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The *FLO10* Gene Product Regulates the Expression Domain of Homeotic Genes *AP3* and *PI* in Arabidopsis Flowers

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We describe a novel mutant of Arabidopsis, Flo10, which is the result of a recessive allele, *flo10*, in the nuclear gene *FLO10*. The first three organ whorls (sepals, petals, and stamens) of Flo10 flowers are normal, but the fourth, gynoecial whorl is replaced by two to eight stamens or stamen-carpel intermediate organs. Studies of ontogeny suggest that the position of the first six of these fourth-whorl organs often resembles that of the wild-type third-whorl organs. To determine the interaction of the *FLO10* gene with the floral organ homeotic genes *APETALA2* (*AP2*), *PISTILLATA* (*PI*), *AP3*, and *AGAMOUS* (*AG*), we generated lines homozygous for *flo10* and heterozygous or homozygous for a recessive allele of the homeotic genes. On the basis of our data, we suggest that *FLO10* functions to prevent the expression of the *AP3/PI* developmental pathway in the gynoecial (fourth) whorl.

INTRODUCTION

Wild-type flowers of Arabidopsis consist of four types of organs arranged on the receptacle in a characteristic pattern of concentric whorls, as illustrated in Figures 1A and 1B (Muller, 1961; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989; Smyth et al., 1990). Each organ type develops from primordia with a unique time and place of initiation (Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989). For example, primordia in the outermost whorl, initiated first (lowest on the receptacle), always develop as sepals, whereas primordia in the innermost whorl, initiated last (highest on the receptacle), always develop as carpels. Thus, the invariant pattern of floral organs is dependent on the ability of primordial cells to recognize their unique position and/or time of initiation on the floral receptacle and thereby develop into the appropriate organ type.

Recently, rapid progress has been made toward understanding the mechanisms by which the fates of floral organ primordia are determined. In Arabidopsis, four genes, *APETALA2* (*AP2*), *PISTILLATA* (*PI*), *AP3*, and *AGAMOUS* (*AG*), whose products appear to play a direct role in this process, have been identified by mutation (Komaki et al., 1988; Bowman et al., 1989, 1991; Hill and Lord, 1989; Kunst et al., 1989). Based on the mutant phenotypes, a model has been developed (Haughn and Somerville, 1988; Bowman et al., 1989, 1991; Kunst et al., 1989; Meyerowitz et al., 1991) that proposes that each of the genes is expressed in two of the four floral organ whorls and acts alone or in combination to specify floral organ type as follows: *AP2* for sepals and petals, *PI* and *AP3* for petals and stamens, and *AG* for stamens and carpels. Moreover, *AP2* and *AG* appear to negatively regulate each other, such that the absence of one allows the expression domain of the other to expand. Double mutant studies (Bowman et al., 1989, 1991; Kunst et al., 1989; Drews et al., 1991; Meyerowitz et al., 1991; L. Kunst and G.W. Haughn, unpublished results) and molecular analyses (Yanofsky et al., 1990; Drews et al., 1991) have all been consistent with these hypotheses. Carpenter and Coen (1990), Schwarz-Sommer et al. (1990), and Coen (1991) have suggested similar models based on their analyses of homeotic genes in snapdragon. Figure 1C is consistent with all these models, the representation being closest to that of Bowman and Meyerowitz (1991), Bowman et al. (1991), and Meyerowitz et al. (1991).

We report here a novel mutant of Arabidopsis, Flo10 (**flo**ral mutant **10**; Schultz and Haughn, 1989a, 1989b, 1990), in which the gynoecium is replaced by stamens. Thus, unlike the other homeotic mutants, Flo10 shows homeotic conversion of only a single whorl. Based on phenotypic characterization of Flo10 and of double mutants between *flo10* and the other homeotic loci, we propose that the *FLO10* gene is required to switch the floral meristem from a stamen-producing to a carpel-producing mode by negatively regulating *AP3/PI* expression in the gynoecial whorl.

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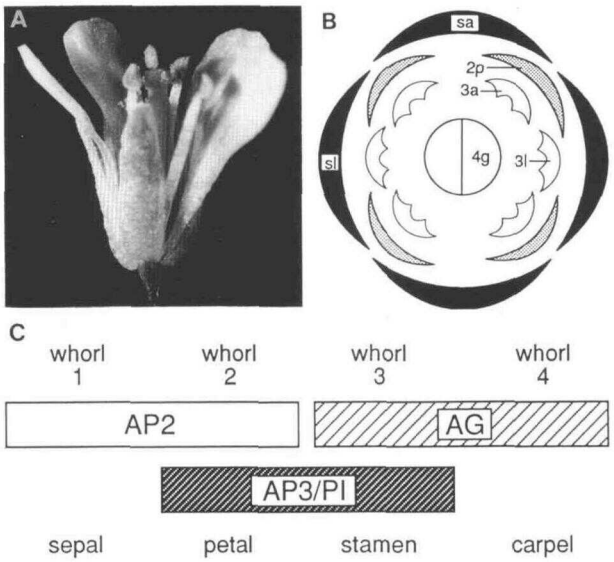

Figure 1. Morphology of Wild-Type Arabidopsis Flowers and the Domains of Expression of Homeotic Genes Controlling Organ Identity in These Flowers.

(A) Wild-type Arabidopsis flower. Magnification ×20.
(B) Floral diagram of a wild-type Arabidopsis flower showing the four concentric whorls of organs. First whorl, two axial sepals (sa) and two lateral sepals (sl); second whorl, four petals (2p); third whorl, four long, axial stamens (3a) and two short, lateral stamens (3l); fourth whorl, bicarpellate gynoecium (4g).
(C) Diagrammatic representation of the spatial expression of the genes *AP2*, *AG*, *AP3*, and *PI* in floral whorls of wild-type Arabidopsis flowers.

RESULTS

Genetic Analysis

A mutant plant isolated from an ethyl methanesulfonate-mutagenized population of the ecotype Columbia was found to produce flowers lacking carpels and having an increased number of stamens (Flo10, Schultz and Haughn, 1989a, 1989b, 1990; see phenotypic description below). To determine the genetic basis of the Flo10 phenotype, mutant plants were used as male parents in a cross to the wild type. All 83 of the F_1 progeny analyzed from such a cross had wild-type phenotypes. The F_2 progeny consisted of wild-type and Flo10 plants, segregating in a ratio of approximately 3 to 1 (406 wild-type:128 Flo10, $\chi^2 = 0.3$, $P > 0.6$). Thus, the Flo10 phenotype appears to be the result of a single recessive nuclear allele (*flo10*) of a gene we designate *FLO10*.

We were interested in determining whether the *FLO10* gene is closely linked to any other mutations that alter floral morphology. For this reason we determined the chromosomal linkage of *FLO10* by scoring 400 F_2 progeny of the cross W100 (a line carrying two genetic markers per chromosome; Koornneef et al., 1987) × Flo10. *FLO10* showed genetic linkage only to the markers *glabra1* (*gl1*) *long hypocotyl2* (*hy2*) on chromosome three. This position was confirmed by analyzing 932 F_2 progeny of the cross MSU22 (a line carrying three markers, *hy2*, *gl1*, *transparent testa5* [*tt5*], on chromosome three) × Flo10 and 512 F_2 progeny of the cross *auxin resistant2* (*axr2/axr2*, Wilson et al., 1990) × Flo10. Figure 2 shows the linkage data from these crosses. These data suggest that *FLO10* lies on chromosome 3 between *HY2* and *GL1* at approximately position 24.1 with respect to the current linkage map for Arabidopsis (Koornneef, 1990). None of the floral morphology mutants described previously is genetically linked to *FLO10*.

Phenotypic Analyses

Wild-Type Morphology

The morphology, cell surface features, and ontogeny of wild-type Arabidopsis flowers has been described extensively in previous studies (Muller, 1961; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989; Smyth et al., 1990). Here, we provide only a brief overview to serve as a basis for comparison to the phenotype of Flo10.

The wild-type flower is typical of the Brassicaceae, being regular, hypogynous, and consisting of four concentric whorls of organs (Figures 1A and 1B). Figure 3 shows a

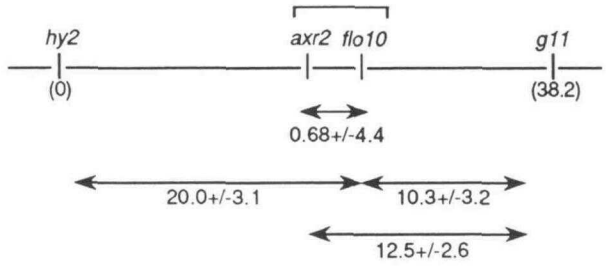

Figure 2. Location of *flo10* on Chromosome 3 of Arabidopsis Relative to Several Closely Linked Markers.

Arrows span distance between pairs of markers, and the numbers beneath these arrows represent the map distances and standard error in centimorgans, as calculated from our data. Bracket indicates that the order within the bracketed group could not be conclusively established. Numbers in parentheses are the chromosomal location of the markers as assigned by Koornneef (1990). The distance relative to the *axr2* gene was based on data from Wilson et al. (1990).

