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Function of the *apetala-1* Gene during *Arabidopsis* Floral Development

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We have characterized the floral phenotypes produced by the recessive homeotic *apetala 1-1* (*ap1-1*) mutation in *Arabidopsis*. Plants homozygous for this mutation display a homeotic conversion of sepals into bracts and the concomitant formation of floral buds in the axil of each transformed sepal. In addition, these flowers lack petals. We show that the loss of petal phenotype is due to the failure of petal primordia to be initiated. We have also constructed double mutant combinations with *ap1* and other mutations affecting floral development. Based on these results, we suggest that the *AP1* and the *apetala 2* (*AP2*) genes may encode similar functions that are required to define the pattern of where floral organs arise, as well as for determinate development of the floral meristem. We propose that the *AP1* and *AP2* gene products act in concert with the product of the *agamous* (*AG*) locus to establish a determinate floral meristem, whereas other homeotic gene products are required for cells to differentiate correctly according to their position. These results extend the proposed role of the homeotic genes in floral development and suggest new models for the establishment of floral pattern.

INTRODUCTION

Vegetative meristems form a reiterated series of vegetative leaves with associated axillary buds and have the capacity for indefinite growth (McDaniel, 1980). In contrast, floral meristems are strictly determinate and form a species-specific pattern of floral organs. In response to various cues, cells in the vegetative shoot apical meristem can initiate floral development. Once the floral signal is perceived, florally determined apical meristems are competent to carry out a floral program of development, even when isolated from the rest of the plant (Hicks and Sussex, 1970; Singer and McDaniel, 1986). These results indicate that after floral evocation floral development of the apical meristem is independent of the rest of the plant. Therefore, the development and differentiation of floral organs depend on localized interactions within the apical meristem. The biochemical nature of the substances involved in these interactions is unknown, but certain conclusions regarding their action have been inferred from a variety of surgical manipulations of flower primordia. Bisections of young floral buds result in the formation of two nearly complete flowers, indicating that cells within the developing meristem can reassess their position and differentiate accordingly (Cusick, 1956; Hicks and Sussex, 1971). These experiments suggest that cells within the apical meristem differentiate in response to a combination of as yet uncharacterized signals, which results in the organization of floral organs in a stereotypic pattern.

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To define the gene products involved in specifying where and how floral organs develop, we are studying mutations that specifically alter floral development. A number of such mutations have been isolated in *Arabidopsis thaliana* (Koornneef et al., 1982; Haughn and Somerville, 1988; Komaki et al., 1988; Bowman et al., 1989; Kunst et al., 1989). These include a number of homeotic floral mutations in which one organ type is replaced by another that normally develops in a different location. Extensive analyses of homeotic mutations in *Drosophila* have provided a genetic and molecular framework for understanding how cells correctly differentiate according to their position (Lewis, 1978; Akam, 1987; Ingham, 1988). The discovery of the homeobox, a DNA-binding motif present in many of the *Drosophila* homeotic genes, has provided the basis for models in which these genes function to regulate transcription (Laughon and Scott, 1984; McGinnis et al., 1984). The conservation of similar homeobox sequences in vertebrate genes suggests that a common mechanism of transcriptional control is an integral part of positional specification in animal systems (Shepherd et al., 1984; McGinnis, 1985). The identification of a different DNA-binding motif in both the homeotic *deficiens A* gene of *Antirrhinum* and the homeotic *agamous* gene of *Arabidopsis* indicates that transcriptional control may be involved in positional specification in plants as well (Sommer et al., 1990; E. Meyerowitz, personal communication). An analysis of floral-specific genes in *Arabidopsis*, where both genetic and molecular studies are feasible (Meyerowitz and Pruitt, 1985), will

facilitate a better understanding of how organ position and identity are specified during floral development.

We have characterized the phenotype of a homeotic floral mutation in *Arabidopsis* that appears to affect some of the earliest stages of floral development. This mutation, *apetala1-1* (*ap1-1*), is recessive and maps to a single locus on chromosome 1 (Koornneef et al., 1983). We present a detailed description of the mutant phenotype and of the development of mutant flowers. We also describe double mutant studies in which we combined *ap1-1* with other mutations affecting floral development. These genetic analyses suggest that the *AP1* wild-type gene product is required to specify the location at which floral organ primordia will develop, as well as for the appropriate differentiation of specific cell types.

RESULTS

Wild-Type Morphology

Wild-type plants (ecotype Landsberg *erecta*) germinate in 3 to 5 days and produce a rosette of about eight to 10 leaves. At about 16 days post-germination, the conversion from vegetative growth to flowering becomes apparent with the appearance of the rapidly bolting floral meristem. Anthesis first occurs at about 21 days. By 28 days, the mature plant is in full flower and secondary floral branches appear: usually three or four axillary floral stems branch off from the main stem, each subtended by a cauline leaf, or bract, as illustrated in Figure 1. On each axillary floral branch two secondary cauline leaves arise at transverse positions relative to the primary cauline leaf. Later in development inflorescences develop in the axils of these cauline leaves and other inflorescences can arise from the basal rosette.

The *Arabidopsis* inflorescence is a raceme, and flowers arise in a helix around the axis. Each flower arises directly from the axis without a subtending bract. Flowers develop acropetally, with older flowers near the base of the inflorescence and progressively younger flowers toward the apex. *Arabidopsis* flower morphology has been extensively described and is similar to that of other crucifers (Vaughan, 1955; Polowick and Sawhney, 1986; Bowman et al., 1989; Hill and Lord, 1989). The wild-type flower consists of four whorls of morphologically distinct organs, as shown in Figures 2A and 2B. We would like to emphasize that we use "whorl" to indicate a position in the flower rather than the organ that develops at that location. In wild-type flowers, the first, or outer, whorl is composed of four sepals that alternate with the second whorl of four petals. The third whorl consists of six stamens: two short outer stamens on the transverse plane and four long inner stamens on the median plane. The central pistil has two carpels that compose the fourth whorl.

The *apetala 1* Mutant Phenotype

The *apetala 1* (*AP1*) locus maps at 103.5 centimorgans on chromosome 1 (Koornneef et al., 1983). A number of recessive mutations at the *AP1* locus, all with apparently similar phenotypes, have been isolated and briefly characterized (Koornneef et al., 1982). Here we describe the phenotype of plants homozygous for the *ap1-1* mutation. The *ap1-1* mutation affects the development of flowers; we do not observe any disruption of normal vegetative growth or in the formation of floral axillary branches (data not shown).

ap1-1 flowers show a transformation of the first-whorl organs into bract-like structures based on both epidermal morphology and the development of flower buds in the axil of each bract-like organ. Figures 2C and 2D show that this pattern of development is reiterated in the axillary secondary flowers so that tertiary buds can be formed in the axils of each secondary bract-like organ. Secondary and tertiary flowers can be incomplete or irregular, with mosaic organs or with fewer organs than normal. The secondary flowers are oriented with respect to the primary flower axis. This is most clearly seen in the orientation of the septum of each secondary pistil, which lies on a median plane with respect to the primary flower axis (Figure 2D). In wild-type plants, floral internodes are compressed, whereas in *ap1-1* flowers, the internodes between the medial and the lateral first-whorl organs elongate. In general, the lateral first-

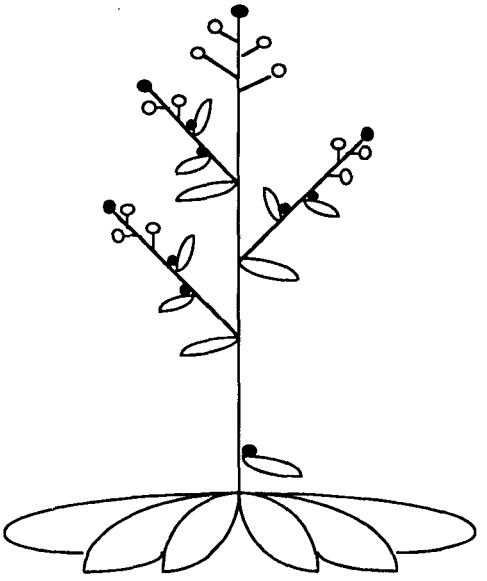


Figure 1. Diagram of Wild-Type *Arabidopsis* Morphology.

