1 lfy-26 double-mutant plants terminated in leaflike and carpeloid organs (Figure 6D). Further, in all three double-mutant combinations, secondary inflorescence meristems gave rise only to filamentous structures before terminating. The nearly total inability of ffo lfy-26 double-mutant meristems to produce flowers indicates that the FFO genes function in similar processes but in a separate pathway from UFO and LFY in control of early floral patterning events.

**DISCUSSION**

The FFO genes make up a subclass of the filamentous structures group of genes: In a genetic screen to identify novel genes required for early flower development, we recovered four mutants with similar floral phenotypes that fell into three complementation groups (Table 1). Mutations in any of the three FFO genes strongly enhance the ufo filamentous structures defect, placing them in the large class of filamentous structures genes that affect very early floral initiation events. Identification in our screen of a pin allele as an additional ufo enhancer indicates that pin is also a member of this class, bringing the current total to more than 10. The fact that the genes that make up this group play widely divergent roles in early flower patterning suggests that disruption of more than one genetic regulatory pathway may result in insufficient developmental information to carry out the flower-specific program.

We observed several similarities between FFO double-mutant phenotypes that lead us to propose that the FFO genes form a subclass of the filamentous structures group affecting meristem structure and floral organ separation. First, double mutants generated by crossing the various ffo alleles among themselves did not form filamentous structures. Their phenotypes can therefore be interpreted as being additive—although in the absence of known null alleles, an interaction between them cannot be ruled out. Second, double mutants constructed with any of the ffo alleles and lfy or ufo alleles generated very few flowers (Figure 1, A–D, and Figure 6). This strong enhancement of the ufo and/ or lfy meristem identity phenotype, which occurred with all six ffo alleles, suggests that the FFO genes may function in a similar process but in a separate pathway from LFY and UFO. Third, the ffo mutants displayed only weak to moderate interactions with cv3. This result suggests that...
only a subset of ff0 double-mutant combinations with
genes in the filamentous structures class has dramatic
effects on floral meristem formation, although in the
absence of known ff0 null alleles this interpretation must
be viewed with caution. Finally, ff0-2 clv3-1 and ff0-3
double mutants displayed moderate synergism in
old inflorescence meristems, revealing that FF02 and
FF03 have redundant roles with CLV3 in controlling
cell proliferation at later stages of inflorescence develop-
ment.

Some filamentous structures most closely resemble
leaves: The organs formed by most double mutants of
the filamentous structures class, including ufo-2 ff0-1
and ufo-2 ff0-3, consisted of thin green cylindrical pro-
trusions from the stem. Earlier work raised the issue of
whether these structures are more closely related to
leaves or flowers. Previous SEM analysis of the epidermal
cells of these organs suggested a resemblance to cells
in elongated pedicels or along the midvein of adaxial
leaf surfaces (Levin and Meyerowitz 1995). Neither
AP1 nor LFY are required to produce these structures,
nor are the homeotic genes AP3 or PI expressed in
filamentous structures formed by ufo-2 inflorescences
(Levin and Meyerowitz 1995). However, our observa-
tion that ufo-2 ff0-1 inflorescences formed carpeloid
filamentous structures suggested that these organs re-
tained some floral character.