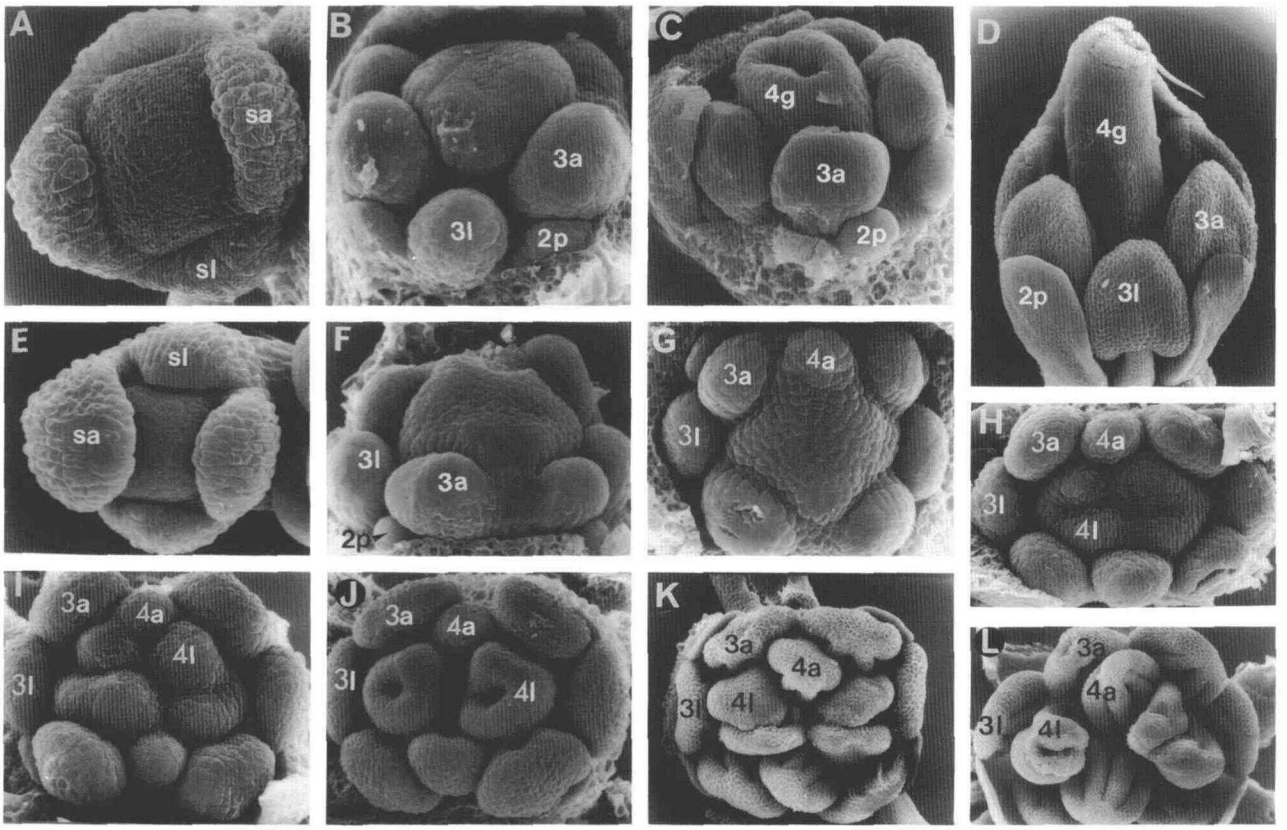

Figure 3. SEM Micrographs Showing Several Developmental Stages of Wild-Type and Flo10 Flowers.

(A) Very early developmental stage of a wild-type flower with axial (sa) and lateral (sl) sepal primordia. Magnification ×539.
(B) Early developmental stage of a wild-type flower, sepals removed. Two young petal primordia (2p) are visible in the second whorl. One lateral (short) stamen (3l) and all four axial (long) stamen primordia (3a) are visible in the third whorl. Magnification ×682.
(C) Later developmental stage of a wild-type flower with sepals partially removed and lateral stamen removed; three young petal primordia (2p), four developing axial stamens (3a), and gynoecial cylinder (4g) are visible. Magnification ×352.
(D) Immature wild-type flower with sepals removed, developing petals (2p) and developing axial and lateral stamens (3a and 3l) having well-differentiated filament and anther, and gynoecium (4g) closed with the beginnings of stigmatic papillae. Magnification ×108.
(E) Very early developmental stage of a Flo10 flower with adaxial and abaxial sepal (sa) and lateral sepal (sl) primordia. Magnification ×382.
(F) Early developmental stage of a Flo10 flower with sepals removed, two young petal primordia (2p), six third-whorl stamen primordia (3a and 3l), and the beginnings of fourth-whorl primordial initiation. Magnification ×405.
(G) Early developmental stage of a Flo10 flower with sepals removed, six third-whorl stamen primordia (3a and 3l), and two axial fourth-whorl primordia (4a). Magnification ×494.
(H) Later developmental stage of a Flo10 flower with six third-whorl stamen primordia (3a and 3l), two axial fourth-whorl primordia (4a), and four lateral fourth-whorl primordia (4l). Magnification ×306.
(I) Later developmental stage of a Flo10 flower with six third-whorl stamen primordia (3a and 3l), two axial fourth-whorl primordia (4a), and four lateral fourth-whorl primordia (4l). Magnification ×339.
(J) Later developmental stage of a Flo10 flower with six third-whorl stamen primordia (3a and 3l), two axial fourth-whorl primordia (4a), and primordia in fourth whorl lateral positions (4l) forming structures similar to small gynoecial cylinders. Magnification ×240.
(K) Flo10 flower close to maturity with six third-whorl stamens (3a and 3l) and all stamen-like organs of the fourth whorl (4a, 4l) unfused. Magnification ×152.
(L) Flo10 flower close to maturity in which six third-whorl stamens (3a and 3l) and two fourth-whorl axial stamens are unfused (4a); stamen-carpel intermediate organs in the fourth-whorl lateral position (4l) appear to be two pairs of fused organs. Magnification ×84.

few stages of development of the wild-type flower. The first primordium initiated is the abaxial sepal, which is immediately followed by the adaxial and two lateral sepals (Figure 3A). Although the positions occupied by the four sepals may be considered two whorls (Jones, 1939; Lawrence, 1951; Kunst et al., 1989), for the purposes of this paper we describe the sepal whorl as whorl one. Next and apparently simultaneously, four petal primordia, whorl 2 (Figure 3B), are initiated in positions alternating with the sepals. Two pairs of long inner stamens appear simultaneously opposite the axial sepals, followed soon afterward by two short outer stamens (Figure 3B) that arise in positions opposite the lateral sepals and further from the floral apex than the long stamens. The stamens may also be considered two whorls, but we will describe all six stamens as whorl 3. The remainder of the floral apex develops into the gynoecium, a two-carpellate, bilocular pistil (whorl 4). During this process, a ridge of cells forms around the periphery of the floral apex, and this ridge then extends to form an open cylinder (Figure 3C). The cylinder closes to form a stigma in the final stages of gynoecial development (Figure 3D). Floral organs of mature wild-type flowers are distinct and easily recognized based on their position and morphology (Kunst et al., 1989). In addition, each organ type comprises cells with unique surface features (Kunst et al., 1989) that can be used in conjunction with morphology to identify organ types unambiguously.

Flo10 Morphology and Ontogeny

The morphology of Flo10 flowers is shown in Figure 4. The organs within whorls 1 (four sepals), 2 (four petals), and 3 (six stamens) of Flo10 flowers are identical with that of the wild type in morphology and relative position (Figure 4A). In contrast, Flo10 flowers with a normal bicarpellate pistil in the fourth whorl have not been observed. Instead, there are a variable number of organs (two to eight, but typically four to six) that can be morphologically normal, pollen-producing stamens (Figure 4D), organs with morphological and cell-surface characteristics of both carpels and stamens (stamen-carpel intermediate organs; Figures 4C and 4D), and/or organs that appear to be morphologically normal carpels (Figure 4B). Table 1 summarizes some of the variability in organ type and number observed in the fourth whorl of Flo10 flowers. The variation in organ number in the fourth whorl of Flo10 flowers is not independent of organ type. An increase in staminoidy of the fourth whorl is correlated with an increase in the number of organs. For example, when only two or three fourth-whorl organs develop in a flower, the organs are very carpel-like (Figure 4B). Conversely, when six to eight fourth-whorl organs develop, the organs are very stamenlike (Figure 4D). In all cases, the last organs to develop are the most carpel-like. Thus, as in the wild type, carpel development is associated with a termination of floral shoot growth. For the sake of clarity, we will define the fourth whorl of Flo10 flowers as including all organs that develop after the six third-whorl stamens.

The stamen-carpel intermediate organs at one extreme resemble normal stamens with small sectors of carpel tissue and/or fusion with adjacent organs (Figures 4C and 4D). At the other extreme, they resemble normal carpels having small sectors of stamen tissue (Figure 4B). The most carpel-like organs sometimes produce seed when pollinated. As in wild-type stamens and carpels, only those cells in the apical regions of the intermediate organs have characteristics of anther or stigmatic cell types (Figure 4C). Thus, cells in an intermediate organ differentiate into cell types appropriate to their position along the longitudinal axis of the organ.

Flo10 flowers at various stages of early development were examined by scanning electron microscopy (SEM) to determine the relative time and position of floral organ initiation. Figures 3E to 3L show the results of this analysis. The ontogeny of organs in the first three whorls (sepals, petals, and stamens) seems to be identical with that of wild-type flowers (compare Figures 3E and 3F with Figures 3A and 3B). After the initiation of the six third-whorl stamens, the Flo10 floral apex usually fails to form the open cylinder associated with normal gynoecial development (compare Figures 3F to 3J with Figure 3C). Instead, a variable number of distinct organ primordia can usually be observed (Figures 3F to 3J). The organs arising from these primordia vary in shape and degree of fusion to one another, although certain trends are apparent. When distinct primordia are observed, the two in axial positions (opposite the axial sepals and the four long third-whorl stamens) and closer to the apex than the third-whorl, long-stamen primordia (Figures 3F and 3G) appear to develop first. We will refer to this pair of organs as fourth-whorl axial. Four distinct primordia may develop shortly afterward, slightly closer to the apex than the previous two, and in lateral positions (opposite the lateral sepals and the third-whorl short stamens) (Figures 3H and 3I). We will refer to any organs appearing in these positions as fourth-whorl lateral. Thus, the primordia appear to be arranged in a pattern analogous to that of the third whorl, but rotated 90°. Mature Flo10 flowers with more than six fourth-whorl organs occasionally occur; however, six distinct primordia was the maximum number observed in the sample of flowers examined by SEM.

