AP2 Gene Determines the Identity of Perianth Organs in Flowers of Arabidopsis thaliana

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We have examined the floral morphology and ontogeny of three mutants of Arabidopsis thaliana, Ap2-5, Ap2-6, and Ap2-7, that exhibit homeotic changes of the perianth organs because of single recessive mutations in the AP2 gene. Homeotic conversions observed are: sepals to carpels in all three mutants, petals to stamens in Ap2-5, and petals to carpels in Ap2-6. Our analysis of these mutants suggests that the AP2 gene is required early in floral development to direct primordia of the first and second whorls to develop as perianth rather than as reproductive organs. In addition, our results support one of the two conflicting hypotheses concerning the structures of the calyx and the gynoecium in the Brassicaceae.

INTRODUCTION

According to the classical theory originally proposed by Goethe, the flower can be considered a shoot with a determinate growth pattern, compressed internodes, and specialized organs arranged in concentric whorls (Arber, 1950). Evidence from comparative morphology, ontogeny, and vascular anatomy suggests that floral organs are morphological equivalents, or homologs, of leaves (Arber, 1937). During development of the flower, the type, number, and position of the organs are strictly regulated, although as yet little is understood about the underlying molecular mechanisms.

In an attempt to determine the number and function of genes involved in these developmental processes, we have identified a large number of Arabidopsis thaliana mutants with altered floral morphologies (Haughn and Somerville, 1988). A subset of these mutants is particularly interesting because their homeotic phenotype (one floral organ type is replaced by a homologous type; Bateson, 1894) suggests that they carry mutations in genes required during development to specify the identity of floral organs. Furthermore, because such homeotic mutants are not unique to species of the Brassicaceae (e.g., Masters, 1868), it is likely that an analysis of this aspect of floral development in Arabidopsis will also contribute to our understanding of floral organogenesis in other flowering plants.

Initially, we chose to study three morphologically distinct and independently isolated mutants, Flo2, Flo3, and Flo4, each of which has homeotic transformations of the perianth (calyx and corolla), but not reproductive organs (androecium and gynoecium). Our preliminary genetic anal-

yses demonstrated that all three mutant phenotypes are due to mutations at the AP2 locus (Haughn and Somerville, 1988; Komaki et al., 1988; Bowman, Smyth, and Meyerowitz, 1989). We have investigated in detail the phenotypic variation within the AP2 allelic series to try to elucidate the role of the AP2 gene product in flower development.

RESULTS

Three independent mutant lines of A. thaliana, Flo2, Flo3, and Flo4 (Haughn and Somerville, 1988), with distinct abnormal floral morphologies were selected for analysis because all three appeared to have homeotic transformations of the perianth organs. Each mutant phenotype segregated as a single recessive nuclear mutation in genetic crosses (data not shown). Two lines of evidence indicated that flo2, flo3, and flo4 are allelic to ap2-1, another mutation that causes homeotic changes of the perianth (Haughn and Somerville, 1988; Komaki et al., 1988; Bowman et al., 1989). First, pairwise reciprocal crosses between the four mutants always yielded F_1 progeny with mutant floral morphology. Second, the flo2 mutation was found to map to the AP2 locus on chromosome 4 (data not shown). For these reasons we designate the new mutations ap2-5 (flo2), ap2-6 (flo3), and ap2-7 (flo4) to distinguish them from several alleles described previously (ap2-1, Koornneef, de Bruine, and Goetsch, 1980; Koornneef et al., 1983; ap2-2, Bowman et al., 1989; ap2-3, ap2-4, Komaki et al., 1988).

To determine the extent of the phenotypic variation, we

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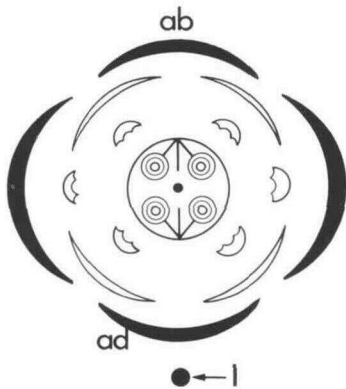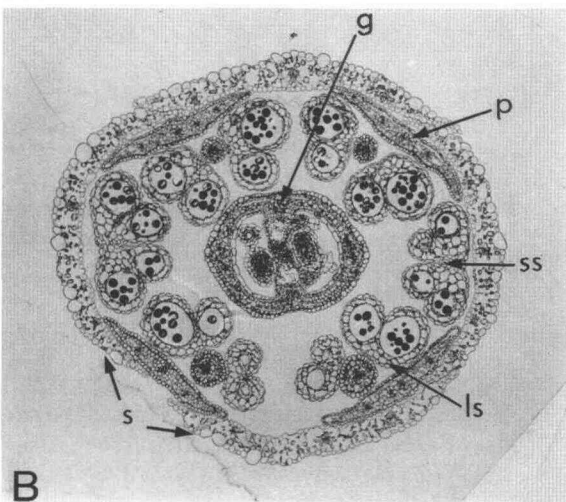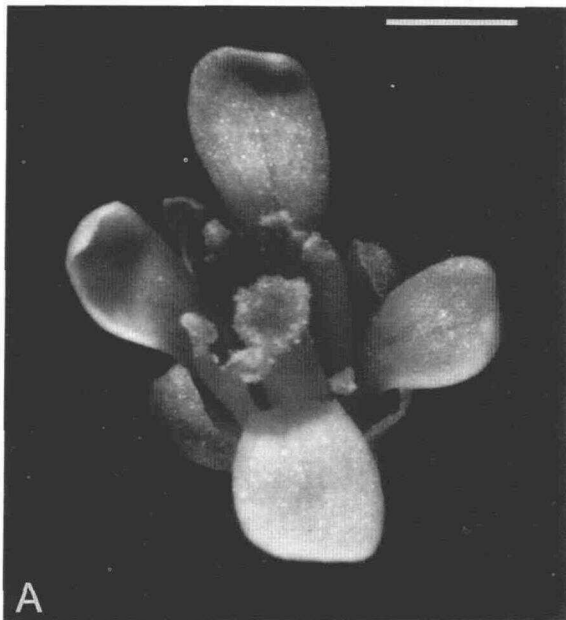
examined the floral morphology of wild type and each of the three mutants.