Open circles represent floral meristems, and closed circles are inflorescence meristems.

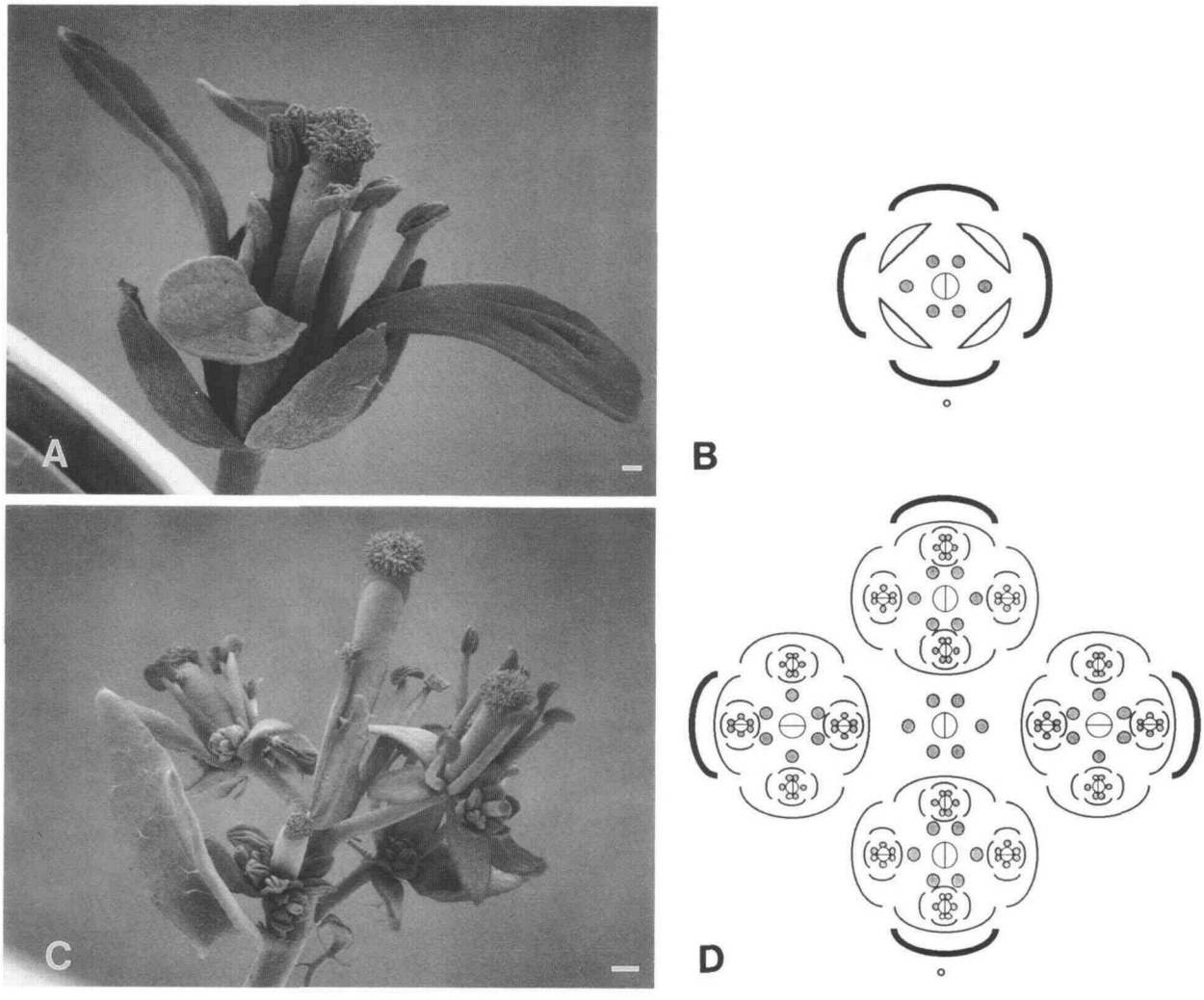


Figure 2. Morphology of Wild-Type and *ap1-1* Mutant Flowers.

(A) Wild type. Bar = 100 μm.
(B) Diagram of wild type. The adaxial sepal is adjacent to the inflorescence axis (indicated by small circle).
(C) Homozygous *ap1-1* mutant flower. Bar = 200 μm.
(D) Diagram of *ap1-1* flower.

whorl organs appear opposite each other and the abaxial and adaxial bract-like organs are located more apically.

The transformation of first-whorl organs into more leaf-like bracts can be seen at the cellular level in Figure 3. Wild-type sepals have characteristic elongated epidermal cells and a few simple trichomes on their abaxial surface. Wild-type cauline leaves, on the other hand, have irregularly shaped epidermal cells and stellate trichomes on their abaxial surface. In *ap1-1* plants, the epidermal cells of the first-whorl organs appear similar to those of cauline leaves and do not have any of the epidermal features associated with sepal tissue. This bract-like epidermal phenotype is also characteristic for the first-whorl organs of the secondary *ap1-1* flowers.

In addition to the aberrant development of first-whorl organs as bract-like structures, *ap1-1* mutants also lack petals. Very rarely in *ap1-1* mutants some petal epidermal cells differentiate as part of a mosaic organ. Occasionally, on secondary and tertiary flower buds, organs develop that are mosaics of leaf-like and stamen-like tissue; these organs appear to arise from a region in the meristem where the first- and third-whorl primordia are closely apposed.

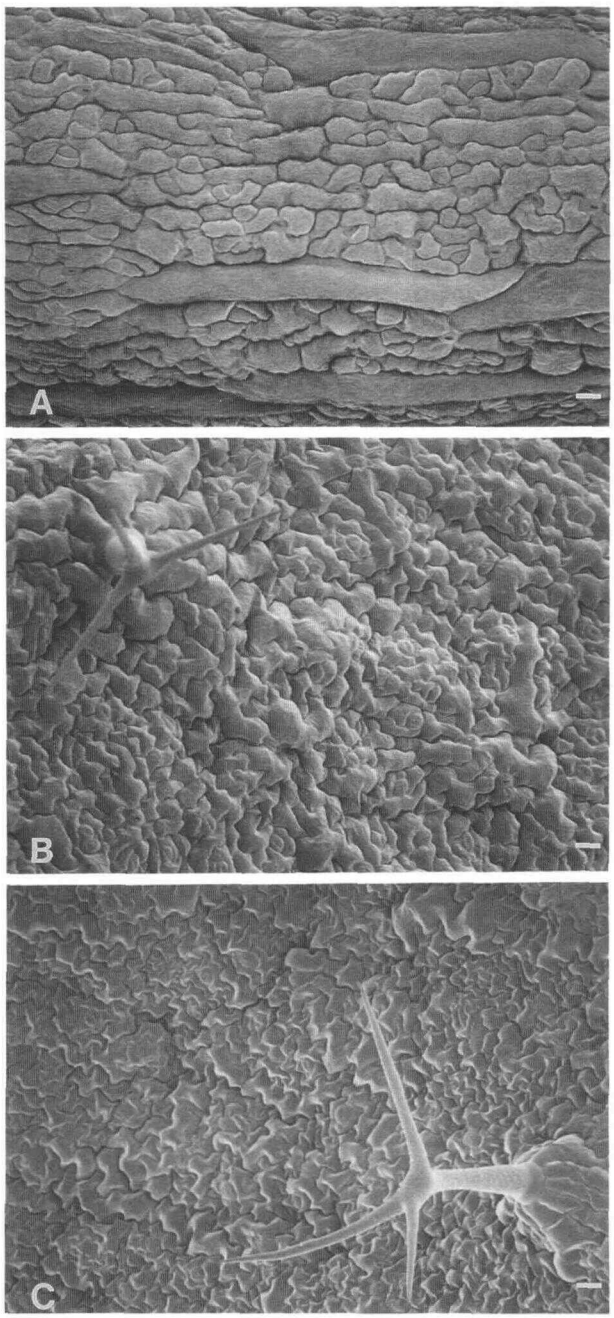


Figure 3. Epidermal Cell Phenotypes of Wild-Type and *ap1-1* Tissues.