From analysis of developing Flo10 flowers, it was evident that with increasing fourth-whorl staminoidy, fusion between organs is less frequent (compare Figure 3I with 3J and Figure 3K with 3L). In some cases, primordia develop as separate stamenlike organs (Figures 4D and 3L). More frequently, fused stamen-carpel organs form in these positions (Figures 3J and 3K). The organs arising in the axial positions are always more stamenlike than those in the lateral positions. Occasionally, several fourth-whorl

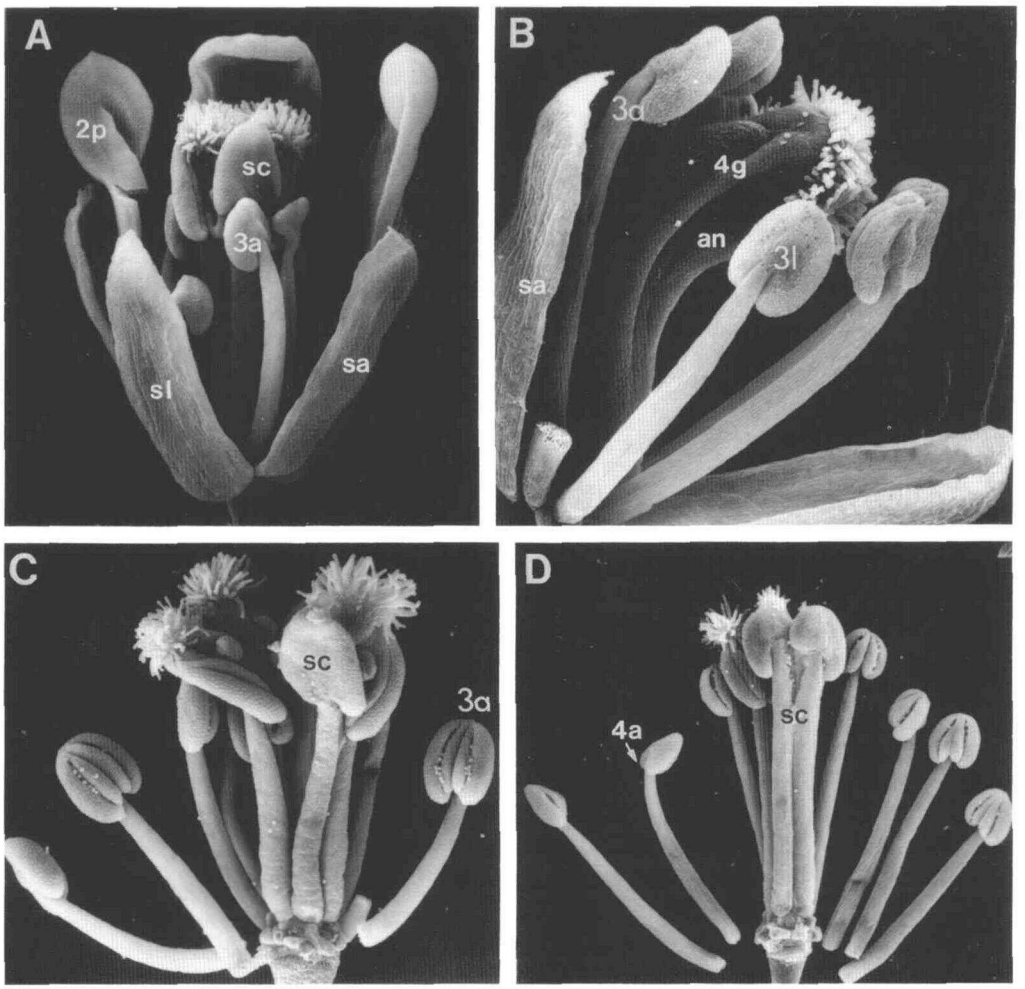

Figure 4. SEM Micrographs of Mature Flo10 Flowers.

(A) Flo10 flower, one petal removed. Axial sepals (sa), lateral sepals (sl), petals (p), and third-whorl stamens (e.g., 3a) are wild type, but in the fourth whorl, four fused stamen-carpel intermediate organs (sc) have formed. Magnification ×33.

(B) Less extreme Flo10 flower with all perianth organs except axial sepals (sa) removed. Six third-whorl stamens (3a and 3l) enclose a gynoecium (4g) that appears almost normal, except for the anther lobe (an), which results in the pistil curving. Magnification ×48.

(C) More extreme Flo10 flower with entire perianth removed. Six stamens (e.g., 3a) in the third whorl. In the fourth whorl, four partially fused stamen-carpel intermediate organs (sc) have formed. Magnification ×39.

(D) Most extreme Flo10 flower with entire perianth removed. Four third-whorl stamens have been removed from the receptacle and lie beside the flower, as well as one unfused fourth-whorl stamen (4a). The additional four stamens (sc) occupying the fourth whorl are fused, but otherwise well developed. Magnification ×30.

organs fuse, resulting in a gynoecial-like cylinder (Figure 4B). Although an increase in the degree of fusion is correlated with a decrease in the number of fourth-whorl organs, we believe that the two processes are distinct. In the following discussion, the "degree of staminoidy" refers not only to the variation in organ type, but also to the associated changes in organ number and fusion relative to the wild-type gynoecium.

Double Mutant Phenotypes

The phenotypic analysis of the Flo10 mutant indicated that the *FLO10* gene plays an important role in the determination of organ number and type in the fourth whorl of Arabidopsis flowers. To determine the interaction of *FLO10* with other Arabidopsis genes known to alter floral organ type and/or number, we identified and analyzed the floral

Table 1. The Phenotype of the Fourth Whorl of Flowers Homozygous for *flo10* and Homozygous for Heterozygous for Other Loci

Genotype		*flo10/flo10* (no.[a] = 330)	*flo10/flo10 ap2-5/ap2-5* (no.[a] = 92)	*flo10/flo10 AP2/ap2-6* (no.[a] = 30)	*flo10/flo10 AP3/ap3-1* (no.[a] = 25)	*flo10/flo10 PI/pi-1* (no.[a] = 50)
Percentage of flowers with "n" fourth-whorl stamens	n = 0	29	89	70	100	64
	1	28	11	27	0	28
	2	20	0	3	0	4
	3	8	0	0	0	0
	4	6	0	0	0	0
	5	5	0	0	0	4
	6	2	0	0	0	0
	7	2	0	0	0	0
	8	0.3	0	0	0	0
Percentage of flowers with "n" unfused or partially fused carpel-like organs	n = 0[b]	12	52	57	45	22
	1	2	0	0	0	0
	2	20	5	3	0	12
	3	30	16	10	4	26
	4	35	11	30	52	40
	5	1	0	0	0	0
	x[c]	0	16	0	0	0
Percentage of flowers with "n" organs fused to form pistil-like structure	n = 0[d]	88	48	43	55	78
	1	2	10	0	0	0
	2	5	8	13	4	4
	3	4	10	14	9	6
	4	1	11	30	32	12
	x[c]	0	13	0	0	0

[a] "no." indicates the number of flowers examined for each genotype.
[b] n = 0 refers to any flower in which no carpel-like organs developed in the fourth whorl, or in which all fourth-whorl, carpel-like organs were completely fused to form a pistil-like structure.
[c] "x" indicates that although the degree of fusion was analyzed, the number of carpel-like organs was not determined.
[d] n = 0 refers to any flower in which no carpel-like organs developed in the fourth whorl, or in which not all fourth-whorl, carpel-like organs were completely fused to form a pistil-like structure.

phenotypes of plants homozygous for *flo10* and either homozygous or heterozygous for a recessive allele of the genes *AP2*, *AG*, *PI*, or *AP3*. In addition, the floral phenotype of plants homozygous for the three mutations *flo10*, *ag-1*, and *pi-1* was examined. The genotype of putative double mutants was verified genetically by testcrossing to the parental lines and/or constructing lines homozygous for a mutant allele of one gene and segregating for a mutant allele of the second gene as outlined in Methods. The phenotypes of the double and triple mutants are described below.

flo10-ap2. Recessive mutations in the *AP2* locus cause homeotic transformations of perianth organs to reproductive organs and, depending on the allele, a decrease in organ number in the first three whorls (Komaki et al., 1988; Kunst et al., 1989; Bowman et al., 1991). For example, the *ap2-5* allele (a "weak" allele) causes sepal-to-carpel and petal-to-stamen transformations. The *ap2-6* allele (a "strong" allele) causes sepal-to-carpel and petal-to-carpel transformations, as well as a reduction in organ number in the first three whorls (Kunst et al., 1989).

As shown in Figure 5A, flowers of plants doubly homozygous for *ap2-5* and *flo10* have perianth organs indistinguishable from the Ap2-5 parent. Stamens or stamen-carpels were never observed in the first whorl, suggesting that *flo10* is not effective in transforming carpel to stamen in the first whorl. The third whorl of double mutant flowers is wild type (Figure 5A). As in the Flo10 parent, the fourth whorl comprises additional stamens and stamen-carpel intermediate organs (Figure 5A). However, relative to the Flo10 parent, the number of organs in the fourth whorl is reduced, and organ type is noticeably more carpelloid, with fewer stamens and an increased frequency of organ fusion and fertility (Table 1). For example, the number of double mutant flowers having a fourth whorl with at least one separate stamen (11% of 92) and/or incompletely fused stamen-carpel intermediates (48% of 92) decreased relative to Flo10 single mutants (71% and 88% of 330,

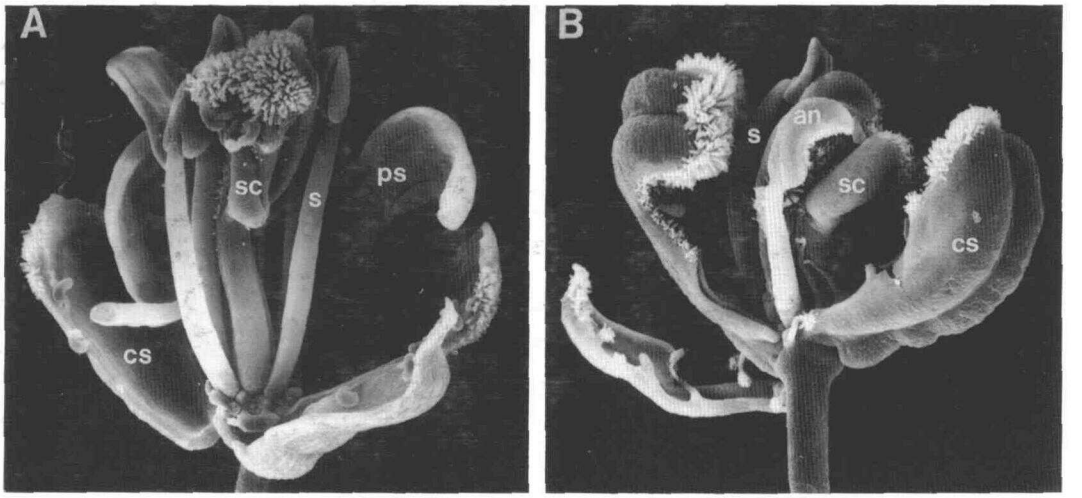

Figure 5. SEM Micrographs of Flowers Doubly Homozygous for *flo10* and *ap2-5* or *ap2-6*.

