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

Joshua Z. Levin,[1,2] Jennifer C. Fletcher,[2] Xuemei Chen and Elliot M. Meyerowitz

Division of Biology, California Institute of Technology, Pasadena, California 91125

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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*, *lfy*, 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-

Corresponding author: Elliot M. Meyerowitz, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125.
E-mail: meyerow@cco.caltech.edu

[1]*Present address:* Novartis Crop Protection, Biotechnology and Genomics Center, Research Triangle Park, NC 27709.

[2]These authors contributed equally to this work.

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; Skowyra *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*), *leunig* (*lug*), and *hanaba taranu* (*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 *lfy clv1* and *lfy 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 lfy* 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. Sakai, 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-6* 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 M_1 plants

germinated and produced M_2 seeds, which were collected from each individual M_1 plant. Approximately 40 M_2 plants were screened from each of 975 M_1's.

Genetic and phenotypic analysis: The isolated enhancer mutants were crossed to wild-type Landsberg *erecta* (L*er*) plants. F_2 plants from this cross were screened to identify the mutant phenotype in the absence of the *ufo-6* allele. The single-mutant F_2 plants were backcrossed two additional times to L*er* 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 L*er* before analysis. The *ffo2-2* allele was identified in an EMS mutagenesis of *ag-4* mutants in the L*er* ecotype and was backcrossed three times to L*er* 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 F_1 plants from each cross were scored for the presence of the mutant phenotype and F_2 seeds collected in bulk. To confirm the F_1 results, approximately 60 F_2 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*, *cuc2*, *pinoid-8*, or *pin-formed-5* mutation (Okada *et al.* 1991; Bennett *et al.* 1995; Aida *et al.* 1997). The F_1 plants from each cross were wild type, suggesting that the mutations were not allelic. To confirm these results, approximately 60 F_2 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, F_3 seeds 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 *aintegumenta* (Elliot *et al.* 1996; Klucher *et al.* 1996), which lies near *apetala2 (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 F_1 and F_2 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 F_2 plants scored to identify the double mutant. F_3 seeds from 28 single-mutant F_2 plants were collected and sown, and the F_3 families analyzed for segregation of doubly mutant plants. According to the frequency of recombination in the F_3 generation, *FFO3* 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 50 seeds/plate, incubated at 4° for 5 days, placed under lights (600 f.c. of constant cool-white fluorescent) at 23° 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 F_3 generation by analyzing the segregation of the double mutant among the progeny of an F_2 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 Philips 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 M_2 seeds from each M_1 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 M_2 seeds from individual M_1 plants. This approach allowed us to recover the enhancer mutation of interest from the heterozygous M_2 sibling plants. By screening the progeny of approximately 975 *ufo-6* M_1 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**

Allele	Isolation No.	Inheritance	Allele strength
ffo1-1	971	Recessive	Stronger
ffo1-2	607	Recessive	Weaker
ffo1-3	6.1	Recessive	Weaker
ffo2-1	3-4	Semidominant	Stronger
ffo2-2	870	Recessive	Weaker
ffo3-1	833	Recessive	—

All mutants were isolated in a Landsberg *erecta* (L*er*) genetic background with the exception of 6.1, which was isolated in Nossen and backcrossed three times to L*er* 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 Meyerowitz 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