Our analysis of the internal cellular composition of
filamentous structures from three different ufo-2 double
mutants by TEM suggests that in some genotypes these
organs are somewhat leaflike, while those of other gen-
types contain highly vacuolated cells with neither a dis-
tinct leaflike nor floral character. Although the arrange-
ment of the vascular bundles in angiosperm pedicels
can differ slightly from that in leaves (Fahn 1990), we
were not able to classify the filamentous structures as
“leaflike” or “flowerlike” on the basis of this criterion
because those organs analyzed did not contain any vis-
ible vasculature (Figure 3). Indeed, serial sections taken
at the base of one ufo-2 ff0-1 filamentous structure sug-
gest that vascular bundles may extend up to but not
into these organs. The cell types observed in some fil-
amentous structures, particularly those from ufo-2 ff0-1
double mutants, most closely resemble the spongy pa-
renchyma found in leaf blades. Both sets of cells are
irregularly shaped and have a similar cellular content,
including chloroplasts, and may be separated by large
intercellular spaces. Bracts, which subend flowers in
many plant species but which have been lost in most
 cruciferous taxa, are considered modified leaves (Gif-
ford and Foster 1988) and are often observed subend-
ting the first flowers of ly, ufo, ff01, and ff03 mutant plants
(Weigel et al. 1992; Levin and Meyerowitz 1995; this
study). Our TEM results are therefore consistent with
the idea that some filamentous structures may be more
similar to reduced bracts than to reduced flowers. In
contrast, the cell types observed in ufo-2 clv3-1 filamen-
tous structures are neither overtly leaflike nor floral,
suggesting that such structures may arise as meristem
projections without undergoing differentiation into pe-
ricular organ types.

Comparison with other genes affecting organ bound-
daries: The fused floral organ phenotypes of the FF0
genes indicate that they are members of a newly identi-
fied set of genes involved in establishing and main-
taining boundaries during plant development. This
class also includes the Arabidopsis CUC1 and CUC2
genes and the petunia nam gene, which appear to have
functions similar to those of the FF0 genes. CUC1 and
CUC2 are partially functionally redundant with each
other, as mutations in either gene have slight effects in
both seedlings and flowers, while double mutants dis-
play synergistic phenotypes including fused cotyledons,
absent shoot apical meristems, and fused sepals and
stamens in flowers on adventitious shoots (Aida et al.
1997). nam mutants also have fused cotyledons and lack
a shoot apical meristem. Occasional shoots produced
from nam seedlings form flowers with increased petal
number and mosaic and/or deformed second-, third,-
and fourth-whorl organs (Sower et al. 1996). The Arabi-
dopsis PIN and PID genes also affect floral organ bound-
dary determination, but their pleiotropic mutant pheno-
types suggest that their floral defects have different
underlying causes than the ff0 mutants (Goto et al. 1987;
Okada et al. 1991; Bennett et al. 1995). Mutations in
the Arabidopsis fddlhead (FDH) gene cause postgenital
fusion between floral organs (Lolle et al. 1992), but
in fdh mutants, unlike ff0 mutants, fusion also occurs
between floral buds and leaf surfaces. Fusion events in
fdh mutants appear to be due to adherence between
epidermal cells upon contact with other surfaces (Lolle
et al. 1992), indicating that the underlying cause of the
fdh phenotype differs from that of the ff0 mutants.

The floral phenotypes of the ff01 and ff03 mutants are
similar to, but much stronger than, those of the cuc1
and cuc2 mutants. In ff01 and ff03 flowers, sepal and
stamen fusion occurred at a high frequency, and partial
to complete fusion between adjacent sepals was often
observed (Tables 2 and 4). In contrast, sepal and stamen
fusion occurs at a low frequency in cuc1 and cuc2 flowers
and affects only a small section of the entire organs
(Aida et al. 1997). Fused sepals are undetectable in cuc1
cuc2 double-mutant buds prior to stage 6 (Aida et al.
1997), suggesting that the CUC genes act like FF01 in
the maintenance of organ boundaries but perhaps at a
slightly later stage. Whether these differences in floral
phenotype between ff01 and ff03 and the cuc mutants
are due to differences in allele strength is currently an
open question, as the null phenotypes of these genes
have yet to be defined.