(A) Intact Flo10/Ap2-5 flower. The organs of the first whorl are sepal-carpels (cs) and those of the second whorl are petal-stamens (ps). The third whorl consists of six stamens (s), whereas the fourth whorl contains four fused stamen-carpel intermediate organs (sc). Magnification ×31.

(B) Intact Flo10/Ap2-6 flower. All the perianth organs are carpelloid (cs) and fused to one another. The third whorl contains only one stamen (s), whereas the fourth whorl consists of several stamen-carpel intermediate organs (sc) with anther lobes (an). Magnification ×29.

respectively). At the same time, the number of double mutant flowers in which organs of the fourth whorl fused to form a pistil-like structure (52% of 92) increased relative to Flo10 (12% of 330) flowers. Typically, the pistil-like structure appeared to consist of three or four carpels, although two carpellate pistils were seen occasionally. Thus, although the double mutant phenotype was additive, *ap2-5* appears to suppress partially the effects of the *flo10* allele in the fourth whorl.

A similar additive phenotype was observed for the Flo10/Ap2-6 double mutant (Figure 5B). The first two whorls are indistinguishable from the Ap2-6 parent. As in Ap2-6, the number of stamens in the third whorl is reduced. The fourth whorl comprises stamen-carpel intermediate organs, but these were more carpelloid than those of the Flo10 parent, and an unfused stamen was never observed. Because of the high frequency of split pistils in the Ap2-6 parent (Kunst et al., 1989), the degree of carpelloidy of these organs was more difficult to assess on the basis of fusion than in Flo10/Ap2-5 or Flo10 flowers.

We noticed that in F_2 populations segregating for *ap2-5* and *flo10* or *ap2-6* and *flo10*, many plants with Flo10 phenotype (no changes in perianth whorls) were considerably more fertile than those in the parental Flo10 line. We examined five flowers from each of 10 Flo10 plants chosen at random from the F_2 populations and, as described in Table 2, found that increased fertility occurred in a significant proportion of the flowers as a result of decreased fourth-whorl staminoidy. Because the *AP2* gene was also segregating in the F_2 population (two thirds of the Flo10 plants were *AP2/ap2* heterozygotes), we reasoned that the partial suppression of Flo10 may occur in plants that are also heterozygous at the *AP2* locus. To determine whether the increased fertility correlated with *ap2* heterozygosity, we generated plants heterozygous for *ap2-6* and homozygous for *flo10* by backcrossing double mutants from the F_2 population to *flo10/flo10* plants from the parental line, to generate plants with the genotype *flo10/flo10 AP2-6/ap2-6*. Analysis of flowers from these plants showed that they had, on average, fourth-whorl organs that were fewer in number and significantly less staminoid than Flo10 flowers (Table 1). For example, of 30 *flo10/flo10 AP2-6/ap2-6* flowers examined, 57% had a fourth whorl that was completely fused to form a fertile gynoecium (compare with 12% in *flo10/flo10* plants), whereas only 30% had at least one unfused stamen in the fourth whorl (compared with 71% in *flo10/flo10* plants). These data suggest that *ap2* heterozygosity is correlated with a decrease in fourth-whorl staminoidy. Thus, it seems that both *ap2* homozygosity and heterozygosity affect organ type in the fourth whorl of Flo10 flowers.

flo10-ap3. Figure 6 shows flowers from Ap3-1 mutants and Flo10/Ap3-1 double mutants in different stages of development. Flowers of plants homozygous for the recessive mutation *ap3-1* when grown at 22°C (Bowman et al., 1989) have four sepals in the second whorl, six stamen-

Table 2. The Fourth-Whorl Phenotype of Flo10 Flowers from F_2 Populations of a Variety of Crosses

Cross		Ler[a] × Flo10 (no.[b] = 121)	*AG/ag-1* × Flo10 (no.[b] = 81[c])	Ap2-5 × Flo10 (no.[b] = 50[d])	Ap2-6 × Flo10 (no.[b] = 50[d])	Ap3-1 × Flo10 (no.[b] = 50[d])
Percentage of flowers with "n" fourth-whorl stamens	n = 0	15	2	74	52	58
	1	12	7	10	36	16
	2	19	7	8	6	6
	3	10	6	4	4	4
	4	12	11	2	0	10
	5	18	12	0	2	6
	6	6	11	0	0	0
	7	5	7	2	0	0
	8	2	2	0	0	0
	>8	0	35	0	0	0
Percentage of flowers with "n" unfused or partially fused carpel-like organs	n = 0[e]	26	22	50	34	44
	1	6	25	0	0	12
	2	24	31	6	0	8
	3	25	16	10	32	10
	4	18	4	32	34	22
	5	2	0	2	0	4
	>5	0	2	0	0	0
Percentage of flowers with "n" organs fused to form pistil-like structure	n = 0[f]	90	100	50	66	60
	1	1	0	0	0	2
	2	8	0	24	4	8
	3	1	0	6	8	14
	4	0	0	20	22	16

[a] Ler, Landsberg *erecta*. This cross was also segregating for *abscisic acid insensitive3*.
[b] "no." indicates the number of F_2 flowers examined for each cross.
[c] Twenty-three plants having a Flo10 phenotype were chosen at random from the F_2 population, and three or four flowers were examined from each plant.
[d] Ten plants having a Flo10 phenotype were chosen at random from the F_2 population, and from each plant five flowers, taken throughout the inflorescence, were analyzed.
[e] n = 0 refers to any flower in which no carpel-like organs developed in the fourth whorl, or in which all fourth-whorl, carpel-like organs were completely fused to form a pistil-like structure.
[f] n = 0 refers to any flower in which no carpel-like organs developed in the fourth whorl, or in which not all fourth-whorl, carpel-like organs were completely fused to form a pistil-like structure.

carpel intermediate organs in the third whorl, and a wild-type pistil in the fourth whorl (Figure 6A).

Analysis of F_2 progeny from an Ap3-1 × Flo10 cross (see Methods) indicated that only Ap3-1, Flo10, and wild-type phenotypes were present. However, after identification of the double mutant by genetic analysis, a more careful phenotypic study of both mature (Figures 6C and 6D) and developing flowers (Figures 6E to 6H) of the double mutant revealed that they differed from Ap3-1 flowers in the reproductive whorls. The double mutant flowers have at most six carpelloid organs in the inner two whorls. The pattern of fusion of these six organs in mature flowers (often the four organs in the position of the long stamens fused in opposite pairs [Figure 6C], or these four organs fused completely, leaving only the two in the position of the short stamens separate [Figure 6D]) suggested that all six organs represent third-whorl stamen-carpels, whereas fourth-whorl organs fail to develop. To test this hypothesis, we examined reproductive organ ontogeny in Ap3-1 and the double mutant flowers. Development of Ap3-1 flowers is similar to wild-type flowers in that six primordia form in the third whorl, followed by a gynoecial cylinder (Figure 6B). In the double mutant flowers, primordia in the positions of the long stamens appear to develop first, followed by two primordia in the positions of the short stamens (Figures 6E and 6F). The four primordia in the position of the long stamens are not always distinct, often developing as two ridges of tissue (Figures 6F to 6H) or, less frequently, as a cylinder. Normally, double mutant flowers appear to lack a fourth whorl completely (Figures 6F to 6H), although very rare flowers (two in approximately

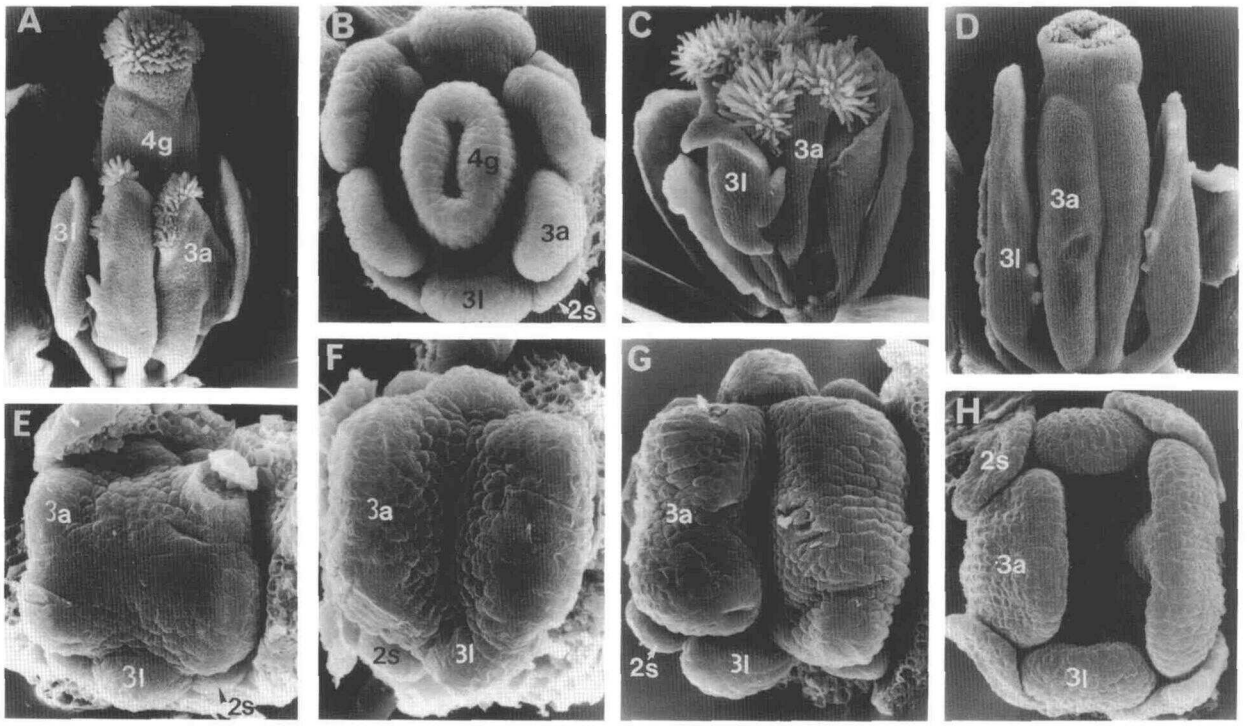

Figure 6. SEM Micrographs of Mature and Developing Flowers of Ap3-1 Mutants and Flo10/Ap3-1 Double Mutants.