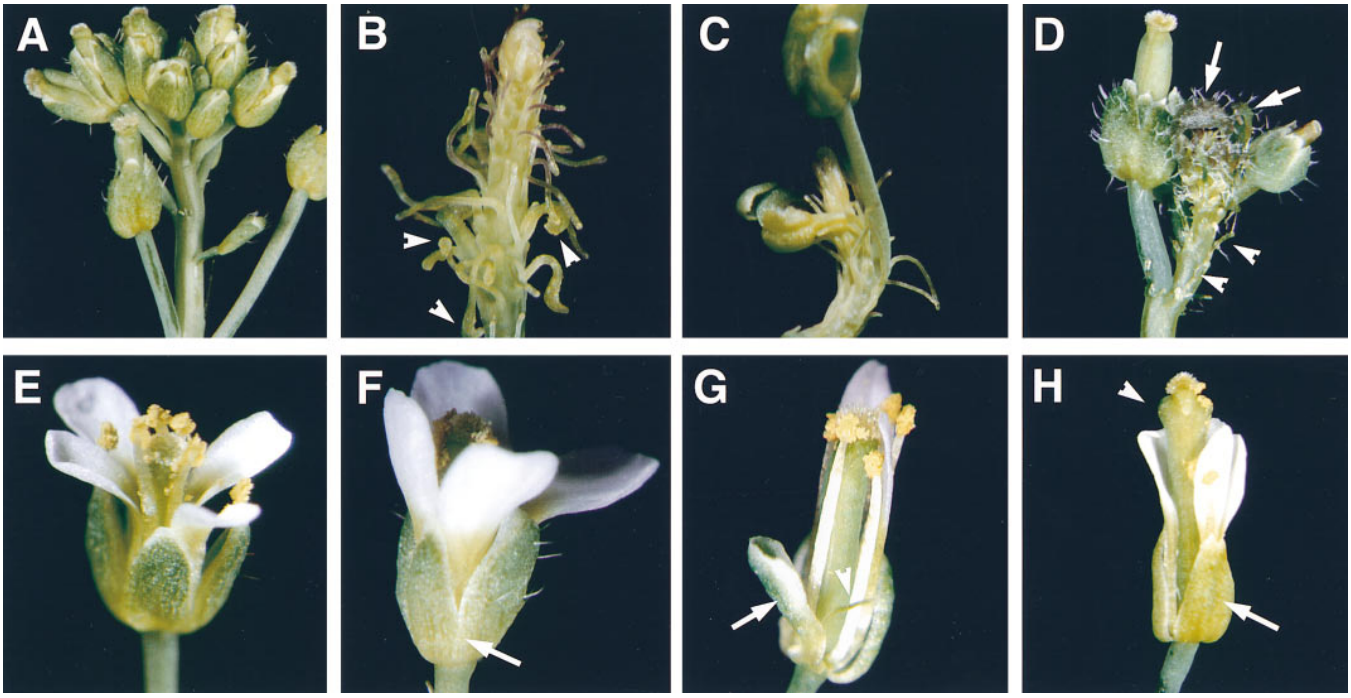

Figure 1.—Phenotypes of *ufo-2 ffo* double-mutant and *ffo* single-mutant plants. (A) A *ufo-2* inflorescence with a reduced flower and several tiny filamentous structures. The flowers contain mosiac organs and consist predominantly of sepals and carpels, reflecting a reduction in class B organ identity 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 L*er* 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).