(A) Wild-type sepal tissue.
(B) Wild-type cauline leaf tissue.
(C) *ap1-1* first-whorl organ tissue.
Bar = 15 μm.

Rarely, we observe mosaics of leaf-like and petal-like tissue, as illustrated in Figure 4. In all cases, the sectors of leaf, stamen, or petal tissue are large, contiguous patches running longitudinally from the base to the apex of the organ. Although the sector boundary is not always straight, we see a relatively discrete transition between cell types, with only one or a few cells of intermediate phenotype spanning the boundary (Figure 4C).

Table 1 shows that the development of first-whorl organs as bract-like structures with associated axillary flower buds is most consistent and complete in the more basal flowers of the inflorescence. Figures 5A and 5B illustrate that the first-whorl organs on more apical flowers can be aborted or can develop into small structures that have some of the epidermal features of cauline leaves. Nonetheless, secondary flowers can develop in the axils of aborted organs. The most common pattern of development of the more apical flowers is for one or both of the lateral first-whorl organs and their associated axillary buds to develop, whereas abaxial and adaxial first-whorl organs and their associated axillary buds tend to abort (Table 1; Figure 5C). Whereas the extent of axillary bud formation varies with the position of the primary flower on the inflorescence, the petal phenotype is constant throughout the plant. That is, we never see petals formed, and the occurrence of the rare mosaic organs containing petal tissue appears to be random with respect to the position of the flower along the inflorescence.

Development of Wild-Type and *ap1* Mutant Flowers

The ontogeny of wild-type flowers has been described both morphologically and histologically (Vaughan, 1955; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989). We will briefly summarize the stages of wild-type floral development. Figure 6A illustrates how wild-type flower primordia arise in a helical sequence as outgrowths of cells on the flank of the apical meristem. In the young flower primordium, the abaxial sepal is initiated first, quickly followed by the development of a ridge of cells destined to become the adaxial sepal. The lateral sepals are then initiated and begin to grow out; the whorls of petal and stamen primordia are also apparent at this stage (Figure 6B). The sepals grow to enclose the developing inner primordia and begin to differentiate the elongated cells and stomata characteristic of the mature sepal epidermis. Petal development is retarded while stamen primordia continue to grow. At this time, the remaining tissue of the meristem elongates and develops a cleft in the center (Figure 6C). This central tube will give rise to the pistil. As the stamens grow, filament and anther primordia differentiate; anthers develop locules prior to the expansion of the filaments (Figure 6D). By this stage, petal primordia begin to elongate, and the beginning of differentiation of the stigmatic papillae on the pistil becomes

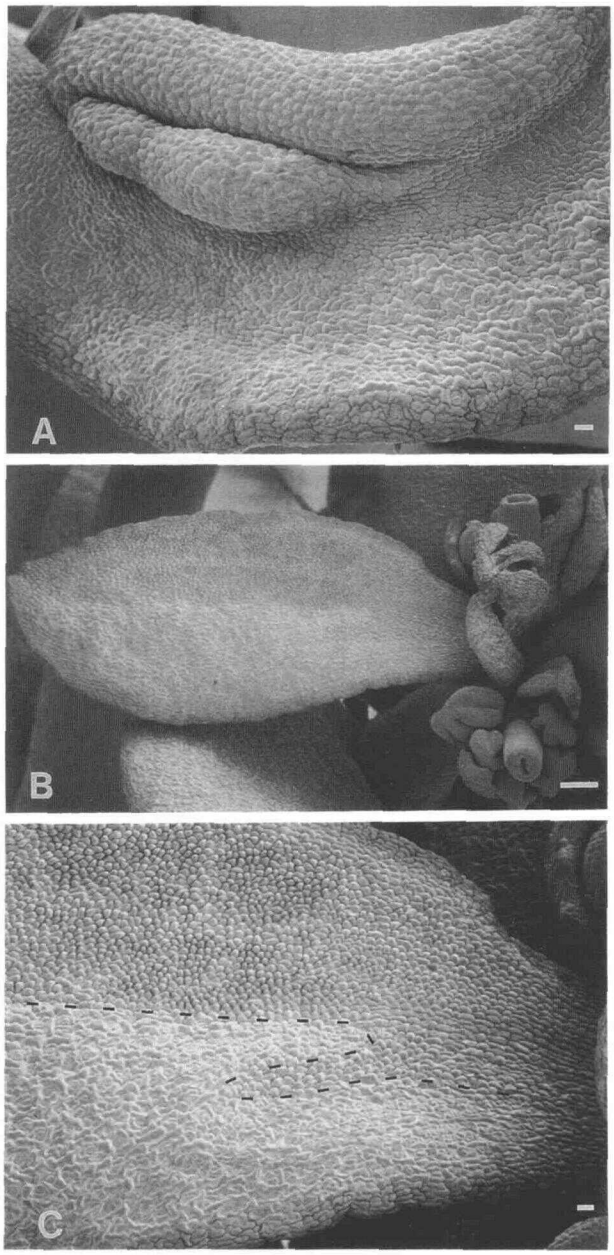


Figure 4. *ap1-1* Mosaic Organs.

(A) Mosaic of stamen and bract tissue. Locules and cellular morphology characteristic of stamens is toward the top; bract-like epidermal cells appear in the lower part of this mosaic organ. Tip of the organ is to the right. Bar = 15 μm.

(B) Mosaic of bract and petal tissue. Epidermal cells with characteristic petal cell morphology occupy approximately the top half of this organ. Tip of the organ is to the left. Bar = 75 μm.

(C) Closeup of organ pictured in **(B)**. The mosaic boundary is indicated with a dotted line. Bar = 10 μm.

apparent. At this point, nectaries at the base of the stamens also develop. The petals then rapidly expand to reach the tip of the pistil. Finally, the filaments of the stamens elongate as the pollen matures.

The apical meristem of *ap1-1* plants appears similar to the wild type in size and shape (Figure 6E). However, deviations become apparent as soon as individual *ap1-1* flower primordia begin to develop. First-whorl organs appear in an order similar to that of wild-type sepals but do not grow to enclose the developing bud. These primordia are more rounded and grow away from the central floral meristem (Figure 6F). As these first-whorl organs mature, stipules are often apparent at the base (Figure 6G). Stipules are characteristic of leaves and normally do not appear on sepals. Primordia do not appear in the second whorl; it appears that the cells that reside in the spaces alternate with the first-whorl primordia are eventually recruited into the growing bract-like first-whorl organs. Stamen and carpel primordia develop normally. Small outgrowths that appear to be nectaries are variably present at the base of the stamens. As the stamen primordia grow, development of buds in the axils of the first-whorl organs becomes apparent. These buds develop in a manner similar to that of the primary flowers, but because they are initiated later, they appear younger than the primary flower (Figure 6H).

Double Mutant Studies

We have generated plants that are homozygous for both *ap1-1* and other recessive mutations affecting floral development. These mutations either affect carpel number or result in homeotic transformations of different floral organs. Our observations are based on examination of the basal flowers formed on the inflorescence of each double mutant combination because the transformation elicited by the *ap1-1* mutation is most complete in these flowers. We find

Table 1. Number of First-Whorl Organs and Axillary Buds that Develop in *apetala 1* Mutant Flowers[a]

	Abaxial	Adaxial	Lateral
First-whorl organ development			
Basal flowers[b] (n = 22)	17	6	25
Apical flowers[c] (n = 12)	6	0	12
First-whorl axillary bud development			
Basal flowers[b] (n = 22)	20	19	41
Apical flowers[c] (n = 12)	2	0	15

[a] The development and location (abaxial, adaxial, and the two lateral positions) of bract-like first-whorl organs and axillary buds were scored in both basal and apical flowers.