(A) Mature Ap3-1 flower, perianth removed. The third whorl is occupied by stamen-carpel intermediate organs, the lateral organs (3l) are more staminoid than the axial (3a), and the fourth whorl is occupied by a normal, two-carpellate gynoecium (4g). Magnification ×47.

(B) Early developmental stage of an Ap3-1 flower with sepals removed. Young second-whorl sepal primordia (2s) enclose six distinct organ primordia (3a and 3l) of the third whorl and the fourth-whorl gynoecial cylinder (4g). Magnification ×392.

(C) Mature Flo10/Ap3-1 flower with perianth partially removed. Six stamen-carpels occupy the third whorl. The stamen-carpels occupying the axial positions (3a) fuse in pairs, whereas those in the lateral positions (3l) remain unfused. Magnification ×35.

(D) Mature Flo10/Ap3-1 flower with perianth removed. Organs seen are those of the third whorl. Stamen-carpel organs formed in lateral positions of the third whorl (3l) remain unfused, whereas those in axial positions (3a) fuse to form what appears to be a four-carpellate gynoecium. Magnification ×71.

(E) Very early developmental stage of a Flo10/Ap3-1 flower with sepals removed. Second-whorl primordia (2s) are visible, two lateral third-whorl primordia (3l) are well formed, and primordia are also developing in the axial positions (3a). Magnification ×500.

(F) Very early developmental stage of a Flo10/Ap3-1 flower with sepals removed. Two lateral third-whorl primordia (3l) remain distinct, but ridges of tissue develop in the axial third-whorl positions (3a). No gynoecial cylinder is evident. Magnification ×600.

(G) Early developmental stage of a Flo10/Ap3-1 flower with sepals removed. Small second-whorl primordia (2s) enclose two third-whorl lateral primordia (3l), which continue to develop as distinct organs, and ridges of tissue, which develop in the axial third-whorl positions (3a). No gynoecial cylinder has formed. Magnification ×425.

(H) Late developmental stage of a Flo10/Ap3-1 flower with sepals removed. Developing second-whorl sepals (2s) enclose the third whorl, which consists of two lateral organs (3l), and two ridges in axial positions (3a). There are no organs in the fourth whorl. Magnification ×342.

100 flowers examined) were observed to have a gynoecial cylinder in the fourth whorl.

We noticed that many Flo10 plants within the F_2 population segregating for *flo10* and *ap3-1* were distinctly more fertile, as a result of decreased fourth whorl staminoidy, than those in a population segregating only for *flo10* (Table 2). This increase in fertility could have been due to the Landsberg *erecta* (Ler) background introduced in the cross to Ap3-1. However, an examination of flowers with a Flo10 phenotype from plants in an F_2 population segregating for *flo10* and Ler (from the Ler background) but not *ap3-1* revealed no such increase in fertility (Table 2). To determine whether the alteration of the Flo10 phenotype was correlated with *ap3-1* heterozygosity, we generated plants homozygous for *flo10* and heterozygous for *ap3-* by backcrossing a sample of the F_2 plants having an Ap3 phenotype to *flo10* (see Methods). Progeny of these crosses having a Flo10 phenotype (*flo10/flo10 AP3/ap3-1*)

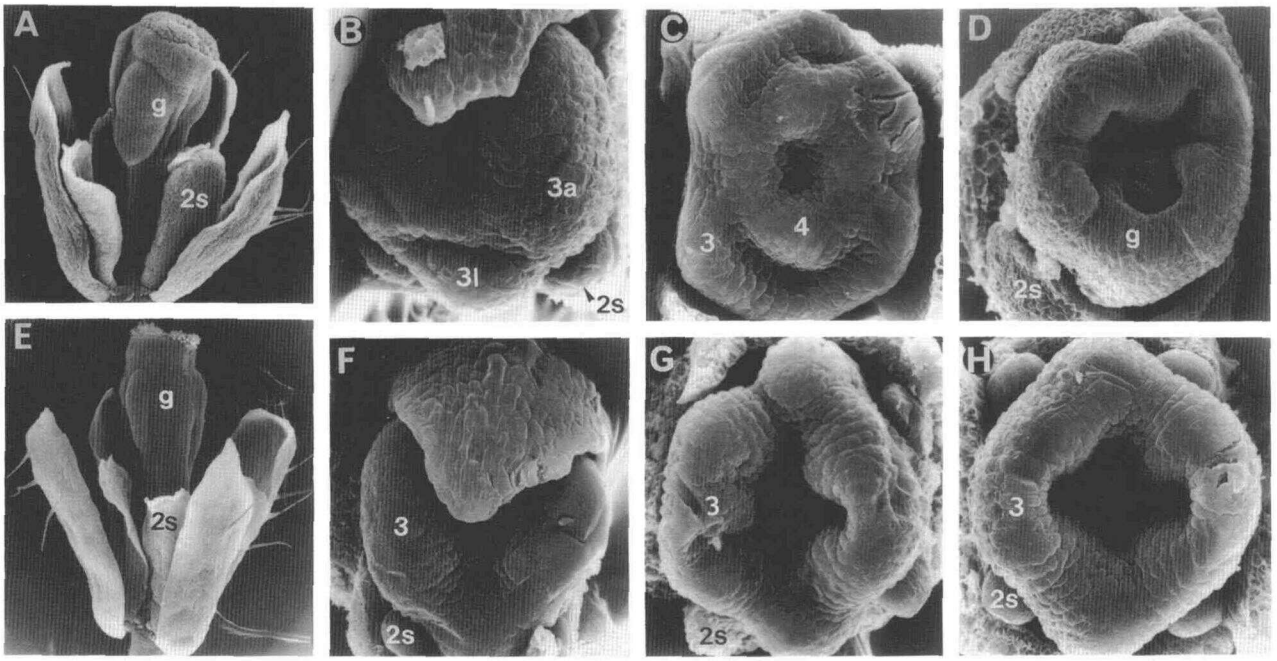

Figure 7. SEM Micrographs of Mature and Developing Flowers of Pi-1 Mutants and Flo10/Pi-1 Double Mutants.

(A) Mature Pi-1 flower. Sepal-like organs (2s) are found in the second whorl and the gynoecium (g) is multicarpellate. Magnification ×28.
(B) Very early developmental stage of a Pi-1 flower with sepals partially removed. Second-whorl primordia (2s) enclose the developing third whorl. A primordium arising in the lateral position is distinct (3l), and inside this is a ring of tissue (3a). Magnification ×629.
(C) Early developmental stage of a Pi-1 flower, sepals removed. The ring of tissue (3) occupying the third whorl continues to develop and encloses the emerging gynoecial cylinder of the fourth whorl (4). Magnification ×392.
(D) Late developmental stage of a Pi-1 flower, sepals removed. Second-whorl developing organs (2s) enclose the third- and fourth-whorl organs, which form a multicarpellate gynoecium (g). Magnification ×287.
(E) Mature Flo10/Pi-1 flower. Sepal-like organs (2s) are present in the second whorl and the gynoecium (g) is multicarpellate. Magnification ×24.
(F) Very early developmental stage of a Flo10/Pi-1 flower with sepals partially removed. Second-whorl organ primordia (2s) enclose a ring of tissue (3) developing in the third whorl. Magnification ×392.
(G) Early developmental stage of a Flo10/Pi-1 flower with sepals removed. Second-whorl organ primordia (2s) enclose the ring of tissue (3), which continues to develop in the third whorl. No obvious organ primordia are developing in the fourth whorl. Magnification ×436.
(H) Late developmental stage of a Flo10/Pi-1 flower with sepals removed. Second-whorl organ primordia (2s) enclose the ring of tissue (3) of the third whorl. No gynoecial cylinder has formed in the fourth whorl. Magnification ×392.

were found to produce flowers in which the organs of the fourth whorl were, on average, fewer in number, more carpel-like, more completely fused, and had a much higher seed set per plant than occurs in Flo10 in either Columbia or Ler segregating populations. For example, of 25 *flo10/flo10 AP3/ap3-1* flowers (from 10 plants) examined, none had a normal fourth-whorl stamen compared with 71% in Flo10 with a Columbia background, (Table 1, *flo10/flo10*) or 85% in Flo10 plants segregating for Columbia and Ler backgrounds, Table 2, Ler × Flo10). Furthermore, 45% of the *flo10/flo10 AP3/ap3-1* flowers had a fourth whorl that was completely fused, compared with 12% in Flo10 with a Columbia background (Table 1) or 10% in Flo10 plants segregating for Columbia and Ler backgrounds (Table 2). These observations suggest that the fourth-whorl staminoidy of Flo10 is sensitive to heterozygosity at the *AP3* locus.