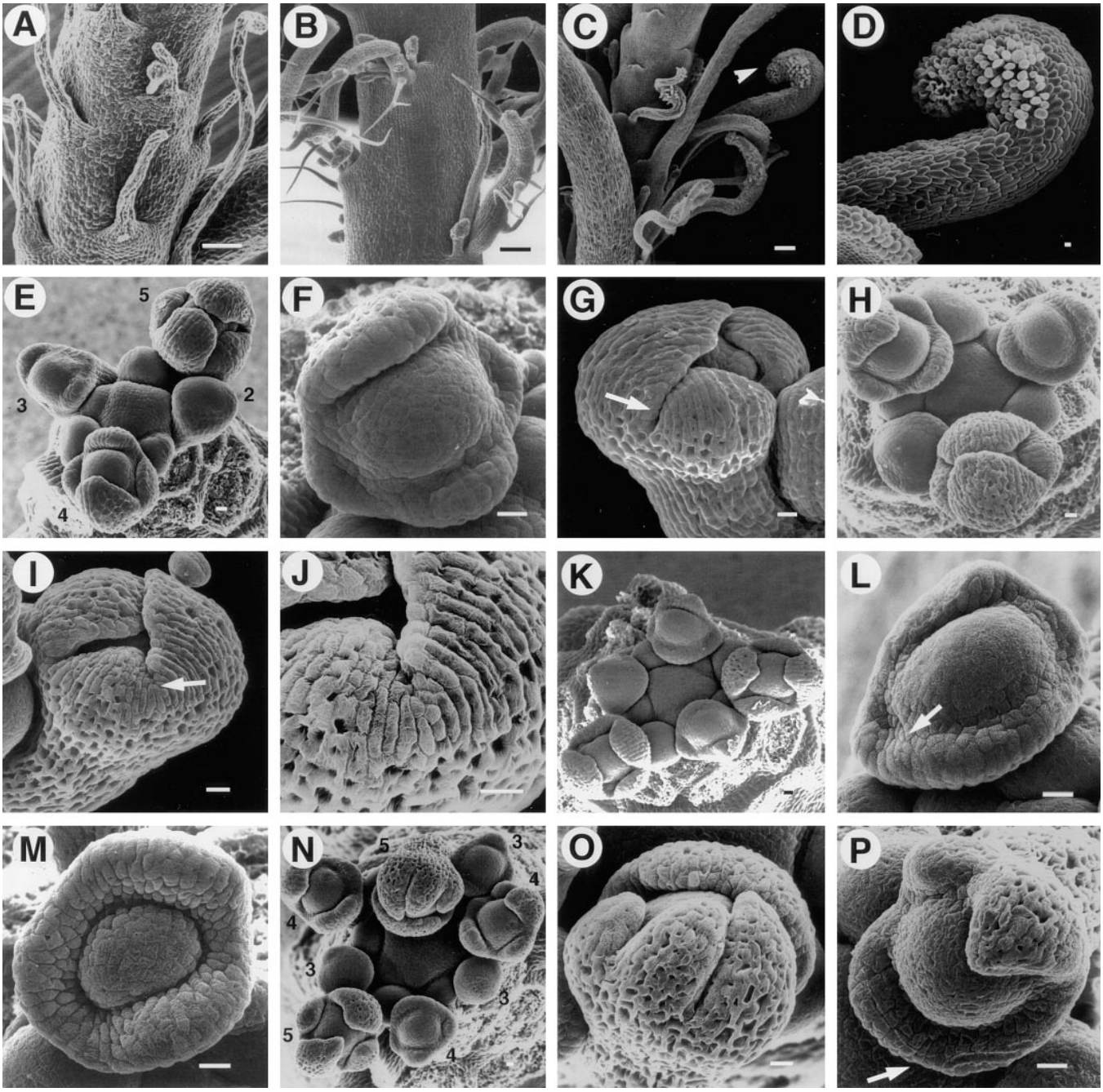

Figure 2.—Phenotypes of wild-type, *ffo* mutant, and *ufo-2 ffo* double-mutant inflorescences and flowers visualized by SEM. Numbers refer to stages of floral development, which are according to Smyth *et al.* (1990). (A) A *ufo-2 ffo2-1* inflorescence. (B) A *ufo-2 ffo3-1* inflorescence. (C) A *ufo-2 ffo1-1* inflorescence with basal filamentous structures topped by stigmatic tissue (arrowhead). (D) Higher magnification of a *ufo-2 ffo1-1* filamentous structure from C. The apical adaxial surface is made up of stigmatic cells. (E) A wild-type L*er* 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 L*er* stage 3 flower. (G) An L*er* 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 *ffo1-1* inflorescence. The floral primordia at these early stages of development appear indistinguishable from the wild type. (I) An *ffo1-1* 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 *ffo3-1* inflorescence. (L) An *ffo3-1* stage 3 flower with two adjacent first-whorl organ primordia fused to one another (arrow). (M) Another *ffo3-1* stage 3 flower in which the first-whorl sepal primordia have arisen fused together into a ring. (N) An *ffo2-2* 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 *ffo2-2* stage 5 flower viewed from the side. The first-whorl sepals 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 *ffo2-2* stage 4 flower. Congenital fusion between two adjacent sepals 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 mutants 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 clv3-1*, *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 clv3-1* 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 clv3-1* cells to *ufo-2 ffo1-1* cells, which resembled those in wild-type leaves. The cells of *ufo-2 clv3-1* 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 clv3-1*, 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*