Flower Morphology

Wild Type

Figure 1 shows a mature flower of *A. thaliana* (Haughn and Somerville, 1988; Komaki et al., 1988; Bowman et al., 1989; Hill and Lord, 1989) that shares common characteristics with flowers of other members of the family *Brassicaceae* (Jones, 1939; Vaughan, 1955; Muller, 1961; Polowick and Sawhney, 1986). It is regular, hypogynous, and consists of five concentric whorls of organs. The four sepals of the calyx are usually considered to constitute a single whorl (whorl 1), although it has also been proposed that the calyx consists of two whorls of two sepals each (Jones, 1939; Lawrence, 1951). Inside and alternate to the sepals is a single whorl of four petals (whorl 2), followed by two whorls of stamens: an outer whorl, containing two short stamens (whorl 3), and an inner whorl containing four long stamens (whorl 4). The gynoecium (whorl 5), in the center of the flower, has a two-locule ovary, each locule of which contains two vertical rows of ovules growing from the placental tissue at the base of the septum.

Floral organs of wild-type flowers are distinct and easily recognized based on their position and appearance. Figure 2 illustrates the unique surface features, shapes, and sizes of cells comprising each organ that, in addition to morphology, can be used for unambiguous identification of organ types.

Ap2 Mutants

Figures 3, 4, and 5 show typical floral phenotypes associated with *ap2-5*, *ap2-6*, and *ap2-7* alleles, respectively, and Table 1 summarizes the major features of these phenotypes. It should be pointed out that there are substantial morphological variations among flowers even in a single inflorescence. In the Ap2-5 and Ap2-7 inflorescences, there is a gradual acropetal change in floral morphology. Early Ap2-5 flowers are almost wild type in morphology, late Ap2-5 flowers resemble early Ap2-7 flowers, and late Ap2-7 flowers are similar to some Ap2-6 flowers. The simplest explanation for the cause of such a pheno-

Figure 1. Morphology of *A. thaliana* Flower.

(A) Mature wild-type flower.
(B) Cross-section of wild-type flower. Four petals (p) are in positions alternate to four sepals (s). Short stamens (ss), long stamens (ls), gynoecium (g).
(C) Diagram of wild-type flower. Inflorescence axis (l), adaxial sepal (ad), abaxial sepal (ab).
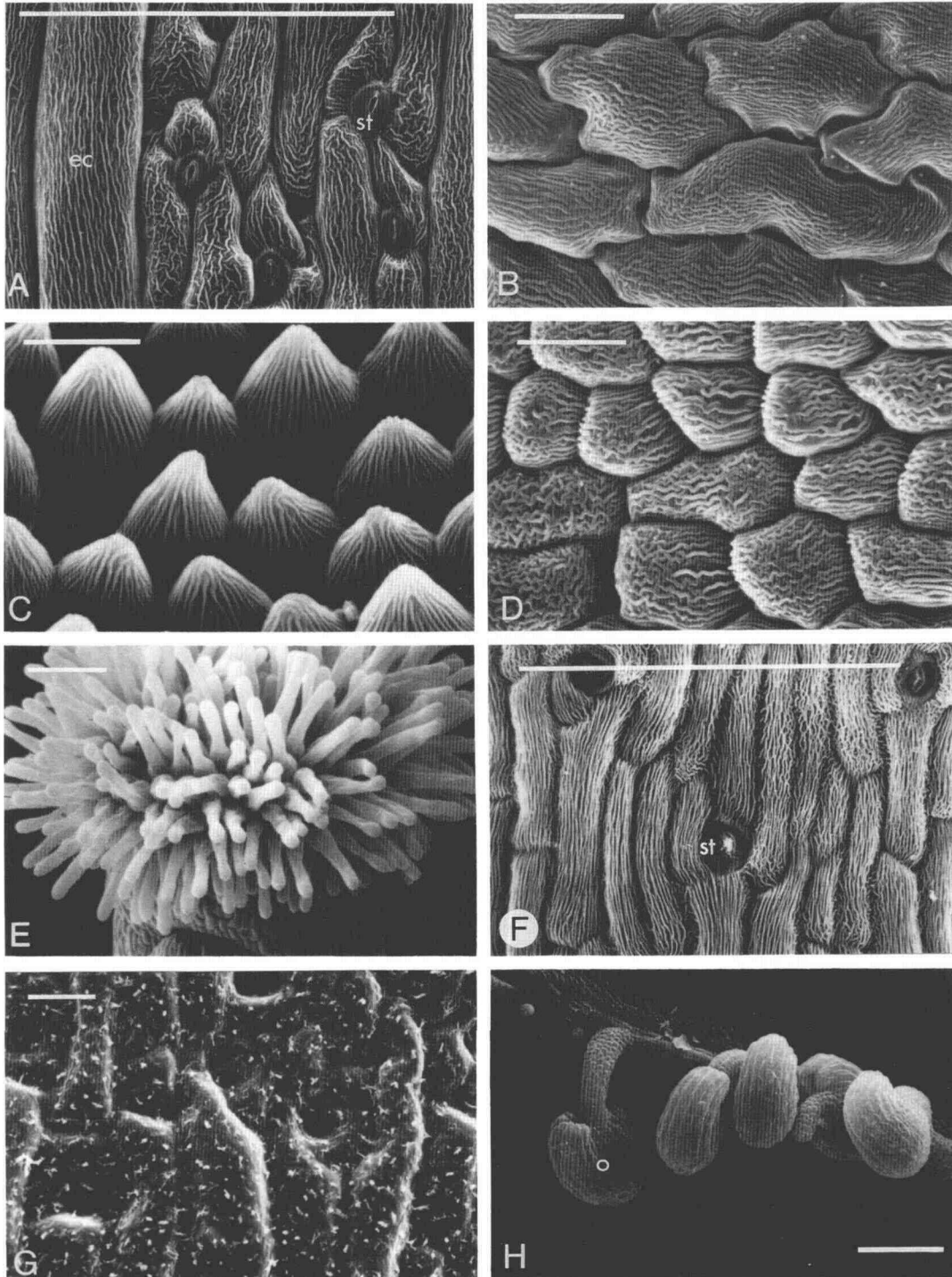
Figure 2. Scanning Electron Micrographs of Epidermal Cells Associated with Various Floral Organs.