flo10-pi. Figure 7 shows that the flowers of plants homozygous for recessive mutations in *PI* resemble those of Ap3-1. The second whorl consists of sepal-like organs. In the third whorl, filamentous or carpel-like organs form, and these may become fused to the fourth-whorl organs to form a multicarpellate gynoecium (Figure 7A). The apparent number of third-whorl organs is usually reduced (Bowman et al., 1989, 1991; Hill and Lord, 1989; E.A. Schultz and G.W. Haughn, unpublished observations). In

double mutant combinations with recessive alleles of other homeotic genes (*ag*, *ap2*), Ap3-1 and Pi-1 have comparable phenotypes (Bowman et al., 1991; L. Kunst and G.W. Haughn, unpublished observations). We anticipated that Flo10/Pi-1 double mutants would also have a phenotype similar to the Flo10/Ap3-1 double mutant. Indeed, in the F_2 population, we found only wild-type, Flo10, and Pi-1 plants. Genetic analysis of the Pi-1 plants from this population indicated that one quarter of such plants were double mutants. Analysis of the general morphology (Figure 7E), cell surface features, and internal anatomy (data not shown) of mature double mutant and Pi-1 flowers using a combination of SEM, serial sectioning, and light microscopy revealed no obvious differences between them. However, as shown in Figure 7, examination of the ontogeny of these flowers indicated that the development of the fourth whorl was different. In Pi-1 flowers, the third whorl often takes the form of a ring of up to six partially fused primordia (Figure 7B), which usually enclose a smaller gynoecial cylinder occupying the fourth whorl (Figure 7C). In later stages of development, the ring of tissue in the third whorl obscures the presence or absence of a developing gynoecial cylinder in the fourth whorl (Figure 7D). In double mutant flowers, a similar ring of what appears to be six partially fused primordia forms (Figure 7F), but we have never observed an inner cylinder (Figures 7G and 7H).

In contrast to the population segregating for *ap3* and *flo10*, the F_2 population segregating for *pi* and *flo10* did not appear to have any Flo10 plants with significantly increased fertility. However, we analyzed plants having the *flo10/flo10 PI/pi-1* genotype (Table 1, constructed as in Ap3-1, see Methods), and although not as fertile as *flo10/flo10 AP3/ap3-1* plants, they produce flowers with a fourth whorl that is somewhat more carpelloid than that of *flo10/flo10* flowers. Of 50 flowers examined, 22% had a fused pistil, compared with 12% in Flo10 with a Columbia background (Table 1, *flo10/flo10*) or 10% in Flo10 plants segregating for Columbia and Ler backgrounds (Table 2, Ler × Flo10), whereas only 36% have any stamens in the fourth whorl, compared with 71% in Flo10 with a Columbia background (Table 1) or 85% in Flo10 segregating for Columbia and Ler backgrounds (Table 2). These data suggest that as for *AP3*, the fourth-whorl staminoidy of Flo10 is sensitive to heterozygosity at the *PI* locus.

flo10-ag. Figure 8 shows that plants homozygous for recessive mutations in the *AG* gene form indeterminate flowers that produce normal first and second whorls and petals in the third whorl and then repeat this pattern of four sepals and 10 petals. Plants homozygous for at least some alleles (Yanofsky et al., 1990), including the *ag-1* allele in the Columbia ecotype, have flowers in which internode elongation occurs prior to each inner sepal whorl (Figure 8A). Among the Flo10 plants in the F_2 population of an *AG/ag-1*, *fca/fca* (*late flowering* MSU10 line, see Methods) × *flo10/flo10* cross, we observed that many flowers had an increased number of stamens relative to those in a population segregating for *flo10* alone (Table 2). A relatively low frequency (2%) of Flo10 F_2 plants had flowers with an extreme Flo10 phenotype in which the fourth whorl consisted of an indeterminate number (50 to 70) of stamens or stamenlike organs (Figure 8B). A higher frequency of Flo10 plants had flowers with an increased number of fourth whorl stamens (15 to 20) relative to Flo10 plants in other populations (Tables 1 and 2). These extreme Flo10 phenotypes were not correlated with the late flowering phenotypes. Fourteen F_2 plants with a large number of fourth-whorl stamens (including those with an indeterminate number of stamens) were testcrossed to *AG/ag-1* heterozygotes. Approximately 25% of the progeny of each cross were Ag in phenotype, suggesting that all 14 of the Flo10-like parents were heterozygous for *ag-1*. These results suggest that the degree of Flo10 fourth-whorl staminoidy is sensitive to heterozygosity at the *AG* locus, although we cannot rule out possible Ler background effects or heterozygosity at the *FCA* locus.

A second novel floral phenotype among the F_2 population (1 of 16) consisted of a whorl of four sepals followed by multiple whorls of petals (Figure 8C). In contrast to Ag-1 flowers, neither sepals nor sepaloid sectors ever occurred in the inner floral whorls and internode elongation was never seen. Frequently, the flowers had a floral meristem that was enlarged and often split into two or three separate meristems. The same floral phenotype was observed in one quarter of a population of plants homozygous for *flo10* and segregating for *ag-1*, indicating that it is the phenotype of Flo10/Ag-1 double mutants. These data suggest that in Ag-1 flowers, *FLO10* is required for sepal development and internode elongation in the inner whorls and is expressed in a periodic manner, for the first time in the fourth whorl and every three whorls thereafter.

***flo10, pi, and ag* Triple Mutant.** The analysis of Flo10/Ag-1 double mutants indicated that *FLO10* is required for sepal development in the inner whorls of Ag flowers. Plants homozygous for both *ag-1* and *pi-1* are known to produce flowers with an indeterminate number of sepal whorls (Bowman et al., 1989). To determine whether *FLO10* is required for sepal development in any of the whorls of Ag-1/Pi-1 double mutants, we constructed a line homozygous for *flo10* and segregating for *ag-1* and *pi-1*. The Flo10/Ag-1/Pi-1 triple mutant floral phenotype (one sixteenth of the population) consisted of multiple whorls of sepals (Figure 8D) closely resembling the Ag-1/Pi-1 double mutant floral phenotype. *FLO10* is not, therefore, required for sepal development in inner whorls of Ag-1 flowers in the absence of *PI*.

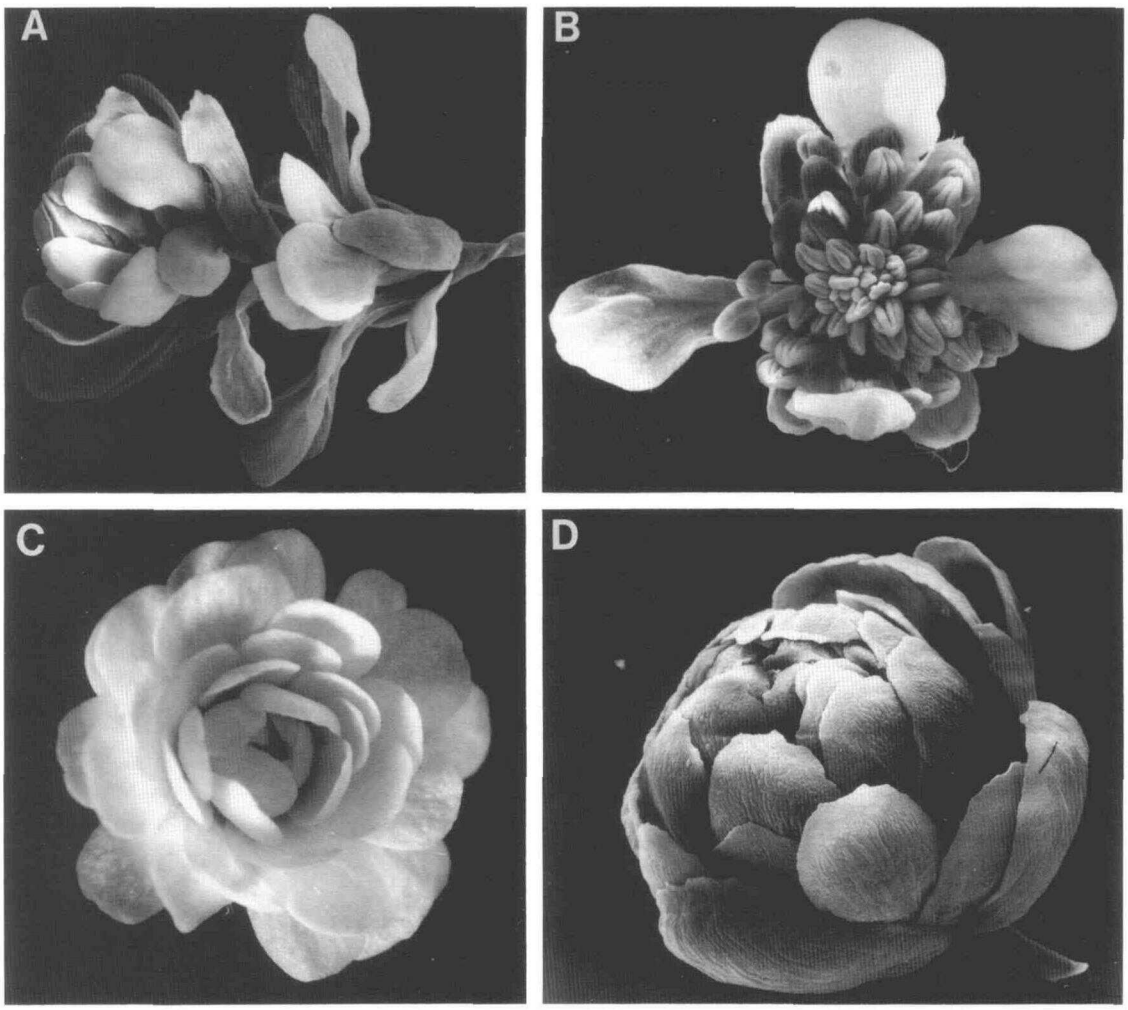

Figure 8. SEM Micrographs of Mature Flowers from Plants with Genotypes *ag-1/ag-1*, *flo10/flo10 AG/ag-1*, *flo10/flo10 ag-1/ag-1*, and *flo10/flo10 ag-1/ag-1 pi-1/pi-1*.