[b] Flowers 1 to 4 on the main inflorescence.

[c] Flowers 10 to 20 on the main inflorescence.

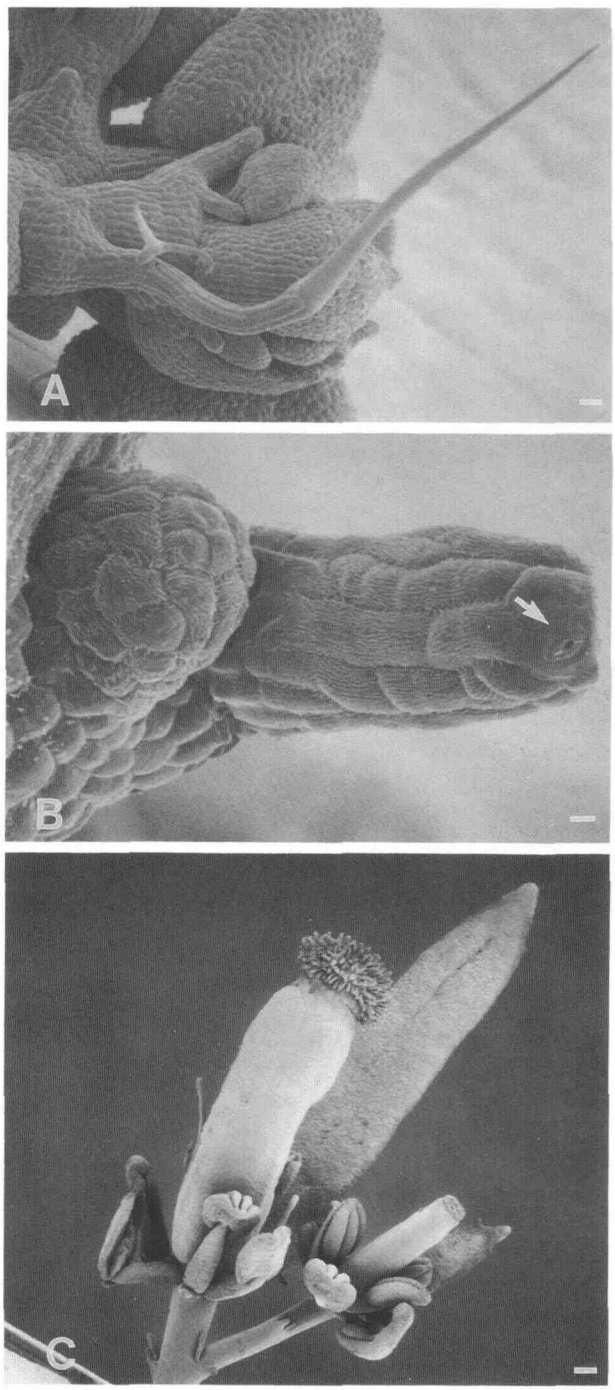


Figure 5. *ap1-1* Aborted and Irregular Organs.

(A) Reduced first-whorl organ that has differentiated a long, hair-like structure at its tip. Bar = 15 μm.
(B) Aborted first-whorl organ with an undeveloped axillary bud. Arrow indicates a mature stomate. Bar = 2 μm.
(C) Flower from the more apical part of an *ap1-1* inflorescence. Only one secondary flower has developed. Bar = 100 μm.

both epistatic and additive interactions, and in the case of *ap1 ap2* double mutants, we see a synergistic effect.

ap1 clv1

Mutations at the *clavata 1* locus (*CLV1*; Koornneef et al., 1983; Okada et al., 1989) alter the carpel number from two to four, resulting in a fat, club-shaped pistil, as shown in Figure 7A. We have combined *ap1-1* with the *clv1-1* allele, and the resulting flowers display a nearly additive phenotype. As in *ap1-1* alone, petals are missing and bracts with axillary flowers are formed, but both the primary and secondary flowers have four carpels instead of two (Figures 7B and 7C). However, the loss of the *CLV1* function appears to result in a greater tendency toward carpelloidy of organs in other whorls of the flower. For instance, mosaic carpelloid bracts are occasionally observed in secondary *ap1-1 clv1-1* flowers (Figure 7C). This type of mosaic organ has not been observed in plants homozygous for *ap1-1*.

ap1 pi and *ap1 ap3*

Figure 8 illustrates that mutations at both the *pistillata* (*PI*; Koornneef et al., 1983) and the *apetala 3* (*AP3*; Bowman et al., 1989) loci have similar phenotypes. Plants homozygous for *ap3-1* display a transformation of second-whorl organs into structures with the epidermal characteristics of sepals, whereas third-whorl organs differentiate as carpels that can fuse to the central pistil (Bowman et al., 1989). Homozygous *pi-1* flowers also have two outer alternate whorls of sepal-like organs surrounding a central enlarged pistil. The loss of stamens has been attributed to the lack of development of the third whorl (Bowman et al., 1989). Closer histological and developmental examination indicates, however, that primordia do arise in the region of the meristem that normally gives rise to the stamens but differentiate into carpelloid organs that fuse with the central pistil (Hill and Lord, 1989). Therefore, the phenotypic effects of the *ap3-1* or *pi-1* mutations appear to be nearly identical.

ap1-1 in combination with either *ap3-1* or *pi-1* results in flowers with very similar phenotypes (Figure 8). In the primary flower, four bracts with their associated axillary buds are formed, but organs derived from the second whorl do not appear. In both *ap1-1 ap3-1* and *ap1-1 pi-1* flowers, the pistil is enlarged and appears to result from the fusion of third- and fourth-whorl organs. This pattern of development is also seen in the secondary flowers of both double mutant combinations. In terms of the second-whorl organs, then, *ap1* appears to be epistatic to both *ap3* and *pi* mutations because second-whorl organs do not develop.

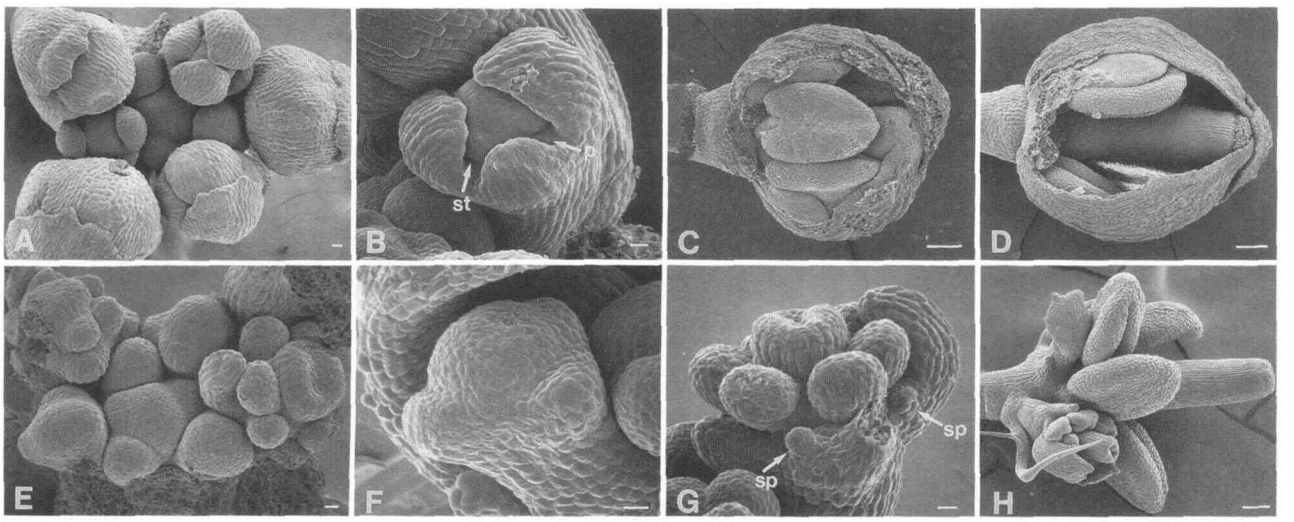


Figure 6. Development of Wild-Type and *ap1-1* Mutant Flowers.