(A) Adaxial view of a mature sepal. Note the elongated cells (ec), numerous stomata (st), and well-defined cuticular thickenings.
(B) Surface cells of a mature anther.
(C) Conical epidermal cells from the adaxial surface of a mature petal with cuticular thickenings radiating from the apex toward the base.
(D) Slightly elongated cells from the base of the adaxial surface of a petal.
(E) Capitate stigma with numerous papillae.
(F) Epidermal cells from the stylar region of the pistil with stomata (st) scattered throughout.
(G) Irregularly shaped cells of the ovary.
(H) Ovules (o) attached to the placental tissue.
Bar = 0.1 mm in **(A)**, **(E)**, **(F)**, and **(H)**, and 10 μm in **(B)**, **(C)**, **(D)**, and **(G)**.

typic gradient is a progressive loss of residual *AP2* activity due to changes in physiological conditions within the inflorescence. This observation also implies that the relative level of *AP2* activity associated with each allele is: wild type > *ap2-5* > *ap2-7* > *ap2-6*.

Differences in the type and the extent of homeotic changes explain most of the variation in phenotype between mutants. The median organs of the first (sepal) whorl of all three mutants exhibit morphological and cell surface features of both the sepal and carpel (Figures 3A to 3E, 4A to 4E, 5A to 5D). Such organs can be considered a partial homeotic transformation of sepal to carpel and we will refer to them as sepal-carpels. Besides sepal-carpels, another type of carpelloid organ is present in the Ap2-6 perianth. It is usually fused to sepal-carpels and it consists of only carpel cells (Figures 4A, 4C, 4D, 4F). We will call these organs carpel-like. The fate of lateral sepals is even more variable. They are either wild type (Ap2-5 and early Ap2-7), transformed to carpels (late Ap2-5 and Ap2-7), or fail to develop (late Ap2-7 and Ap2-6). Similarly, the organization of the petal whorl differs in the three Ap2 mutants. In Ap2-5 it consists of petal-stamen intermediate organs, which range from completely normal petals to structurally wild-type stamens. Figure 6 shows the morphologies, and Figure 7 shows the cell-surface features of such organs. The petal whorl in the Ap2-7 mutant appears to be absent, suggesting that the organs do not develop (Figures 4A and 4B). The perianth of Ap2-6 flowers is complex, consisting of both sepal-carpels and carpel-like organs fused along their margins (Figures 5A and 5B). Therefore, the fate of organs in the petal whorl could only be determined through an analysis of floral development (see section on Flower Development).

Organs of the inner whorls (androecium and gynoecium) remain morphologically wild type (Ap2-5 flowers, most Ap2-6 flowers, and early Ap2-7 flowers; Figures 3A, 4A, 5A). The only exception to this was observed in late Ap2-7 flowers and some Ap2-6 flowers, which have gynoecia that are not completely fused (Figure 5B). Thus, the ability of the flower to produce normal stamens and carpels in the inner whorls does not appear to depend directly on the development of sepals and petals in the outer whorls. A reduction of stamen number is typical of Ap2-6 and Ap2-7 flowers, but may be due in part to fusions to carpel-like organs of the perianth (see below). In general, a split gynoecium and a decrease in stamen number are not characteristics associated only with alleles of the *AP2* locus. Rather, they have been described for a wide variety of floral mutants (Haughn and Somerville, 1988; Komaki et al., 1988, Bowman et al., 1989). Therefore, we consider them secondary pleiotropic effects caused by other disturbances in floral structure.

Of special interest is the nature of the "intermediate" organs observed in the perianth of the mutants: sepal-carpels (Ap2-5, Ap2-6, Ap2-7) and petal-stamens (Ap2-5). They are mosaics of cells normally found in organs of two different whorls. The cells in both organ types appear as distinct sectors (Figures 4D, 4E, 5F, 7A to 7D) and include epidermal as well as internal cell layers (Figure 5D; L. Kunst and G. W. Haughn, unpublished results). These data indicate that the identity of individual cells with respect to organ type can be independent of the fate of neighboring cells.

It should be emphasized that the sectors of different cell types are not randomly positioned with respect to each other within the mosaic organs. On the contrary, it is striking that only the cells along the margins of each organ undergo a homeotic conversion (Figures 3C to 3E, 4D), whereas the central portion of the organ remains wild type. Thus, in AP2 mutants, the position of a cell in the developing organ affects its fate with respect to organ type. It is possible that the partially defective *AP2* gene product is affected by physiological differences across the organ (e.g., pH, growth regulators, or other metabolites), causing the cells in each region to adopt different developmental fates.

Each cell also retains its wild-type position and orientation within the composite organ. For example, anther cells always develop in the distal parts of the petal-stamen intermediates, never at the base of the organ (Figures 6 and 7). Thus, mutations at the *AP2* locus do not affect positional information within an organ or the ability of the cell to detect such information. Similar observations have been made for intermediate organs of other homeotic mutants (Bowman et al., 1989).

A remarkable characteristic of the Ap2-6 and Ap2-7 mutants is the presence of fused perianth organs (Figures 4A to 4D, 4F, 5A, 5B, 5E, 5F). These multi-organ structures always include sepal-carpel or carpel-like organs initiated in the first or second whorl of Ap2-6 and Ap2-7 flowers. However, in Ap2-7 and occasionally Ap2-6 mutants, sepal-carpel or carpel-like organs can also be fused to stamen-like organs (Figures 4F, 5B to 5E) that develop from primordia in the second (petal), third, or fourth (stamen) whorls. Fusions between carpelloid organs of the perianth always occur along the placental tissue at the organ margins, sometimes giving rise to a partial septum. Thus, the fused sepal-carpel and carpel-like organs have many features in common with the wild-type pistil. It seems reasonable to suggest that a carpel (or carpelloid organ) has the ability to induce fusions between itself and adjacent organs, and in wild-type flowers this feature of carpel development is necessary for the fusion of the two carpels into a syncarpous gynoecium.