(A) Mature flower of an *ag-1* homozygote. Magnification ×17.
(B) Mature flower of a *flo10* homozygote, heterozygous for *ag-1*. Magnification ×23.
(C) Mature flower of a plant homozygous for both *flo10* and *ag-1*. Magnification ×21.
(D) Mature flower of a plant homozygous for *flo10*, *ag-1*, and *pi-1*. Magnification ×41.

DISCUSSION

We have characterized the phenotype of a mutant of Arabidopsis, Flo10, which is the result of a recessive allele of a novel gene *FLO10*. The pistil of a Flo10 flower is absent and replaced by two to eight stamens or stamen-carpel intermediate organs arranged in a pattern similar to that of the third-whorl stamens. Thus, the Flo10 phenotype can be considered homeotic (carpel whorl replaced by stamens) or heterochronic (the developmental switch from stamen development to carpel development is delayed) and, as discussed below, suggests that the *FLO10* gene plays an important role in the determination of organ development in the fourth (gynoecial) whorl. Based on the recessive nature of the *flo10* allele and the fact that two other recessive alleles of *FLO10* having a similar phenotype have been found (see section on alleles of Flo10), we will assume in the following discussion that it represents a loss of function of the gene *FLO10*.

The Role of the *FLO10* Gene Product in Flower Development

The phenotypic analyses of Flo10 and the double and triple mutants support the following general conclusions

concerning the mode of action of the *FLO10* gene product in wild-type flowers.

(1) *FLO10* is expressed primarily in the fourth floral whorl. This conclusion is supported by the fact that the loss of *FLO10* function both alone and in combination with complete or partial loss of other gene functions has phenotypic effects limited to the fourth whorl. The only exception to this rule is the phenotype of the *flo10 ap3-1* double homozygote, in which the third whorl organs are noticeably more carpelloid than in plants homozygous for *ap3-1* alone. Carpelloidy in Ap3-1 plants is variable and known to be temperature sensitive (Bowman et al., 1989). It is possible that in Flo10/Ap3-1 double mutants, the abnormal development of the fourth whorl indirectly affects the expression of an unstable *ap3-1* gene product, resulting in increased third-whorl carpelloidy.

(2) *FLO10* inhibits the expression of the *AP3/PI* developmental pathway in the fourth whorl. *PI* and *AP3* gene products are required both for stamen development (in combination with the *AG* gene product) and petal development (in combination with the *AP2* gene product) (Figure 1C) (Bowman et al., 1989, 1991). The fourth-whorl staminoidy of *flo10* homozygotes and the fourth-whorl petalloidy of Flo10/Ag-1 double mutants could be explained by ectopic expression of the *AP3/PI* developmental pathway in the fourth whorl. Other observations consistent with this hypothesis are the decreases in fourth-whorl staminoidy associated with Flo10 plants that are heterozygous for the *PI* or *AP3* gene and the requirement of *PI* activity for the petalloidy of the Flo10/Ag-1 double mutant. It is logical to suggest, therefore, that the *FLO10* gene is required to prevent such ectopic expression during the development of wild-type flowers.

(3) *FLO10* is not required to specify organ type in the fourth whorl. All possible organ types can be found in the fourth whorl of plants homozygous for the mutant *flo10* allele: stamens in *flo10* homozygotes, petals in Flo10/Ag-1 double mutants, and sepals in Flo10/Ag-1/Pi-1 triple mutants. Furthermore, the fourth whorls of *flo10/flo10 PI/pi-1*, *flo10/flo10 AP3/ap3-1*, *flo10/flo10 ap2/ap2*, and *flo10/flo10 AP2/ap2* plants are more carpelloid than *flo10* homozygotes, suggesting that fourth-whorl carpelloidy is not directly dependent on the activity of *FLO10*. Curiously, Flo10/Pi-1 and Flo10/Ap3-1 double mutants appear to completely lack fourth-whorl organs, although carpels were expected. One explanation for this observation is that the expression of a defective *AP3/PI* developmental pathway in the fourth whorl interferes with the specification of any organ type.

Figure 9 presents a model for the role of the *FLO10* gene in flower development of wild-type Arabidopsis that is consistent with the conclusions noted above. Briefly, the product of *FLO10* is expressed in the fourth floral whorl, where it negatively regulates the *AP3/PI* developmental pathway. In this way, stamen development is terminated and the *AG* gene product alone specifies carpel development.

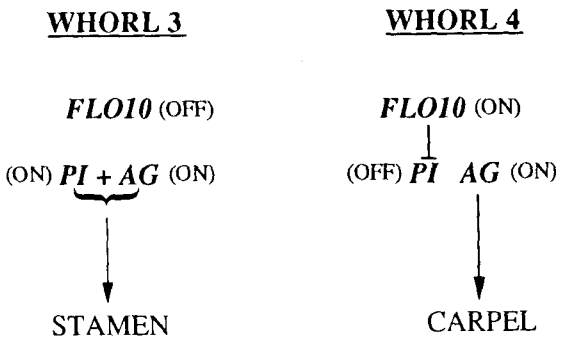

Figure 9. A Model for the Role of the *FLO10* Gene in Determination of Reproductive Organ Type in Flowers of Wild-Type Arabidopsis.

"On" and "off" indicate the expression of each gene in the indicated whorl and the arrows point to the organ type specified. The ⊥ indicates negative regulation of the *AP3/PI* developmental pathway by the *FLO10* gene.

The Regulation of *FLO10* Expression

The mechanism that establishes the fourth whorl as the domain of *FLO10* expression remains to be determined. However, it is worth noting that the fourth-whorl phenotype of mutants homozygous for a recessive mutation in any one of the genes *AG*, *PI*, *AP3*, or *AP2* is altered by a loss of *FLO10* activity (e.g., compare the phenotype of *ap2-5/ap2-5* plants with that of *flo10/flo10 ap2-5/ap2-5* plants). These data suggest that *FLO10* is expressed in the fourth whorl of flowers lacking a functional *AG*, *PI*, *AP3*, or *AP2* gene product and indicate that the activation of *FLO10* expression in the fourth whorl is not dependent upon these genes.

Determinacy of the Floral Shoot

In addition to changes in floral organ type, the loss of *FLO10* activity results in increased fourth-whorl organ number. In extreme cases of the Flo10 phenotype (as observed occasionally in the F_2 population segregating for alleles of both *AG* and *FLO10* genes), the growth of the floral meristem appears to be indeterminate. These data suggest that the *FLO10* gene product plays a role in establishing a determinate floral meristem. Genetic studies have indicated that the *AG* gene product is also required for the termination of meristem proliferation (Bowman et al., 1989, 1991). We propose that the two gene products are required in combination to cause the meristem to terminate in the fourth whorl.

The *FLO10* gene product may have a direct role in establishing floral meristem determinacy that is distinct from its role in the regulation of the *AP3/PI* expression

domain. Alternatively, if the *AP3/PI* developmental pathway is considered to suppress floral meristem determinacy, *FLO10* may be required for floral meristem termination indirectly through its exclusion of *AP3/PI* expression from the fourth whorl. The following logic supports the hypothesis that *AP3* and *PI* act to suppress floral meristem determinacy. In wild-type flowers, the *AG* gene is expressed at the level of transcription in both the third and fourth whorls (Drews et al., 1991), yet the meristem terminates only after the fourth whorl. It is possible that third-whorl expression of the *AP3/PI* developmental pathway prevents the *AG* gene product from terminating floral meristem growth until after the fourth whorl. This proposed role of *AP3/PI* predicts that loss of the *AP3/PI* developmental pathway should lead to premature termination of the floral meristem after the third whorl. Although mutants homozygous for loss-of-function alleles of *PI* and *AP3* genes do have a fourth whorl, none of these alleles may be null alleles. Indeed, a null allele (globifera) of the snapdragon *Deficiens* gene, which appears to have a role in flower development analogous to that of *PI* and *AP3*, does lack a fourth whorl (Sommer et al., 1990; Coen, 1991).

Variability of the Flo10 Fourth Whorl

If the number and type of Flo10 fourth-whorl organs are determined by the relative expression levels of the *AP3/PI* and *AG* developmental pathways in the fourth whorl, then small fluctuations in *AP3*, *PI*, or *AG* expression, whether environmentally or genetically induced, might dramatically alter the phenotype of any given Flo10 flower. For example, a decrease in *AP3* or *PI* expression relative to *AG* should result in primarily carpel-like fourth-whorl organs that are few in number, whereas a decrease in *AG* expression should result in a large number of stamenlike organs. This hypothesis provides an explanation for the smaller number of organs and increased presence of pistil-like structures in the fourth whorls of *flo10/flo10 AP3/ap3-1* and *flo10/flo10 PI/pi-1* flowers relative to *flo10/flo10* flowers. In addition, the hypothesis can explain the increased stamen number in flowers of many Flo10 plants in a population segregating for the *ag-1* allele. Furthermore, the variability in phenotype observed in *flo10/flo10* flowers could be due to environmental influences on the ectopic expression of *PI* or *AP3* in the fourth whorl.

AP2-FLO10 Interactions

The suppression of Flo10 fourth-whorl staminoidy by an *ap2/ap2* or *AP2/ap2* genetic background is surprising because previous genetic analysis has indicated that the *AP2* gene plays no direct role in specifying reproductive organ type in the inner whorls (Bowman et al., 1989; Kunst et al., 1989). As discussed in the previous section, suppression of the Flo10 phenotype can be explained by small increases in the fourth-whorl expression level of *AG*. The *AP2* gene is thought to negatively regulate the *AG* gene in the perianth whorls (Bowman et al., 1991; Drews et al., 1991). Therefore, the apparent *AP2-FLO10* interactions could be the result of *AP2-AG* interactions. Either of the following two hypotheses explains how decreased *AP2* gene expression might cause increased *AG* gene expression in the fourth whorl. First, the loss of *AP2* activity in *ap2* homozygotes results in expression of *AG* earlier in floral meristem development than in wild type, during formation of the perianth whorls (Drews et al., 1991). Such early expression may lead to higher levels of the *AG* gene product in the floral meristem later in development, during formation of the third and fourth whorls. Alternatively, the *AP2* gene may be expressed in the inner two floral whorls of wild-type flowers at a level too low to compete with the *AG* gene product for determination of the reproductive organ type, but high enough to reduce the absolute level of the *AG* gene product. Thus, a decrease in the overall level of *AP2* expression would result in a small increase in *AG* expression in the inner whorls.