In contrast to their similar floral phenotypes, the ff0
and the cuc mutants have nonoverlapping vegetative
phenotypes, with the ff0 mutations affecting primarily
meristem and leaf development (Table 3 and Figure 4B)
and the *cuc* mutations affecting cotyledon development (Aida et al. 1997). The single exception is that a very small percentage of both *ffo1-1* and *cuc2* mutant seedlings have partially fused cotyledons. This phenotype was not enhanced in *ffo* double mutants, nor were the various fused floral organ defects. The additive nature of the *ffo* double-mutant phenotypes therefore indicates that the *FFO* genes, unlike the *CUC* genes, play nonredundant roles in regulating organ separation during plant development.

Both CUC2 and nam have been cloned (Souver et al. 1996; Aida et al. 1997), and while their deduced amino acid sequences shed little light on their possible mechanisms of action, they share a highly conserved N-terminal NAM ATAF1-2, CUC2 (NAC) domain and appear to be members of gene families in their respective organisms. One attractive possibility is that floral organ boundary functions are encoded predominantly by NAC-gene family members, much as floral organ identity functions are encoded mainly by MADS box genes (Coen and Meyerowitz 1991; Ma 1994; Weigel and Meyerowitz 1994; Riechmann and Meyerowitz 1997). However, while more than 20 putative NAC-containing genes have been identified in Arabidopsis database searches (Sablowski and Meyerowitz 1998), none of those mapped as yet corresponds to an *FFO* locus (S. Jacobsen, personal communication).

**Specificity of *FFO1* and *FFO3* organ separation functions:** Our observations indicate that *FFO1* and *FFO3* control a specific subset of the organ separation functions in the developing flower. In *ffo1* and *ffo3* flowers, fusion events occurred exclusively between organs in the same whorl (Tables 2 and 4). Mosaic organs composed of different cell types were rarely if ever observed, suggesting that *FFO1* and *FFO3* are probably not part of a general function specifying boundaries between any two neighboring primordia. In addition, organ fusion in *ffo1* and *ffo3* flowers occurred in a subset of whorls, between sepals in whorl 1 and stamens in whorl 3 but not between petals in whorl 2. Thus, boundary formation between first- and third-whorl organs and between second-whorl organs appears to require distinct gene activities.

We also found that the *FFO1* floral organ separation activity is a function of cell position in the floral meristem and not of cell identity. That is, first-whorl organ fusion occurred in *ffo1* mutant flowers regardless of whether the cells at the margins of the organs were sepal cells, petal cells (as in *ffo1-1 35S:PI* flowers), or leaf cells (as in *ffo1-1 ap2-1* flowers; Figure 4). We predict that the same is true for *FFO3*, as its organ boundary specification requirement occurs at or before floral stage 3. At stage 3, the initial expression of the floral organ identity genes *AP3*, *PI*, and *AG* is observed (Drews et al. 1991; Jack et al. 1992; Goto and Meyerowitz 1994), suggesting that organ primordia separation events regulated by *FFO3* occur concurrently with organ primordia identity specification events and that one is not a direct consequence of the other.

While *FFO1* and *FFO3* activities are required in the same spatial domains during flower development, they appear to be required at different times. *ffo3* mutant flowers display congenital sepal fusion (Figure 2, L and M), suggesting that *FFO3* acts to establish boundaries between the first-whorl organ primordia as they arise. *FFO3* might function transiently during organ initiation or might also be required to maintain separation between the organs as they develop. Once the initial boundaries are established, another function—such as that encoded by *FFO1*—might be enlisted to maintain the sepal/sepal boundaries established by *FFO3*. Such a role for *FFO1* in first-whorl organ boundary maintenance—but not establishment—is consistent with our detection of floral organ fusion in *ffo1* mutants no earlier than stage 5 (Figure 2I). However, *FFO1* likely functions via a separate pathway from *FFO3* rather than directly downstream of it, as the *ffo1-1 ffo3-1* double-mutant phenotype is additive rather than epistatic.