Flower Development

To determine the point at which the floral organogenesis in the mutants deviated from wild type, and to examine the fate of the missing organs from the calyx (Ap2-6, late Ap2-7), corolla (Ap2-6, Ap2-7), and the androecium, we
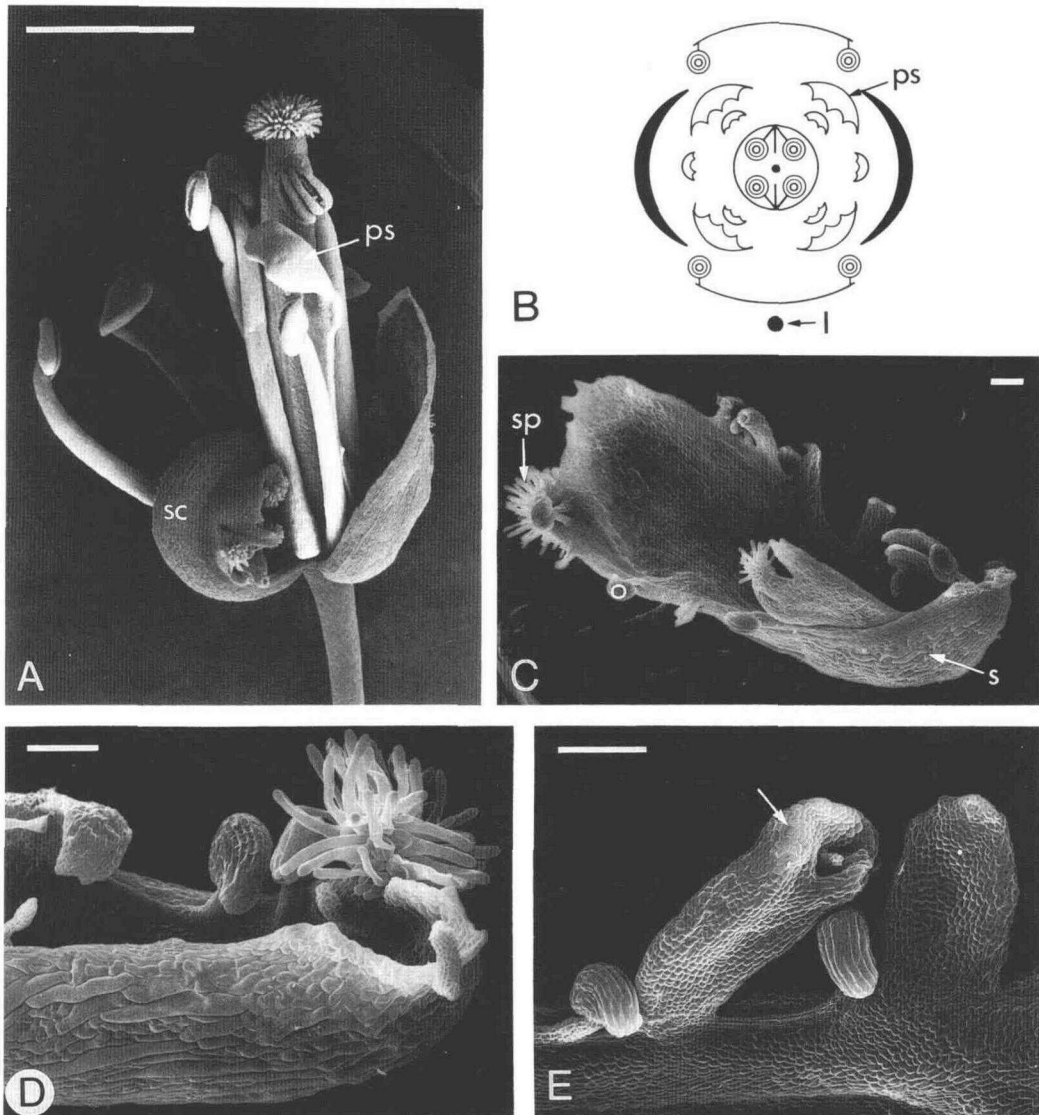
Figure 3. Morphology of Ap2-5 Flower.

(A) Scanning electron micrograph of Ap2-5 floral phenotype showing intermediate organs: sepal-carpel (sc) and petal-stamen (ps).
(B) Ap2-5 floral diagram. Inflorescence axis (l).
(C) Sepal-carpel organ with sepal tissue in the center (s), and placental tissue bearing ovules (o) and stigmatic papillae (sp) along the margins.
(D) An enlarged portion of a sepal-carpel organ showing large sepal-like cells, ovules, and papillae.
(E) A view of the placental tissue extending along the edge of a sepal-carpel organ with two ovules and projections that contain stylar cells (arrow).
Bar = 1 mm in **(A)** and 0.1 mm in **(C)**, **(D)**, and **(E)**.

studied the early ontogeny of all three Ap2 mutants. As a reference point it is necessary to describe in detail wild-type floral organogenesis.

Wild Type

Figure 8 shows the floral ontogeny of wild-type *Arabidopsis*. Organogenesis follows the typical acropetal sequence reported for the crucifer *Cheiranthus cheiri* (Sattler, 1973), with the exception of androecial whorls. The first primordium to appear on the floral apex is the abaxial sepal (Figures 8A and 8B), immediately followed by the adaxial sepal and the two lateral sepal primordia (Hill and Lord, 1989). The four small petal primordia are initiated next and arise apparently simultaneously in positions alternating with sepals (Figure 8C). The subsequent development of petal primordia is delayed until the later stages of flower
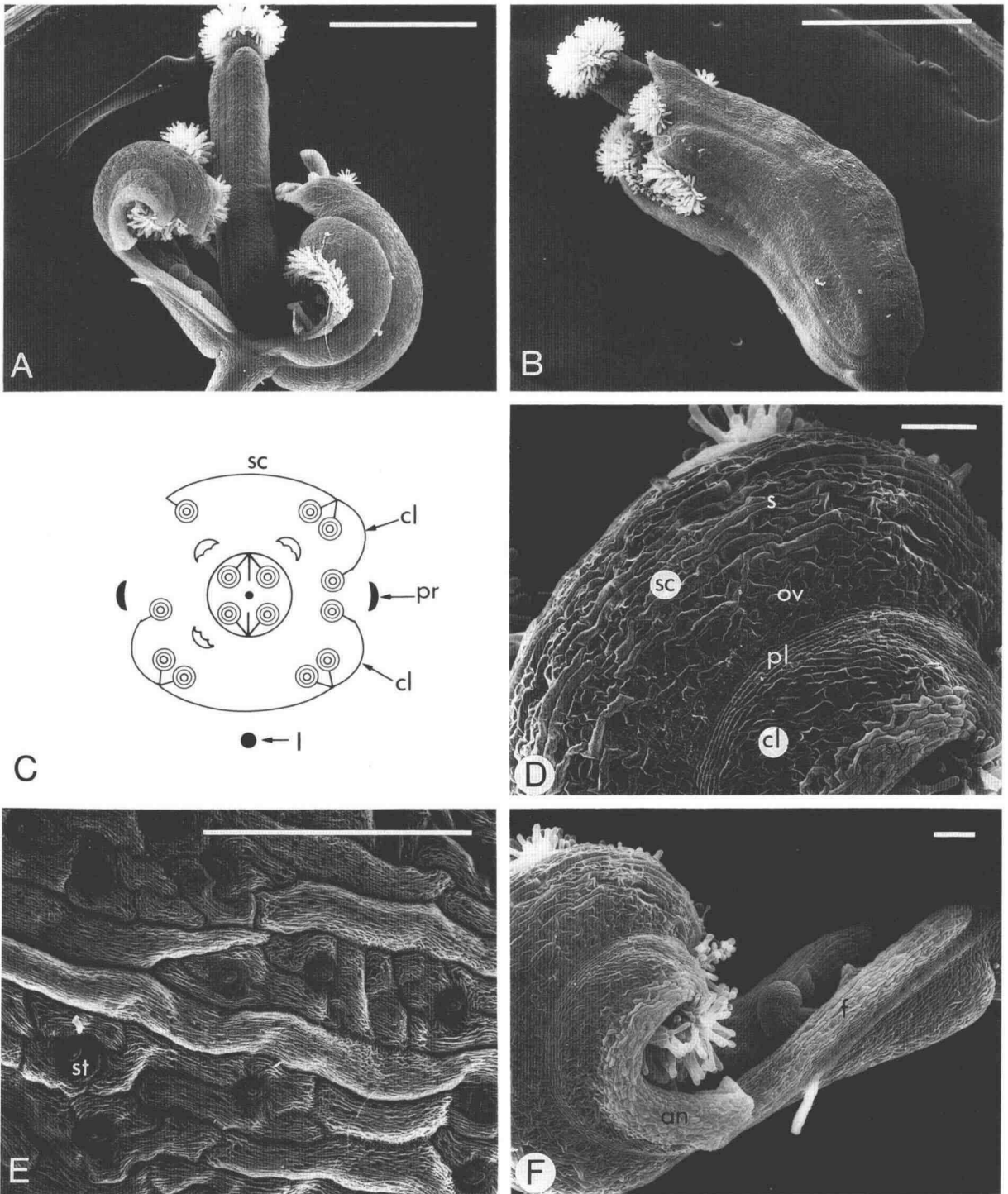
Figure 4. Morphology of Ap2-6 Flower.