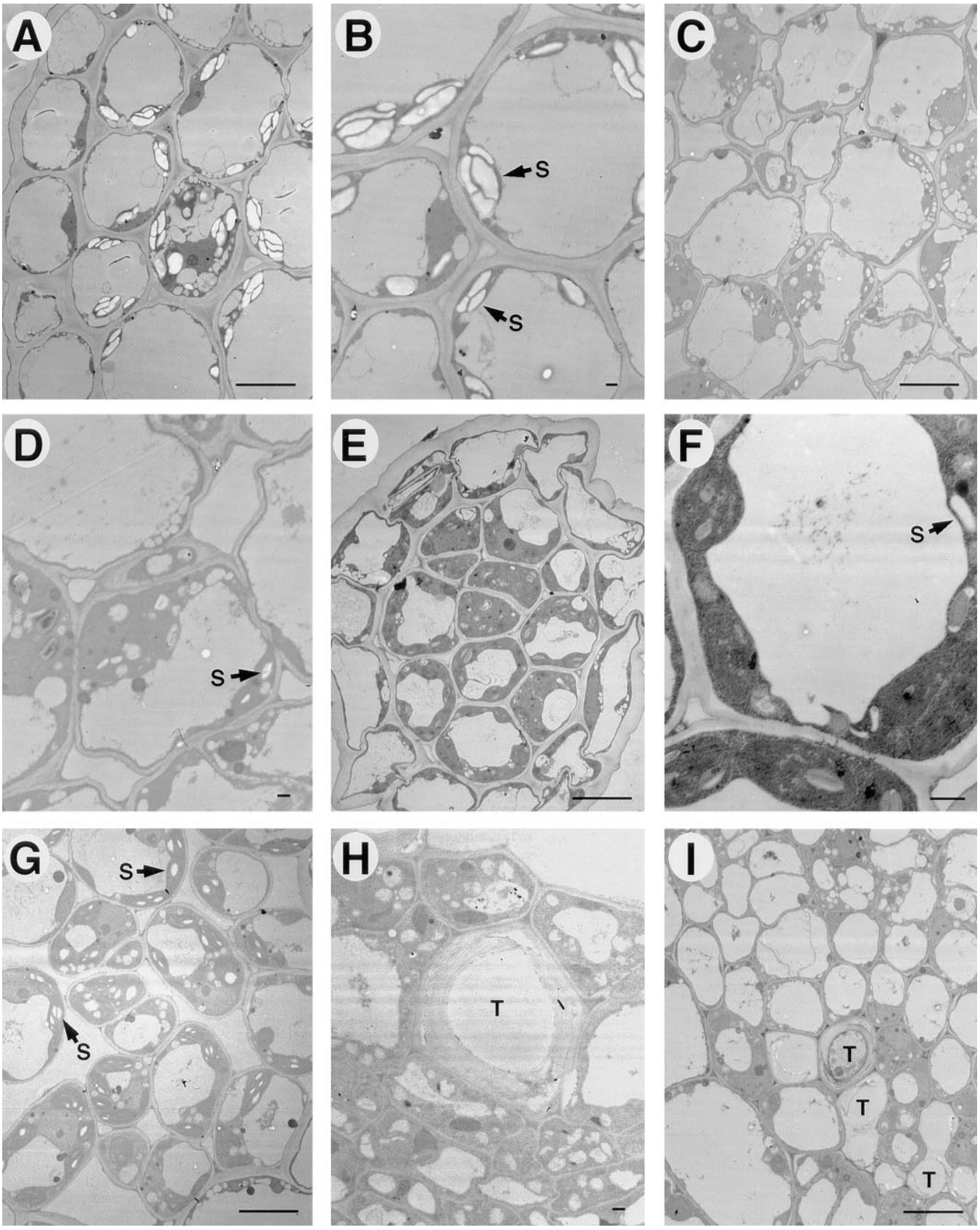

Figure 3.—Transmission electron micrographs of *ufo-2* double-mutant filamentous structures compared with wild-type pedicels and cauline leaves. (A) A *ufo-2 clv3-1* filamentous structure. (B) Higher magnification of the ground tissue cells shown in A. (C) A *ufo-2 fil* filamentous structure. (D) Higher magnification of the ground tissue cells shown in C. (E) A *ufo-2 ffo1-1* 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

***ffo1* organ counts**

	ffo1-1 flower No.[a]						Ler[b]
Positions of flowers	1–5	6–10	11–15	16–20	21–25	1–25	1–25
Whorl 1 (sepal-sepal junction)[c]							
Sepals unfused	3.00	0.20	0.00	0.00	0.00	0.64	4.00
Sepals fused <50%	1.00	2.32	1.04	0.40	0.52	1.05	0.00
Sepals fused >50%	0.04	1.48	2.96	3.60	3.48	2.31	0.00
Total	4.04	4.00	4.00	4.00	4.00	4.00	4.00
Whorl 2							
Petals	4.00	4.00	3.92	4.00	3.88	3.98	3.99
Petals/stamens	0.00	0.00	0.04	0.00	0.00	0.01	0.00
Petals fused	0.00	0.00	0.00	0.00	0.08	0.02	0.00
Total	4.00	4.00	3.96	4.00	3.96	4.01	3.99
Whorl 3							
Stamens	4.96	5.00	4.92	4.84	5.08	5.16	5.84
Stamens fused	1.28	0.96	0.88	0.36	0.24	0.77	0.00
Other (rSt, FSt)	0.04	0.08	0.08	0.04	0.08	0.07	0.00
Total	6.28	6.04	5.88	5.24	5.40	6.00	5.84
Whorl 4							
Carpels	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Whorl 1 fused organs[d]	0.60	1.00	1.00	1.00	1.00	0.92	0.00
Whorl 3 fused organs[d]	0.56	0.44	0.36	0.16	0.12	0.34	0.00

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

[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 sepal-sepal junctions completely unfused, fused along <50% of the margin, or fused along >50% of the margin.

[d] 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

Cauline leaf defect

		Nodes affected[a](%)			
Allele	Plants affected (%)	1st	2nd	3rd	*N*
L*er*	25	25	0	0	16
ffo1-1	100	100	56	22	18
ffo1-2	100	100	67	0	15
ffo1-3	63	63	52	31	19
ffo2-1	0	0	0	0	18
ffo2-2	0	0	0	0	20
ffo3-1	53	53	7	0	15

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

[a] Nodes counted beginning with the most basal on the inflorescence.