It is interesting to note that morphological abnormalities have been observed in the third and fourth whorls of plants homozygous for "strong" *AP2* mutant alleles (Komaki et al., 1988; Kunst et al., 1989; Bowman et al., 1991). Such effects may be the result of the proposed variation in *AG* expression.

Additional Alleles of *FLO10*

Two additional mutants with phenotypes similar to Flo10 have been isolated recently: Superman by Drs. U. Mayer and G. Jurgens, University of Munich, Munich, Germany (Meyerowitz et al., 1991) and BB4 by B. Bernstein and Dr. R. Malmberg, University of Georgia, Athens (unpublished results). Complementation analysis indicates that both Superman and BB4 are allelic to *FLO10* (E.A. Schultz and G.W. Haughn, unpublished results). Because the *flo10* allele described here was the first to be reported (Schultz and Haughn, 1989a, 1989b, 1990) and the first to be described in detail, we designate it as *flo10-1*, Superman as the *flo10-2* allele, and BB4 as the *flo10-3* allele.

Our examination of the Superman and BB4 mutants has indicated that both are very similar in phenotype to that of Flo10. Bowman and Meyerowitz (1991) and Meyerowitz et al. (1991) report that they observed homeotic changes and organ position alterations in the third whorl of Superman. As yet, we have never observed such changes in Flo10-1 flowers nor in a limited sample (50) of Superman flowers. It may be that abnormal development of the third whorl occurs infrequently in Flo10-2 and never in Flo10-1 because of differences in alleles, genetic background, or growth conditions. Further analysis of Flo10-2 flowers should clarify this uncertainty.

METHODS

Plant Material

The mutant line Flo10 (Schultz and Haughn, 1989a, 1989b, 1990) was isolated from an ethyl methanesulfonate-mutagenized population of Arabidopsis. Before being used for analyses reported here, Flo10 was backcrossed to the wild type four times, and individuals with the mutant phenotype were reselected from segregating populations. Lines used for genetic mapping were W100 (*angustifolia* [*an*], *ap1*, *pyrimidine requiring* [py], *erecta1* [*er1*], *hy2*, *gl1*, *eceriferum2* [*cer2*], *brevipedicellus* [*bp*], *male sterile* [*ms*], *tt3*) (Koornneef et al., 1987) and MSU22 (*gl1*, *hy2*, and *tt5*) (gifts from Maarten Koornneef, Department of Genetics, Wageningen Agricultural University, The Netherlands). Lines used for construction of double mutants were SAS 1-2-6 (homozygous for *ap2-5*, backcrossed three times to the wild type) and SAS 1-3-7 (homozygous for *ap2-6*, backcrossed two times to the wild type) (Kunst et al., 1989), MSU10 (*cer4*, *compacta3* [*cp3*], *fca*, and *er*, heterozygous for *ag-1*) (Koornneef and Hanhart, 1983), SAS 1-12-5 (homozygous for *pi-1*), and SAS 1-11-0 (homozygous for *ap3-1*) (gifts from Maarten Koornneef).

Plants were normally grown at 22°C under continuous fluorescent illumination supplemented with incandescent light (100 to 150 μE m^{-2} sec^{-1} PAR) on Tera-lite Redi-earth prepared by W.R. Grace & Co. Canada Ltd., Ajax, Ontario, Canada. Several of the floral homeotic mutants analyzed to date have displayed temperature-sensitive phenotypes. To test if this is also the case for *flo10* homozygotes, a population segregating for *flo10* was grown at 16 and 27°C. The phenotype of *flo10* homozygotes grown at these temperatures was not significantly different from those grown at 22°C.

Genetic Mapping

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

Light Microscopy and SEM

Morphological characterization of the Flo10 phenotype and the phenotype of each of the doubly homozygous lines was performed on at least 300 (single mutant) or 50 (double mutant) individual flowers using the dissecting microscope. Flowers were taken from various positions within the inflorescence at different stages of inflorescence development.

For SEM, mature flowers or young inflorescences were vacuum infiltrated with 3% glutaraldehyde in 0.02 M sodium phosphate buffer (pH 7.2) and fixed in the same solution overnight at 4°C. Samples were then rinsed in buffer, and inflorescences were postfixed in OsO_4 in the same buffer for 2 hr. All samples were dehydrated through a graded acetone series at 4°C before critical point drying in liquid carbon dioxide and mounting on stubs. Perianth organs were removed from very young flowers using pulled glass needles. Flowers were then coated with gold in an Edwards S150B sputter coater and examined with a Philips 505 scanning electron microscope at an accelerating voltage of 30 kV.

Construction of Double and Triple Mutants

Flo10 plants were crossed to plants homozygous for *ap2-5*, *ap2-6*, *pi-1*, and *ap3-1*, and heterozygous for *ag-1*. The double mutants were identified and verified for each cross as follows:

flo10 ap2-5 and flo10 ap2-6

Among the F_2 progeny of both crosses, a novel phenotype with characteristics of both Flo10 and Ap2 was observed. Phenotypes in the F_2 population were in a ratio of 144 wild type: 62 Flo10:48 Ap2-5:15 Ap2-5/Flo10 (9:3:3:1, $\chi^2 = 3.55$, $P > 0.25$) and 216 wild type: 56 Flo10:68 Ap2-6:24 Ap2-6/Flo10 (9:3:3:1, $\chi^2 = 2.7$, $P > 0.25$). Three Ap2-5/Flo10 F_2 plants and three Ap2-6/Flo10 F_2 plants were shown to be homozygous for *ap2-5* (*ap2-6*) and *flo10* by testcrossing to *ap2-5/ap2-5* (*ap2-6/ap2-6*) and *FLO10/flo10* plants.

flo10 pi-1

Among the F_2 progeny examined, we found the phenotypes to be that of the wild type, Flo10, and Pi-1 in a ratio of 265:63:80 (9:3:4, $\chi^2 = 12.6$, $P > 0.005$). This ratio suggested that Pi is epistatic to Flo10. Thirty-one of the phenotypically Pi-1 F_2 plants were crossed to *flo10* homozygotes. One quarter (9 of 31) of these crosses resulted in progeny all with the Flo10 phenotype, indicating that the Pi parents were homozygous for *flo10*. The testcross progeny (homozygous for *flo10*, heterozygous for *pi-1*) were examined for moderations of the Flo10 phenotype and allowed to self-pollinate. (Seed set was relatively low because of poor Flo10 fertility.) Three quarters of the resulting progeny had the Flo10 phenotype, whereas one quarter had the Pi-like phenotype (56 Flo10:13 Pi, 3:1, $\chi^2 = 1.7$, $P > 0.5$). Pi plants in this population were homozygous for both *flo10* and *pi-1*, and were used in subsequent analysis of the double mutant phenotype.

flo10 ap3-1

Among the F_2 progeny analyzed, three phenotypes were found, wild type , Flo10, and Ap3-like, in a ratio of 335:145:144, respectively. These results suggested epistasis, although the ratio was too ambiguous to determine the epistatic phenotype. Anticipating that Ap3-1 would be epistatic, we testcrossed a sample of the F_2 plants having Ap3-1 phenotype to *flo10* homozygotes. One quarter of these crosses (7 of 22) resulted in progeny that were all Flo10 in phenotype, indicating epistasis of *ap3-1* to *flo10*. We examined these Flo10 plants for variation in the phenotype and allowed the plants (*flo10* homozygous, *ap3-1* heterozygous) to self (seed set relatively high due to fertility of *ap3-1* heterozygote), and the progeny showed a 3:1 ratio of Flo10 to Ap3-1 phenotype (94 Flo10:31 Ap3, 3:1, $\chi^2 = 0.0027$, $P > 0.995$). The phenotypes of the 31 Ap3-like plants (*flo10/flo10 ap3-1/ap3-1*) were analyzed in detail to determine the phenotype of the double mutant.

flo10 ag

AG/ag-1 heterozygotes in an MSU10 background (homozygous for *fca*, therefore late flowering) were crossed with pollen from *flo10/flo10* plants. F_1 plants were harvested individually and only those F_2 lines segregating for Ag-1 plants were examined further. Among the F_2 progeny wild type, Flo10 (including those with increased numbers of stamens), Ag-1, and Ag-like (four sepals followed by multiple whorls of petals) plants were observed in a ratio of 373:114:122:36 (9:3:3:1 ratio, $\chi^2 = 1.3$, $P > 0.5$). These results suggest that the Ag-like phenotype represents the double mutant. Because the putative double mutant could not be crossed, we crossed 14 late flowering, Flo10-like plants to *AG/ag-1* heterozygotes. All of these crosses produced progeny with a phenotypic ratio of 3 wild-type:1 Ag-1 plants, suggesting that all parental F_2 lines tested were heterozygous and the double mutant had either a wild-type or Ag-like phenotype. The double mutant phenotype was confirmed as being Ag-like during construction of the Flo10/Pi-1/Ag-1 triple mutant.

flo10 pi-1 ag-1

A plant heterozygous for *ag-1* and homozygous for *flo10* was crossed to a plant doubly homozygous for *pi-1* and *flo10*. The resulting progeny (homozygous for *flo10*, heterozygous for *pi-1* and *ag-1*) was allowed to self (seed set was very low because of low Flo10 fertility). The phenotypic ratio of the progeny, 47:19:19:2, Flo10:Pi:Ag-like:novel phenotype (9:3:3:1, $\chi^2 = 3.1$, $P > 0.25$) established the novel phenotype as the triple mutant and confirmed that the Flo10/Ag-1 double mutant had an Ag-like phenotype.

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