(A) and **(B)** Scanning electron micrographs of Ap2-6 floral phenotypes. Note that the organs of the perianth are carpelloid. They are fused along the margins, but the degree of fusion is variable in different flowers.

(C) Ap2-6 floral diagram showing median sepal-carpel (sc) organs, lateral sepal primordia (pr), carpel-like (cl) organs of the petal whorl, and inflorescence axis (l).

(D) Higher magnification of fused sepal-carpel and carpel-like organ showing sectors with sepal epidermal morphology (s) and sectors of cells with the morphology of wild-type ovary (ov), placenta (pl), and style (sy).

(E) Enlargement of epidermal cells with sepal-like cuticular thickenings and stomata (st) from the center of the sepal-carpel organ.

(F) A sepal-carpel organ with a stamenoid structure fused to its margin. Anther-like cells (an), filament-like cells (f).

Bar = 1 mm in **(A)** and **(B)**, and 0.1 mm in **(D)**, **(E)**, and **(F)**.
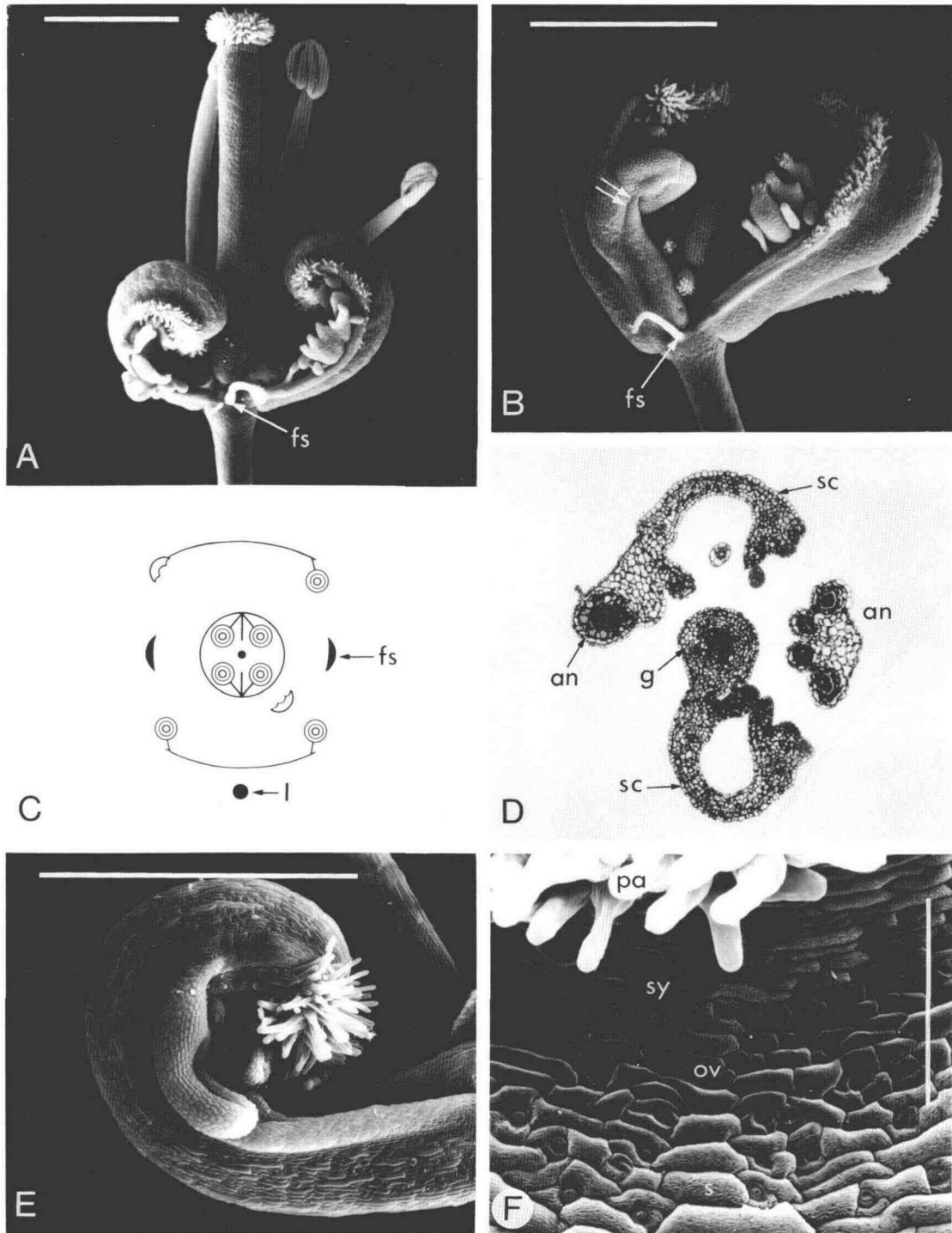
Figure 5. Morphology of Ap2-7 Flower.