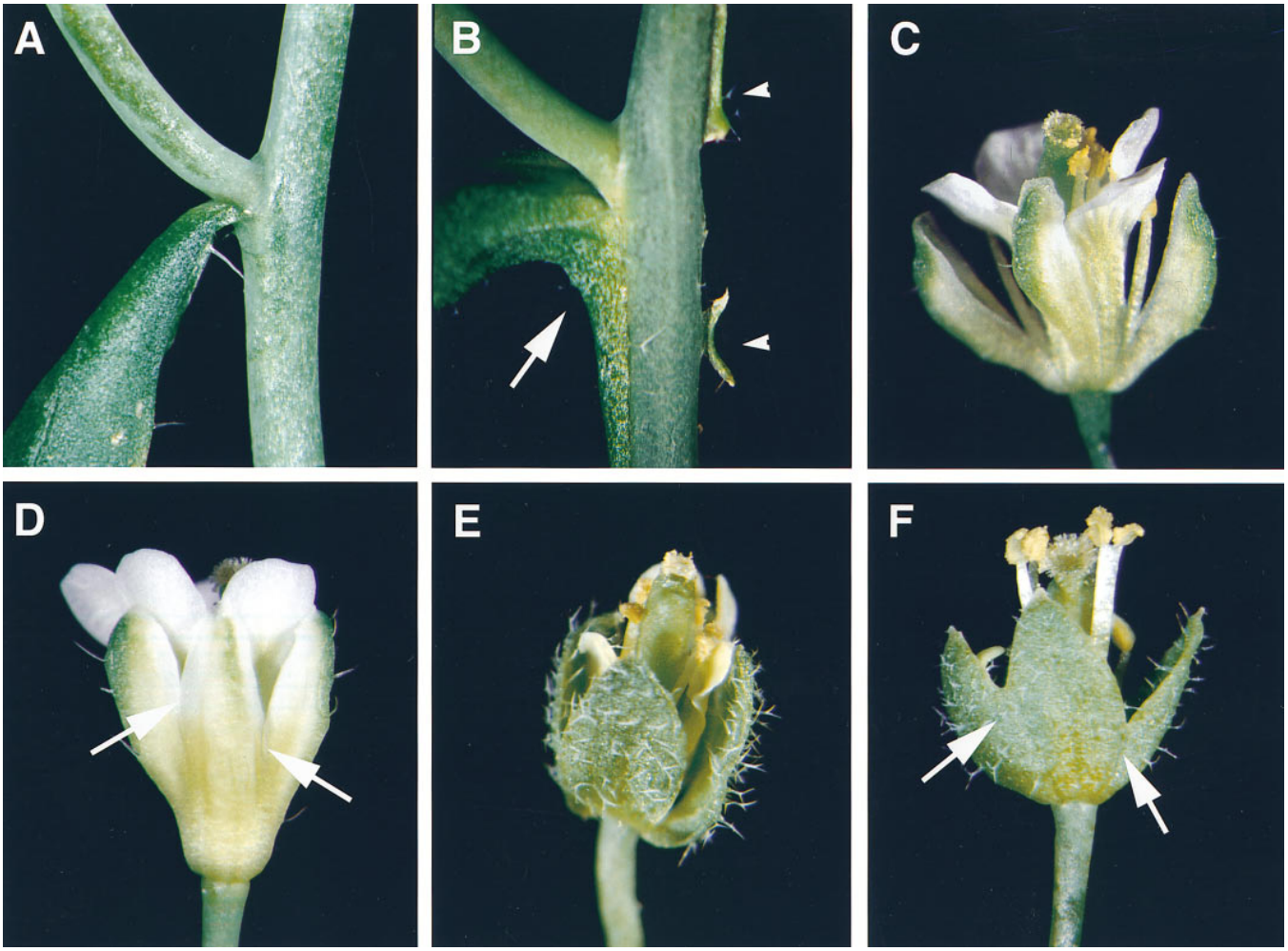

Figure 4.—Phenotypes of *ffo1* single-mutant and double-mutant plants. (A) An L*er* cauline leaf/stem boundary. (B) An *ffo1-1* cauline leaf/stem boundary. The margin of the cauline leaf is fused at the base down along the stem (arrow). Fused cauline leaf margin tissue from the node above the one shown appears to have torn in several places, leaving strips of tissue along the stem (arrowheads). (C) A 35S::PI flower with unfused, first-whorl petaloid sepals. (D) An *ffo1-1* 35S::PI flower. Three of the first-whorl petaloid sepals are partially fused to one another (arrows). (E) An *ap2-1* flower with unfused, first-whorl leaflike organs. (F) An *ffo1-1 ap2-1* flower. Three of the first-whorl, leaflike organs are partially fused to one another (arrows).

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 *ffo1-1* 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 *ffo1* mutants were observed.

ffo3: Like *ffo1* mutants, *ffo3-1* mutant plants showed floral defects primarily in the first and third whorls. *ffo3-1* 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 *ffo3-1* flowers compared to wild type. Only minor defects were observed in the second whorl of *ffo3-1* flowers (Table 4). The gynoecia of *ffo3-1* 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 *ffo1-1* mutants, the first flower formed by *ffo3-1* mutants was subtended by a leaf or filamentous structure on about 20% of primary inflorescences. Overall, the *ffo3-1* floral phenotype was similar to that of *ffo1*, although *ffo3-1* seemed to be slightly weaker.