**Models for *FFO1* and *FFO3* gene activity:** The specific fused floral organ phenotypes displayed by the *ffo1* and *ffo3* mutants suggest several possible models for establishing and maintaining organ separation in developing flowers. In one scenario, *FFO1* and *FFO3* might be required to limit cell division at or near floral organ boundaries. Organ separation could be achieved by *FFO1* and *FFO3* acting in a cell-autonomous manner—in cells of enlarging primordia to spatially restrict their proliferation in the lateral direction and/or in intervening cells to prevent their division and incorporation into developing floral primordia. Alternatively, the two *FFO* genes might have a non-cell-autonomous function, as components of a signaling system(s) required for transducing a signal from the enlarging organ primordia to restrict the division of the intervening cells, or vice versa. These models are consistent with the RNA expression patterns of cloned plant genes known to affect floral-organ boundaries. *Nam* is expressed in petunia flowers in a ring around the stamen primordia and between the two carpel primordia (Souver et al. 1996). *UFO* expression at later stages of flower development is restricted to the base of the petal primordia at the boundary between whorls 1 and 2 (Ingram et al. 1995; Lee et al. 1997), and *FIM* expression also becomes restricted to rings around the petal primordia (Simon et al. 1994).

An alternative model is that these two *FFO* genes might regulate the absolute number of cells allocated to each floral primordium. Misallocation of too many precursor cells to a developing organ might cause those cells to undergo proliferation and expansion, leading to organ fusion. We do not favor this model, however, because if misallocation of additional cells to initiating primordia is the primary cause of the *ffo1* and *ffo3* organ fusion.
phenotype, this outcome does not result in larger floral organs overall. But the models presented are not mutually exclusive; and indeed, a combination of some or all of these mechanisms might contribute to organ boundary specification.

Model for FFO2 gene activity: While ffo2 mutant flowers, like ffo1 and ffo3 flowers, formed fused sepals and stamens, there are several reasons that failure of whorl-specific organ separation does not appear to be the major defect in these plants. First, ffo2 flowers had defects in all four whorls (Table 5). Second, FFO2 affected organ number, size, and positioning as well as boundary specification (Figures 1G, 2O, and 2P). Third, the formation of mosaic organs by ffo2 mutant flowers revealed a defect in organ separation between as well as within whorls (Figure 1G). Therefore, the phenotypes caused by mutations in FFO2 suggest that it functions via a different mechanism than FFO1 and FFO3.

Our analysis of the ffo2 sepal phenotypes by SEM indicates that FFO2 activity is required at the earliest stages of flower development. FFO2 also appears to be required prior to the initiation of the floral meristems, as ffo2 inflorescences initiated more floral meristems than wild-type inflorescences (Figure 2, E and N). All of these defects may be a consequence of a requirement for FFO2 in regulating the spacing between both floral meristem primordia and floral organ primordia. FFO2 might function as part of the mechanism used to initiate floral meristems a sufficient distance apart so that each consists of enough cells to initiate the appropriate number of floral organs. Once the floral meristems begin to generate organ precursors, the FFO2 spacing function might be reapplied to obtain the correct spacing between the primordia, again ensuring that the appropriate number of precursor cells is allocated to each.

In ffo2 inflorescences, according to this model, loss of this spacing function would result in floral meristems being initiated closer together than normal and consequently being allocated fewer precursor cells. Additional mechanisms would presumably ensure that approximately normal numbers of organ primordia are initiated in each whorl; but again, a disruption in spacing between them would cause variation in their position relative to one another and would potentially also cause variation in the number of cells allocated to each. Organs initiating too close to one another within or between whorls would ultimately fuse, while those allocated an insufficient number of cells—perhaps due to their proximity to another primordium—would be reduced or filamentous. Petal primordia, perhaps because of their smaller initial size, might be more sensitive to local spacing disruptions and thus be reduced or absent at a higher frequency than sepal or stamen primordia. Cloning of the FFO2 genes and comparison of their expression patterns should help to refine these models and shed light on these little-understood flower patterning events and on their relationship to organ initiation and cell proliferation.

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