(A) and **(B)** Scanning electron micrographs of Ap2-7 floral phenotype. Median sepals are carpelloid, lateral sepals are filamentous (fs), and the number of wild-type stamens is reduced to one or two. Stamen-like organs are frequently fused to carpelloid organs (double arrow). The pistil is occasionally split in the median plane **(B)**.

(C) Diagram of a typical Ap2-7 flower. Filamentous lateral sepal (fs), inflorescence axis (l).

(D) A cross-section of an Ap2-7 flower. Sepal-carpels (sc), in the position of median sepals, are fused to an anther (an) and to the gynoecium (g).

(E) Sepal-carpel organ with a morphologically perfect stamen fused to its side.

(F) Scanning electron micrograph of the epidermal cells of a sepal-carpel organ showing sectors of four different tissues: sepal (s), ovary (ov), style (sy), and stigma (pa).

Bar = 1 mm in **(A)**, **(B)**, and **(E)**, and 0.1 mm in **(F)**.

Table 1. Typical Floral Phenotypes of Ap2 Mutants

Mutant	Phenotype	Figure
Ap2-5	Homeotic transformation of median sepals to carpels	3A to 3D
	Lateral sepals wild type	3A, 3B
	Homeotic transformation of petals to stamens	3A, 6A to 6E, 7A to 7D
	Androecium and gynoecium wild type	3A, 3B
Ap2-6	Homeotic transformation of perianth organs to carpels	4, 10C
	Perianth organs fused to a different extent along the margins	4A, 4B
	Stamenoid structures fused to the perianth	4F
	Number of stamens in whorls three and four reduced to three or four	4C
	Gynoecium wild type	4A to 4C
Ap2-7	Perianth reduced to two to four organs	5A to 5C
	Homeotic transformation of median sepals to carpels	5A to 5C
	Lateral sepals reduced or absent	5A to 5C, 11A, 11C, 11D
	Organs of the second whorl absent	4A, 4B
	Stamens fused to the perianth	5A, 5E
	Number of stamens in whorls three and four reduced to one to three	5A, 5C
	Incomplete fusion of carpels of the gynoecium	5B

development (Figure 8D), when they expand rapidly. This lag between the initiation and the expansion of petal primordia is also characteristic of the petal whorl in other members of the *Brassicaceae* (Sattler, 1973; Polowick and Sawhney, 1986). After the initiation of petals, the four inner stamen primordia appear simultaneously (Figure 8C). The short outer stamens can be detected soon afterward in positions opposite the lateral sepals. Finally, the remainder of the floral apex develops into a gynoecium. This process involves the formation of a ridge of cells around the periphery of the apical meristem, which extends to form an open cylinder. As development proceeds, the cylinder closes, and the epidermal cells of the stigma start to differentiate into papillae (Figure 8D). The remaining cells of the gynoecium differentiate into the style and ovary.

Ap2 Mutants

Figures 9, 10, and 11 show the ontogeny of flowers of the mutants Ap2-5, Ap2-6, and Ap2-7, respectively. Despite the abnormal structure of the perianth of the mature mutant flowers, the initiation of sepal primordia follows the developmental course of the wild type, appearing in the

correct place at the right time (compare Figure 8A with Figures 9A, 10A, 11A, and 11B). Normal growth and development of floral organ primordia proceed only in the Ap2-5 mutant. However, the pattern of cell differentiation in its perianth organs is altered so that inappropriate cell types and abnormal structures appear (Figure 9B). On the other hand, in Ap2-6 and all but the earliest Ap2-7 flowers, lateral sepal primordia fail to elongate and can be identified as small filamentous outgrowths or projections (Figures 10B and 10C, 11A, 11C, 11D). The abaxial and adaxial (median) sepal primordia continue to grow and develop as sepal-carpel organs.

Petal primordia of all three mutants are morphologically normal and are initiated at the correct time and place. However, their subsequent expansion in Ap2-6 and Ap2-7 flowers is aberrant. Petal primordia fail to elongate in early Ap2-7 flowers (Figure 11C). In Ap2-6 and late Ap2-7 flowers petal primordia start growing immediately after initiation and may be fused to varying extents with each other and with the organs in the position of median sepals (Figures 10B and 10C, 11D). As growth of these organs continues, they gradually acquire carpel-like features. Thus, the Ap2-6 and occasionally the Ap2-7 mutants have carpels in the petal whorl. Stamen primordia are initiated in the correct time and place in the Ap2-5 mutant. Although normal stamen development can be observed in Ap2-6 and Ap2-7 flowers, the number of normal stamens is always less than six. The reduction in stamen number is due in part to the fusion of stamens to the side of the developing organs of the outer whorl (Figures 4A, 4F, 5B, 5E, 11D). The abnormality of the receptacle prevented us from determining whether other stamens were missing because of a defect in stamen primordia initiation or a failure in further elongation of correctly initiated organs.

DISCUSSION

We have extensively characterized three mutant lines of *A. thaliana*, Ap2-5, Ap2-6, and Ap2-7 (Haughn and Somerville, 1988), each of which has a distinct floral morphology due to a recessive mutation in the *AP2* gene. All three mutations affect primarily the structure of the perianth, changing the fate of perianth organs such that they develop morphological characteristics and surface features typically associated with reproductive organs.