However, using SEM we found that *ffo3-1* flowers differed from *ffo1* flowers at the earliest stages of flower development. Some *ffo3-1* 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 *ffo3-1* mutant flowers, we could ob-

TABLE 4

***ffo3* organ counts**

	ffo3-1 flower No.[a]						Ler[b]
Positions of flowers	1–5	6–10	11–15	16–20	21–25	1–25	1–25
Whorl 1 (sepal-sepal junction)[c]							
Sepals unfused	2.76	1.12	1.08	1.64	2.08	1.74	4.00
Sepals fused <50%	1.20	2.08	2.36	1.72	1.36	1.74	0.00
Sepals fused >50%	0.12	0.88	0.56	0.56	0.52	0.53	0.00
Total	4.08	4.08	4.00	3.92	3.96	4.01	4.00
Whorl 2							
Petals	4.04	3.96	4.00	4.00	4.00	4.00	3.99
Petals/stamens	0.08	0.04	0.00	0.00	0.00	0.02	0.00
Filaments	0.00	0.00	0.04	0.00	0.00	0.01	0.00
Total	4.12	4.00	4.04	4.00	4.00	4.03	3.99
Whorl 3							
Stamens	5.80	5.60	5.68	5.88	5.96	5.78	5.84
Stamens fused	0.16	0.28	0.08	0.00	0.00	0.10	0.00
Other (rSt, FSt)	0.04	0.12	0.00	0.00	0.00	0.03	0.00
Total	6.00	6.00	5.76	5.88	5.96	5.91	5.84
Whorl 4							
Carpels	2.08	2.04	2.04	2.00	2.00	2.03	2.00
Whorl 1 fused organs[d]	0.76	0.96	1.00	1.00	0.92	0.93	0.00
Whorl 3 fused organs[d]	0.08	0.16	0.04	0.00	0.00	0.06	0.00

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

[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 sepal-sepal junctions completely unfused, fused along <50% of the margin, or fused along >50% of the margin.

[d] Number of flowers with defect/total number of flowers.

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).

ffo3-1 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%, *ffo3-1* 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% 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 *ffo3-1* 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 1 and a decrease 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). The sepal primordia continue to develop in a regular fashion, with the two medial and the two lateral sepals similar to one another in size (Figure 2F). In *ffo2* mutant flowers, the sepal primordia often did not arise equidistantly from one another, and the presence of an adaxial sepal was not invariant (Figure 2N). The developing 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

TABLE 5

***ffo2* organ counts**

	ffo2-1 flower No.[a]						Ler[b]
Positions of flowers	1–5	6–10	11–15	16–20	21–25	1–25	1–25
Whorl 1[c]							
Sepals	4.20	3.52	3.76	3.66	3.32	3.70	4.00
Sepals fused	0.04	0.72	0.32	0.33	0.72	0.42	0.00
Total[d]	4.24	4.24	4.08	3.99	4.04	4.12	4.00
Whorl 2							
Petals	3.88	3.32	2.56	1.54	1.28	2.52	3.99
Petals/filaments	0.00	0.00	0.04	0.13	0.04	0.04	0.00
Filaments	0.00	0.08	0.08	0.13	0.16	0.09	0.00
Total	3.88	3.40	2.68	1.80	1.48	2.65	3.99
Whorl 3							
Stamens	5.32	5.68	5.52	4.96	4.64	5.23	5.84
Stamens fused	0.12	0.24	0.16	0.50	0.40	0.28	0.00
Other (PSt, rSt,F, StC)	0.16	0.04	0.04	0.08	0.28	0.12	0.00
Total	5.60	5.96	5.72	5.54	5.32	5.63	5.84
Whorl 4							
Carpels	2.04	2.00	2.00	2.00	2.00	2.01	2.00
Whorl 1 fused organs[e]	0.00	0.32	0.16	0.17	0.36	0.20	0.00
Whorl 2 fewer than 4 petals[e]	0.16	0.52	0.60	0.92	1.00	0.64	0.02
Whorl 3 fused organs[e]	0.08	0.08	0.08	0.25	0.36	0.14	0.00

The average number of organs per whorl is given. L*er*, 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.

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 gyneocia 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).

We observed a weaker version of the *ffo2-1* phenotype in plants heterozygous for this mutation, indicating that 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 *ffo1-1* 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 ffo1-1* double-mutant flowers contained fused first-whorl organs (Figure 4F), compared with all of the *ffo1-1* 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.*, carpellody of first-whorl organs and absence of second-whorl organs (Figure 4F).