(A) to **(D)** Wild-type development.
(E) to **(H)** *ap1-1* development.
(A) Top view of a wild-type inflorescence. Developing flower buds 1 through 9 can be seen. Bar = 10 μm.
(B) Young wild-type bud. Sepals are beginning to close over the floral meristem, and the stamen (st) and petal (p) primordia are visible as small outgrowths. Bar = 10 μm.
(C) and **(D)** Progressively later stages of development. Some sepal and petal tissue has been removed. Petals begin to elongate as the central gynoecium matures. Bar = 50 μm in **(C)** and 100 μm in **(D)**.
(E) Top view of an *ap1-1* inflorescence. Developing flower buds 1 through 6 and 8 and 9 can be seen. Bar = 10 μm.
(F) A young *ap1-1* bud of comparable age to the bud shown in **(B)**. Bar = 10 μm.
(G) A slightly later stage of development, where stamen and carpel primordia are apparent. First-whorl organs have stipules (sp). Bar = 10 μm.
(H) Axillary buds develop later than the primary flower but are apparent before the primary flower is mature. Bar = 50 μm.

ap1 ap2 and *ap1 ag*

Both the first- and the second-whorl organs are variably affected by mutations in the *apetala 2* (*AP2*) locus (Komaki et al., 1988; Bowman et al., 1989; Kunst et al., 1989). A number of *ap2* alleles with different phenotypic effects have been generated (Komaki et al., 1988; Bowman et al., 1989; Kunst et al., 1989). Not only do the phenotypes vary from allele to allele, but the *ap2* mutant phenotype varies acropetally within a single homozygous plant. For some alleles, environmental effects such as daylength or temperature affect the severity of the resulting mutant phenotype (Komaki et al., 1988; Bowman et al., 1989). We have used the well-characterized *ap2-1* allele (Bowman et al., 1989) to generate the double mutant combination with *ap1-1*.

Plants homozygous for *ap2-1* show an acropetal change in the mutant phenotype. More basal flowers display a transformation of first-whorl organs into leaf-like structures with stigmatic papillae at their tips. Figure 9A shows that the second-whorl organs develop as white stamenoid petals with infoldings that appear to be rudimentary locules. Stamens and carpels develop normally. The degree of first-whorl carpelloidy and second-whorl stamenoidy increases acropetally although the first whorl never shows a complete transformation to carpels.

Both organ initiation and organ identity are disrupted in *ap1-1 ap2-1* double mutants. These plants are normal in vegetative growth and upon bolting develop normal cauline leaves and associated axillary buds. However, floral meristems that would normally give rise to a flower differentiate in an indeterminate pattern characteristic of an inflorescence (Figures 9B, 9C, and 9D). Lateral structures arise off this transformed meristem in a helical phyllotaxy characteristic of *Arabidopsis* inflorescences, not in the cruciform pattern of an *Arabidopsis* flower. These lateral structures differentiate as a normal bicarpellate pistil associated with one or a few stamens, or more often as a fusion of several carpelloid structures that may or may not have associated stamens. Up to 30 lateral structures can differentiate on one transformed floral meristem. The most basal lateral structures of an *ap1-1 ap2-1* flower can themselves develop as an inflorescence, occasionally subtended by a leaf-like organ (Figure 9D). As the flower matures, inter-

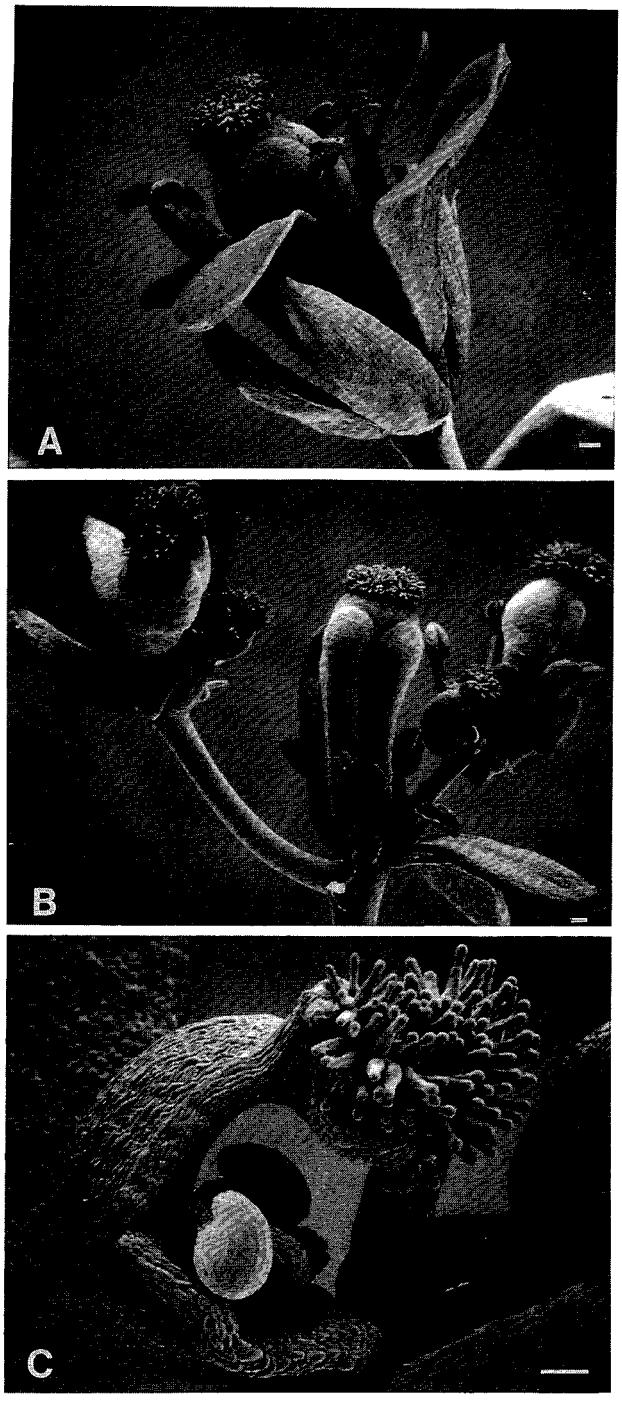


Figure 7. *clv1-1* and *ap1-1 clv1-1* Floral Phenotypes.

(A) *clv1-1* flower.
(B) *ap1-1 clv1-1* flower.
(C) Mosaic *ap1-1 clv1-1* organ composed of bract-like and carpel-like tissue. Stigmatic tissue at the tip of the organ and ovules at the margin are apparent.
Bar = 100 μm.

nodes elongate between lateral structures in a pattern characteristic of an inflorescence. Because an *ap1-1 ap2-1* floral meristem develops as an indeterminate inflorescence when neither *ap1* nor *ap2* flowers alone have a similar indeterminate phenotype, these two mutations interact synergistically.

Mutations at the *agamous* (*AG*) locus (Koornneef et al., 1983) cause a homeotic transformation of third-whorl organs as well as affecting the determinate nature of floral development (Bowman et al., 1989). In homozygous *ag-1* flowers, sepals and petals develop normally, but the third whorl differentiates as six petals. Figure 10A shows that, instead of forming a terminal whorl of carpels, the *ag-1* mutant repeats the abnormal floral program of development so that four sepals develop followed by two more whorls of petals. This indeterminate pattern of development can be reiterated three or four times.