In addition to the three alleles described in this paper, four other *AP2* alleles have been identified to date. The *ap2-1* allele, isolated by Koornneef et al. (1980), remains the most extensively studied (Haughn and Somerville, 1988; Komaki et al., 1988; Bowman et al., 1989), although its phenotype with respect to the sepal whorl is the least typical. In the early flowers of an *ap2-1* homozygote, the outermost whorl contains four morphologically identical organs that resemble leaves on the basis of shape, the timing of senescence, and the presence of stellate tri-
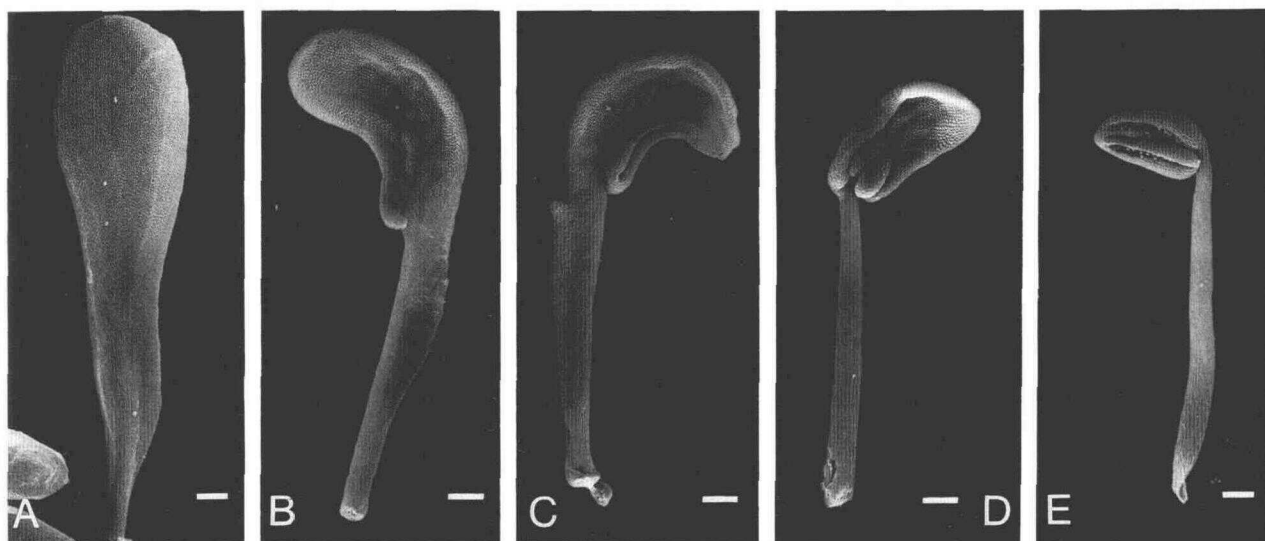
Figure 6. Scanning Electron Micrographs of a Series of Petal-Stamen Intermediate Organs in the Second Whorl of Ap2-5 Flowers.

(A) Wild-type petals occur only in early Ap2-5 flowers.
(B) to **(E)** In later flowers mature organs of the petal whorl are progressively more stamen-like, and have a higher percentage of anther-like cells.
Bar = 0.1 mm.

chomes (Haughn and Somerville, 1988; Komaki et al., 1988; Bowman et al., 1989). However, flowers produced later in the Ap2-1 inflorescence exhibit carpelloidy of the sepal whorl and staminoidy of the petal whorl, closely resembling flowers of the Ap2-5 mutant (Bowman et al., 1989; L. Kunst and G. W. Haughn, unpublished results). The uncharacterized *ap2-2* homozygotes (Bowman et al., 1989) and the partially characterized *ap2-3* and *ap2-4* homozygotes (Komaki et al., 1988) appear to be similar in morphology to Ap2-6 and Ap2-7 mutants. As discussed below, the analyses of the Ap2 mutant phenotypes yield valuable information toward understanding the role of the *AP2* gene product during flower development. In addition, our results provide evidence in support of one of the two conflicting hypotheses concerning the structure of the calyx and the gynoecium in flowers of the *Brassicaceae*.

Structure of the Calyx

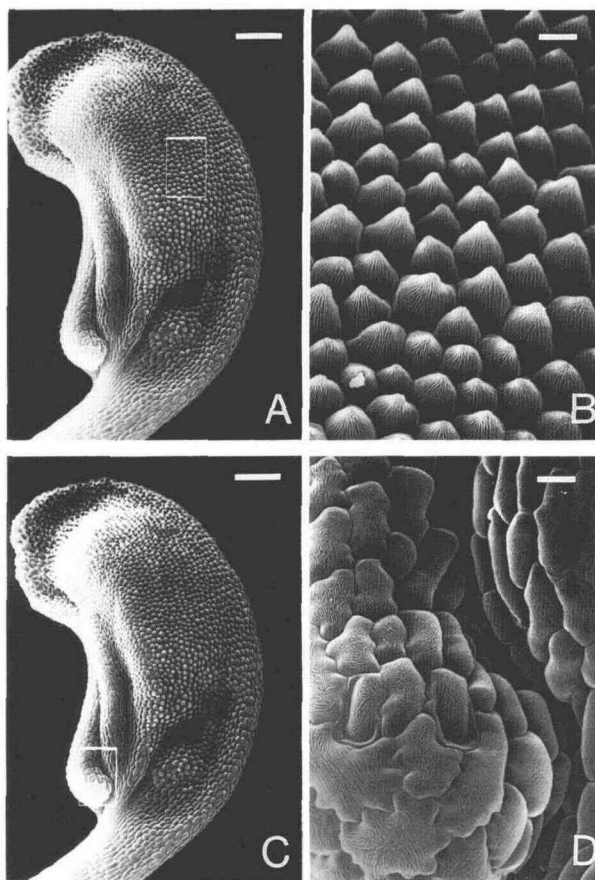One of the long-standing questions of classical morphology concerns the number of sepal whorls in crucifer flowers.

Figure 7. Petal-Stamen Intermediate Organs in Ap2-5 Flowers.