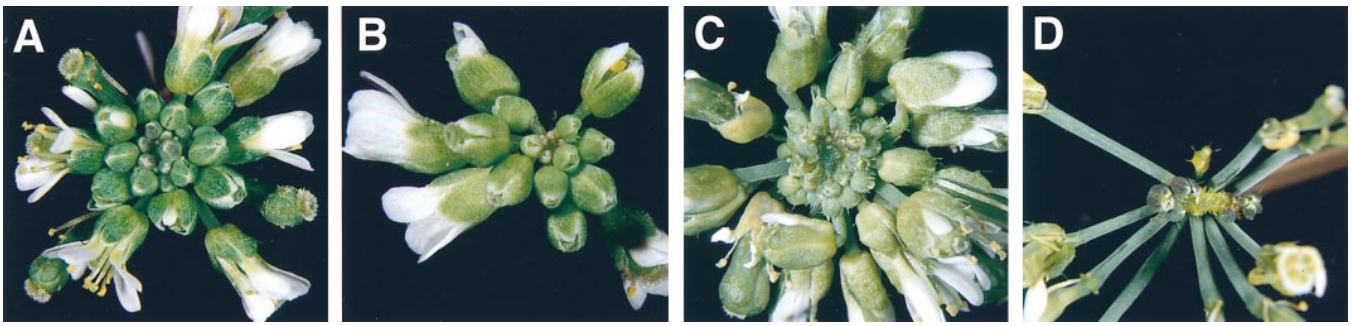

Figure 5.—Phenotypes of *ffo clv3-1* double-mutant plants. (A) A *clv3-1* inflorescence. (B) An *ffo1-1 clv3-1* inflorescence. (C) An *ffo3-1 clv3-1* inflorescence apex, with reduced flowers and overproliferation of apparently undifferentiated meristem tissue. (D) An *ffo2-1 clv3-1* inflorescence apex, with a few reduced flowers and overproliferation of apparently undifferentiated meristem tissue.

Phenotypes of *ffo* double mutants: The similarities observed between the *ffo* mutant floral phenotypes, particularly with respect to sepal fusion, suggested that the products of the three *FFO* genes might act in a common developmental pathway(s). We tested this hypothesis genetically by creating all double-mutant combinations of the *ffo1-1*, *ffo2-1*, and *ffo3-1* alleles. The double-mutant phenotype observed in each case was consistent with an additive interaction between the mutations (data not shown). Both *ffo1-1 ffo2-1* and *ffo3-1 ffo2-1* flowers displayed fused sepals and stamens—characteristic of *ffo1-1* and *ffo3-1* flowers—and also narrow, reduced, and/or mosaic floral organs—characteristic of *ffo2-1* flowers. Fusions between sepals, which occurred at frequencies intermediate between those of the single mutants, were nearly complete along the length of the organs. *ffo1-1 ffo3-1* flowers had an increased frequency of fused sepals and stamens 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 *LEAFY* and *CLAVATA3*: To determine whether the *FFO* genes interact with other members of the filamentous structures class of genes in addition to *UFO*, we constructed plants doubly mutant for an *ffo* mutation and for either a *clv3* (Figure 5) or a *lfy* (Figure 6) allele. Plants homozygous for the *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 *clv3-1* mutant plants are often fasciated, and the floral meristems generate additional whorls of carpels interior to the fourth whorl. The inflorescences of *ufo-2 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, *ffo clv3-1* double-mutant plants had a less severe filamentous structures defect than *ffo ufo-2* double mutants. The phenotype of *ffo1-1 clv3-1* plants was additive early in inflorescence development, with basal flowers displaying fused sepals 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 *ffo3-1 clv3-1* mutant phenotype was also additive early in inflorescence development, with basal flowers displaying fused sepals 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 *clv3-1* single-mutant plants at a similar stage of development (Figure 5A).

The *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 sepals in whorl 1. Later in development, *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 *clv3-1* single-mutant plants at a similar stage of development (Figure 5A). Thus, the moderate synergism of the *ffo2-1 clv3-1* and *ffo3-1 clv3-1* double-mutant phenotypes indicates that both *FFO2* and *FFO3* interact with *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 *LFY* cause a number of inflorescence and flower defects (Schultz and Haughn 1991; Huala and Sussex 1992; Weigel *et al.* 1992), some of which are quite similar to those caused by mutations in *UFO* (Levin and Meyerowitz 1995). *lfy* mutants are characterized by defects