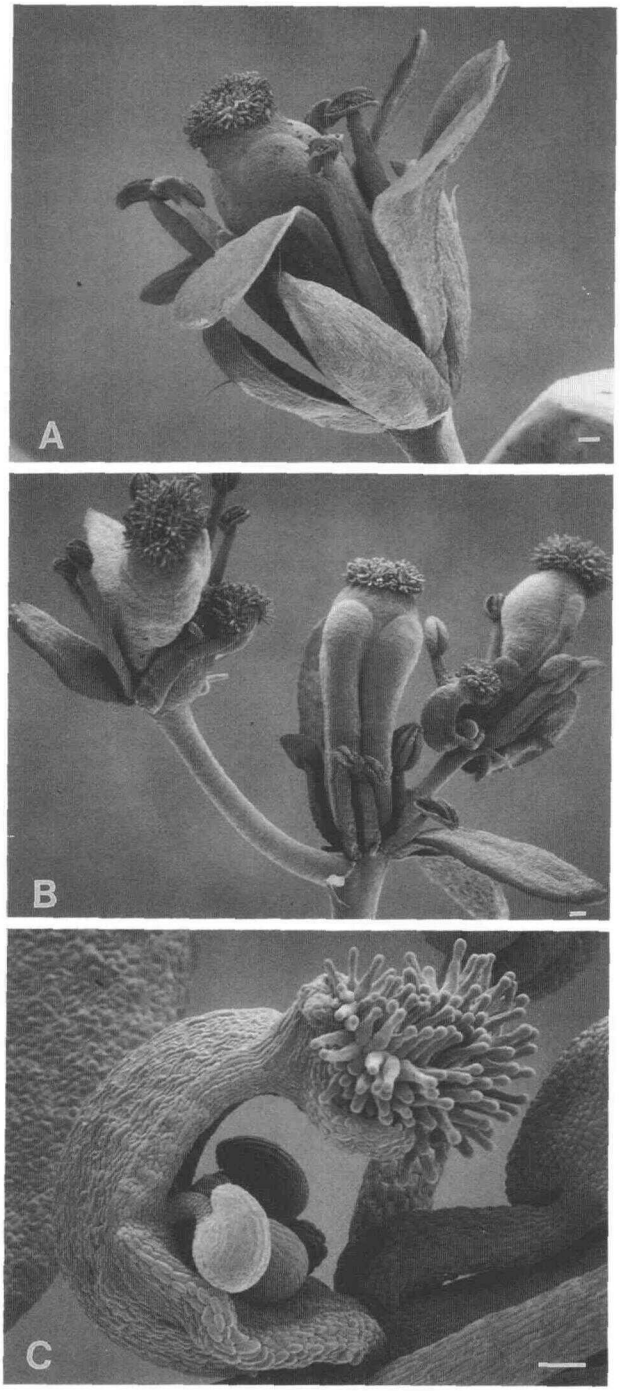
Figure 10B shows that plants doubly mutant for *ap1-1* and *ag-1* do not develop petals. Four bract-like first-whorl organs develop and have buds in their axils. Next, a variable number of green, leaf-like organs arise. The indeterminate nature of the *ag-1* phenotype is still apparent in the *ap1-1 ag-1* double mutants. The pattern of organ development described above reiterates with the appearance of another whorl of bracts with axillary buds, followed by a number of leaf-like organs. The buds formed in the axils of the *ap1-1 ag-1* bracts also repeat this indeterminate pattern of development.

DISCUSSION

We are studying the homeotic mutations of *Arabidopsis thaliana* in an effort to understand how the wild-type products of these genes interact to allow the cells of the meristem to realize their appropriate developmental fates. To this end, we have characterized the phenotype of the homeotic *ap1-1* mutation and of double mutant combinations between *ap1-1* and other mutations involved in floral development. These genetic analyses suggest roles for the *AP1* wild-type gene product in the establishment of both organ position and organ identity.

AP1 Is Required To Promote Sepal Differentiation and Suppress Axillary Bud Initiation

The first-whorl primordia in an *ap1-1* flower develop as bracts, as characterized by their leaf-like epidermal morphology, the presence of stipules, and the formation of buds in their axils. In *ap1-1* flowers, as well as in all the double mutant combinations generated, the axillary buds develop the same phenotype as that of the primary flower. This observation indicates that a new floral meristem is



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AP1 Is Required To Promote Sepal Differentiation and Suppress Axillary Bud Initiation

The first-whorl primordia in an *ap1-1* flower develop as bracts, as characterized by their leaf-like epidermal morphology, the presence of stipules, and the formation of buds in their axils. In *ap1-1* flowers, as well as in all the double mutant combinations generated, the axillary buds develop the same phenotype as that of the primary flower. This observation indicates that a new floral meristem is

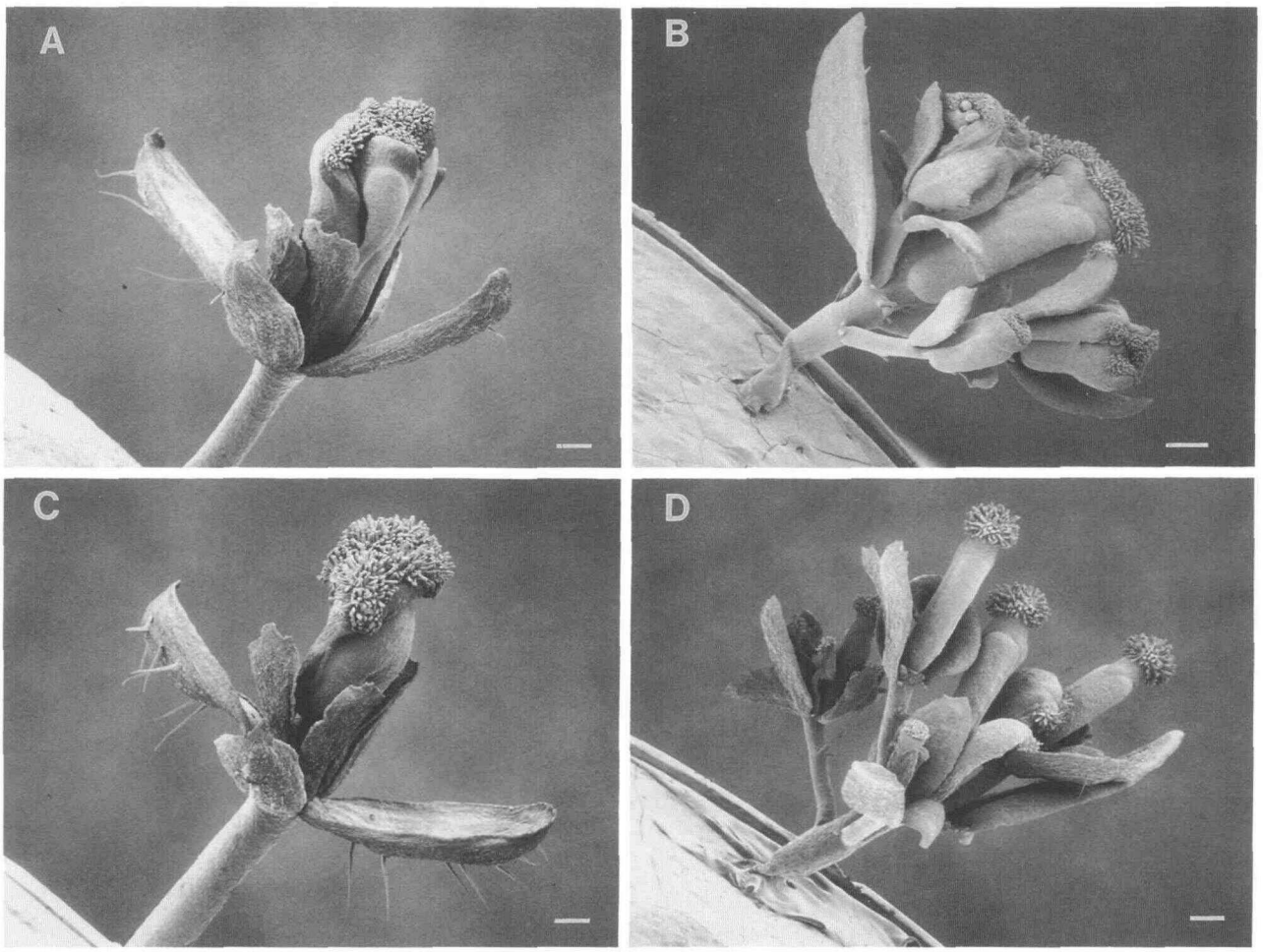

Figure 8. *pi-1*, *ap3-1*, *ap1-1 pi-1*, and *ap1-1 ap3-1* Floral Phenotypes.

(A) *pi-1* flower.
(B) *ap1-1 pi-1* flower.
(C) *ap3-1* flower.
(D) *ap1-1 ap3-1* flower.
Bar = 200 μm.

created in the axil with all the attributes of the primary floral meristem. In other words, whatever interactions are required to define the location and differentiation of floral organ types in the primary meristem, these interactions are reestablished within the axillary meristem. The formation of axillary buds and the concomitant loss of petals are not the result of a homeotic conversion of petal primordia into buds because buds arise in the axils of the first-whorl organs and not in the position of second-whorl primordia. A similar mutation in which axillary flowers develop within the primary flower has been described in another crucifer, *Nasturtium officinale* (Arber, 1931). In this case, petals still develop, demonstrating that in *Nasturtium*, petal development and axillary bud formation are not exclusive processes.

AP1 Is Required for Second-Whorl Development

In homozygous *ap1-1* flowers petal primordia do not arise. Consequently, petal development and differentiation cannot take place. However, there may be a requirement for *AP1* activity for the development of petal tissue. In *ap1-1 ag-1* double mutant plants, phylloid organs arise in the

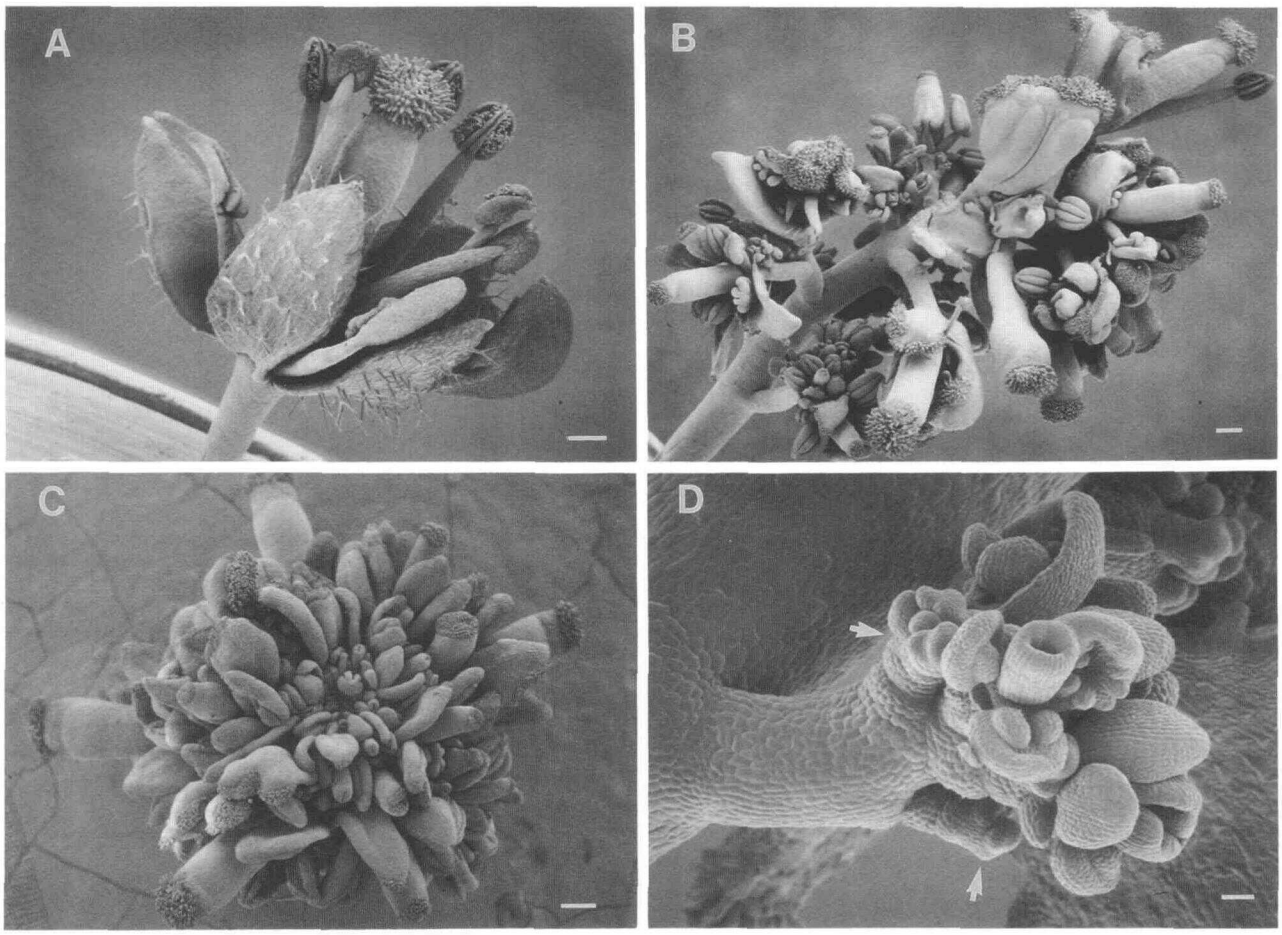

Figure 9. *ap2-1* and *ap1-1 ap2-1* Floral Phenotypes.

(A) *ap2-1* flower from the base of the inflorescence. First-whorl organs have leaf-like trichomes and stigmatic tips; second-whorl organs are white but have some stamenoid characteristics. Bar = 200 μm.
(B) *ap1-1 ap2-1* flower from the side with many lateral structures. Bar = 200 μm.
(C) Top view of an *ap1-1 ap2-1* flower showing the helical phyllotaxy. Bar = 200 μm.
(D) Basal lateral structure with an inflorescence-like phenotype on an *ap1-1 ap2-1* flower. Arrows indicate tertiary structures that also appear to have the morphological characteristics of inflorescences. Bar = 20 μm.

position normally occupied by petals in the *ag-1* mutant alone. These organs do not have any of the epidermal features associated with petals. Thus, it is possible that in addition to being required for second-whorl development, the *AP1* gene product is also required for the differentiation of petal tissue. We occasionally see petal-like epidermal cells in mosaic organs of *ap1-1* plants. These mosaic patches of petal tissue arise along the margins of the first-whorl organs, close to where second-whorl primordia normally arise. This observation suggests that the *ap1-1* mutation has not disrupted the processes required for petal cell differentiation and may only affect the formation of second-whorl primordia. One explanation for this observation is that the *ap1-1* allele may not be a null mutation, and, therefore, we could be seeing the phenotypic effects of residual activity from the mutant allele.

The failure of petal primordia to be initiated in an *ap1-1* mutant argues against any type of relay model in which the identity of each whorl of organs is determined by the preceding whorl (Heslop-Harrison, 1964; McHughen, 1980). Because stamens develop normally in the absence of petals, stamen development cannot be dependent upon petal development. Other models in which the formation of a whorl of organs is dependent upon the biophysical constraints induced by the primordia of the preceding whorl (Green, 1988) also do not seem to be valid.