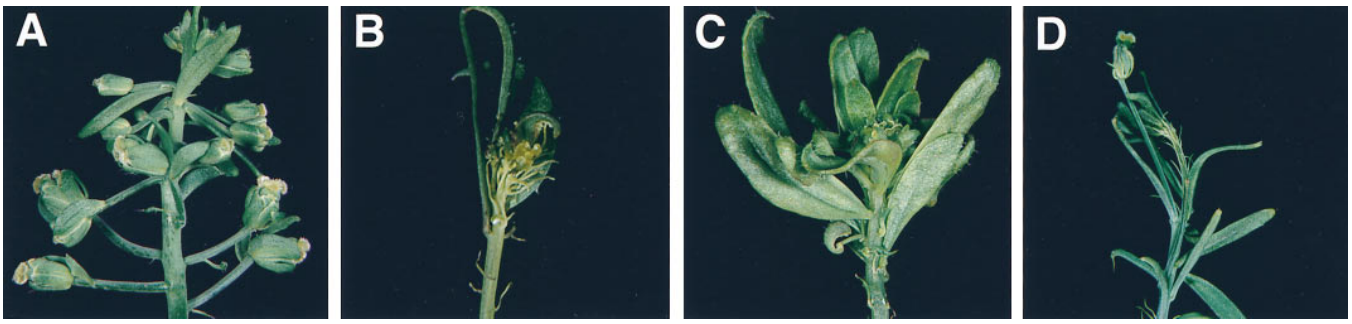

Figure 6.—Phenotypes of *ffo lfy-26* double-mutant plants. (A) An *lfy-26* inflorescence. (B) An *ffo1-1 lfy-26* inflorescence. The floral meristems have been replaced by filamentous structures and by leaflike organs near the apex, which terminates in carpelloid structures. (C) An *ffo3-1 lfy-26* inflorescence, in which the floral meristems have been replaced by filamentous structures and the inflorescence has terminated in a mass of leaflike and carpelloid organs. (D) An *ffo2-1 lfy-26* inflorescence in which a single flower has formed, followed by leaflike organs and filamentous structures.

including an increased number of secondary inflorescences, occasional filamentous structures forming in place of flowers, and premature termination of flowering, with leaflike and carpelloid 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 clv3* double mutants, *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). *ffo1-1 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 carpelloid 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 carpelloid 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 enhanced 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 *clv3*. This result suggests that

only a subset of *ffo* double-mutant combinations with genes in the filamentous structures class has dramatic effects on floral meristem formation, although in the absence of known *ffo* null alleles this interpretation must be viewed with caution. Finally, *ffo2-1 clv3-1* and *ffo3-1 clv3-1* double mutants displayed moderate synergism in older inflorescence meristems, revealing that *FFO2* and *FFO3* have redundant roles with *CLV3* in controlling cell proliferation at later stages of inflorescence development.

Some filamentous structures most closely resemble leaves: The organs formed by most double mutants of the filamentous structures class, including *ufo-2 ffo2-1* and *ufo-2 ffo3-1*, consisted of thin green cylindrical protrusions 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 observation that *ufo-2 ffo1-1* inflorescences formed carpelloid filamentous structures suggested that these organs retained 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 genotypes contain highly vacuolated cells with neither a distinct leaflike nor floral character. Although the arrangement 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 visible vasculature (Figure 3). Indeed, serial sections taken at the base of one *ufo-2 ffo1-1* filamentous structure suggest that vascular bundles may extend up to but not into these organs. The cell types observed in some filamentous structures, particularly those from *ufo-2 ffo1-1* double mutants, most closely resemble the spongy parenchyma 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 subtend flowers in many plant species but which have been lost in most cruciferous taxa, are considered modified leaves (Gifford and Foster 1988) and are often observed subtending the first flowers of *lfy*, *ufo*, *ffo1*, and *ffo3* 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* filamentous structures are neither overtly leaflike nor floral, suggesting that such structures may arise as meristem projections without undergoing differentiation into peripheral organ types.

Comparison with other genes affecting organ boundaries: The fused floral organ phenotypes of the *FFO* genes indicate that they are members of a newly identified set of genes involved in establishing and maintaining 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 *FFO* 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 display 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 (Souer *et al.* 1996). The Arabidopsis *PIN* and *PID* genes also affect floral organ boundary determination, but their pleiotropic mutant phenotypes suggest that their floral defects have different underlying causes than the *ffo* mutants (Goto *et al.* 1987; Okada *et al.* 1991; Bennett *et al.* 1995). Mutations in the Arabidopsis *fiddlehead* (*FDH*) gene cause postgenital fusion between floral organs (Lolle *et al.* 1992), but in *fdh* mutants, unlike *ffo* 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 *ffo* mutants.

The floral phenotypes of the *ffo1* and *ffo3* mutants are similar to, but much stronger than, those of the *cuc1* and *cuc2* mutants. In *ffo1* and *ffo3* 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 *FFO1* in the maintenance of organ boundaries but perhaps at a slightly later stage. Whether these differences in floral phenotype between *ffo1* and *ffo3* 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 *ffo* and the *cuc* mutants have nonoverlapping vegetative phenotypes, with the *ffo* 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 (Souer *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 signalling 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 (Souer *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 that would normally form boundaries to be incorporated into organ primordia. These cells would then 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 *FFO* 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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