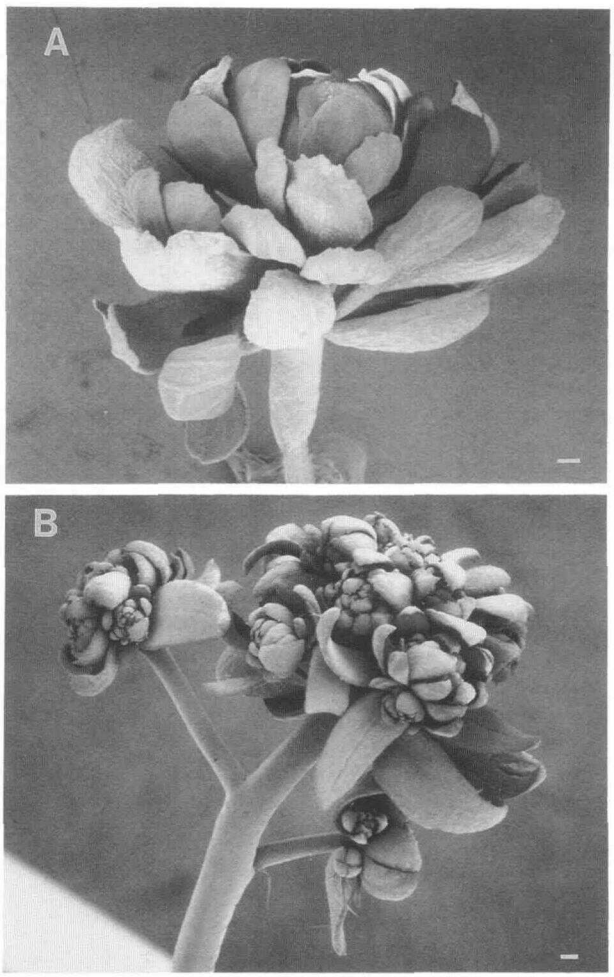

Figure 10. *ag-1* and *ap1-1 ag-1* Floral Phenotypes.

(A) *ag-1* flower.
(B) *ap1-1 ag-1* flower.
Bar = 100 μm.

The occasional development of petal epidermal tissue in large, contiguous mosaic patches suggests two possibilities for how cells in a developing organ assess their position and differentiate accordingly. A cell could acquire a specific fate during the initiation of the organ primordium and then in a heritable fashion pass this decision on to its progeny, resulting in a large clone of cells in the mature organ. A similar model has been proposed to explain the generation of mosaic patches in *ag-1* flowers (Bowman et al., 1989). Alternatively, cells could acquire a particular fate in a nonautonomous manner late in the development of an organ. Cells might normally read their position within a developing organ and then differentiate accordingly. In the case of mosaic organs, cells might read an unstable or threshold signal and then differentiate to form a mosaic pattern. The large size of the mosaic patches would indicate that even though cells are individually reading their position, nonautonomous cell-cell interactions could serve to enhance the acquisition of one cell fate or another. Indirect support for such a model comes from studies on *Impatiens* flowers, in which it appears that cells are not committed to a particular fate until just before their final differentiation (Battey and Lyndon, 1984).

Genetic Interactions of *ap1-1* and *ap2-1*

The *ap1-1* and *ap2-1* mutations interact synergistically, such that doubly mutant flowers develop as indeterminate inflorescences. This indeterminate phenotype may be caused by a partial redundancy of the *AP1* and *AP2* wild-type functions because loss of either gene product alone is insufficient to produce an indeterminate phenotype. We do not know what the *AP1* and *AP2* genes encode so we cannot determine the nature of this functional similarity. These genes may encode similar products, each of which is sufficient to induce determinate growth. Functional redundancy of similar gene products has been demonstrated in yeast, where mutations in different members of the *ras*-related gene family individually are viable, but double mutant combinations result in lethality (Tatchell et al., 1984). Alternatively, the *AP1* and *AP2* gene products may not themselves be homologous but may each participate in functionally similar biochemical pathways. We should emphasize that the products of the *AP1* and *AP2* loci are likely to have unique functions as well because the spectrum of phenotypes produced by mutations at each locus is distinct.

Our interpretation of the similarity of the *AP1* and *AP2* functions rests on our observation of *ap1-1 ap2-1* flowers. However, we do not know how much residual *AP1* or *AP2* activity remains in the double mutant combination. The *ap2-1* allele is only a partial loss of function mutation because more severe alleles exist (Komaki et al., 1988; Bowman et al., 1989). Other *AP2* alleles have been generated whose phenotypes bear some of the features of *ap1-1* mutant flowers. Under short-day conditions, plants homozygous for either *ap2-3* or *ap2-4* occasionally produce flowers in the axils of the leaf-like first-whorl organs (Komaki et al., 1988). This similarity in phenotype produced by mutations at two different loci supports our suggestion that the *AP1* and *AP2* gene products participate in a common developmental pathway.

Role of the Homeotic Genes in Floral Development

The phenotypes of the homeotic floral mutants indicate that the sequential formation of whorls of organs does not appear to depend upon interactions with either floral or-

gans or primordia in the preceding whorl. Instead, it appears that the position of a developing whorl is laid down independently of the formation of a particular organ type. The development of the floral pattern must, therefore, depend on two processes: first, position must be specified within the developing flower bud, and, second, cells must respond to this information and execute a specific genetic program leading to the differentiation of particular cell types. Models in which the formation of floral organs depends upon the establishment of concentric rings of organ-determining substances (Meyerowitz et al., 1989) or in which cells read their position relative to the meristem and not to other whorls (Holder, 1979) are both based upon this premise. However, it is not clear how this positional information is established.

The *AP1* and *AP2* gene products appear to act in concert to promote both determinate growth and cruciform phyllotaxy. The *AG* gene product is also required for determinate development because the *ag-1* mutation causes indeterminate growth of the flower. We propose that interactions between the *AP1* and *AP2* functions together with the *AG* gene product are involved in establishing a determinate floral field. The interactions observed in *ap1-1 ap3-1* and *ap1-1 pi-1* combinations suggest that the *AP1* function is required before the action of *AP3* and *PI*. Similarly, the *AP2* function has been postulated to act earlier than *AP3* and *PI* (Kunst et al., 1989; Meyerowitz et al., 1989). Based on this interpretation, we suggest that the *AP3* and *PI* genes may be activated in certain regions of the floral meristem in response to positional information established by the action of the *AP1*, *AP2*, and *AG* gene products.

Our model of homeotic gene action in *Arabidopsis* differs from that of others (Bowman et al., 1989; Kunst et al., 1989; Meyerowitz et al., 1989) in that we propose a specific role for the *AP1*, *AP2*, and *AG* gene products in establishing position within the developing flower. However, we cannot distinguish whether these gene products are required only for the establishment of position or whether they also play a role in the cellular interpretation of those signals. Other gene products such as *AP3* and *PI* appear to be involved only in the interpretation step and may encode cell-autonomous functions required for the perception or tranduction of positional signals.

METHODS

Plants were grown at 22°C to 24°C, under a 16-hr day/8-hr night regime, with a combination of fluorescent and incandescent light (175 μmol·m^{-2} · sec^{-1} at pot-top). Seeds were planted in a 9:3:1 vermiculite:soil:sand mix and watered daily. The mutant alleles described here are all in the Landsberg ecotype carrying the *erecta* mutation. Mutant alleles were a gift from Maarten Koornneef (Department of Genetics, Wageningen Agricultural University, The Netherlands). Crosses were performed manually with *ap1-1* plants as the pollen donor. Because *ag-1* plants are both male and female sterile, heterozygous *ag-1*/+ plants were used to construct the *ap1-1 ag-1* double mutant line. In all cases, the resulting F1 plants were allowed to self, and double mutant plants were isolated from the F2 population. Morphological characterization of both single and double mutant flowers was based on light microscopic examination of at least 20 flowers and scanning electron microscopy of between five and 10 flowers. For scanning electron microscopy, flowers or buds were fixed in FAA (3.7% formaldehyde, 50% ethanol, 5% acetic acid) for 30 minutes to 1 hr and dehydrated in a graded ethanol series. Dehydrated material was critical point dried in liquid CO_2. Individual flowers were mounted on scanning electron microscope stubs and dissected with glass needles. Specimens were sputter coated with gold-paladium in an SPI sputter coater. Specimens were examined in an ISI SS40 scanning electron microscope with an accelerating voltage of 5 kV.

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