(A) and **(C)** Mature organs.
(B) Enlargement showing petal-like cells from the boxed area in **(A)**.
(D) Enlargement showing anther-like cells from the boxed area in **(C)**.
Bar = 0.1 mm in **(A)** and **(C)**, and 10 μm in **(B)** and **(D)**.
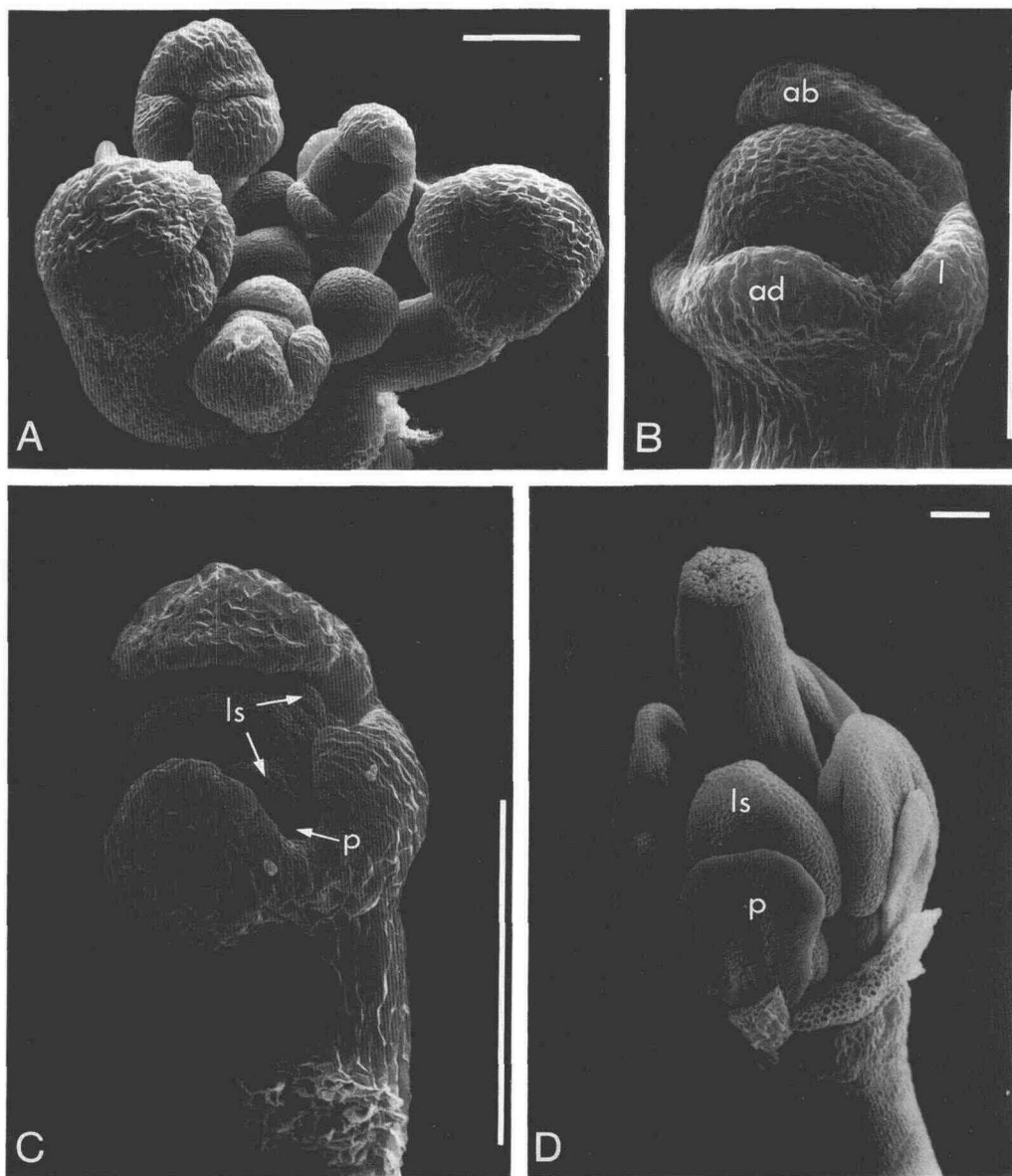
Figure 8. Early Ontogeny of the Wild-Type Flower.

(A) An immature inflorescence showing the helical pattern of initiation of flower buds.
(B) A young flower bud showing the initiation of sepal primordia. Adaxial (ad), abaxial (ab), and lateral (l) sepal primordia.
(C) A young flower bud showing petal primordia (p) and long stamen primordia (ls).
(D) Wild-type flower near maturity. The sepals have been removed. The stamens (ls) have almost reached their full size, whereas the petals (p) are still relatively small.
Bar = 0.1 mm.

On the basis of morphological studies, two hypotheses have been proposed. According to one hypothesis, all four sepals form a single whorl (Lawrence, 1951). Alternatively, it has been suggested that the calyx consists of two whorls of two sepals (Arber, 1931; Jones, 1939). In flowers of

each of the mutants described here, the developmental fates of the median and lateral organs of the sepal whorl are distinctly different. For example, in Ap2-6 flowers the median sepal primordia develop into sepal-carpel organs, whereas the lateral primordia fail to develop at all. Further-
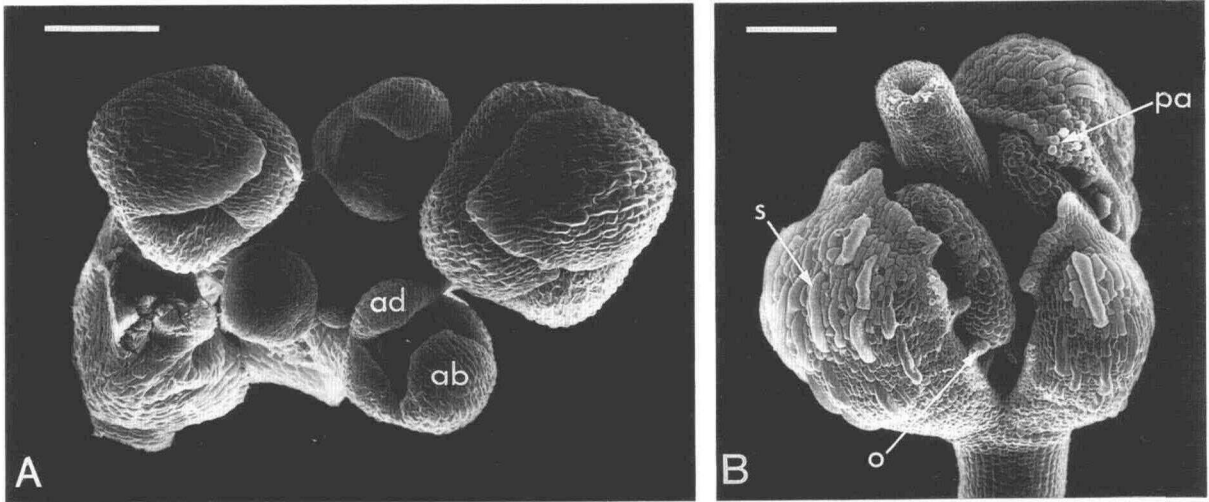
Figure 9. Early Ontogeny of Ap2-5 Flowers.

(A) An immature Ap2-5 inflorescence; adaxial sepal (ad), abaxial sepal (ab).
(B) A developing Ap2-5 flower bud. Sepal-like cells (s) and ovules (o) are visible on the adaxial sepal, whereas stigmatic papillae (pa) are developing on the abaxial sepal.
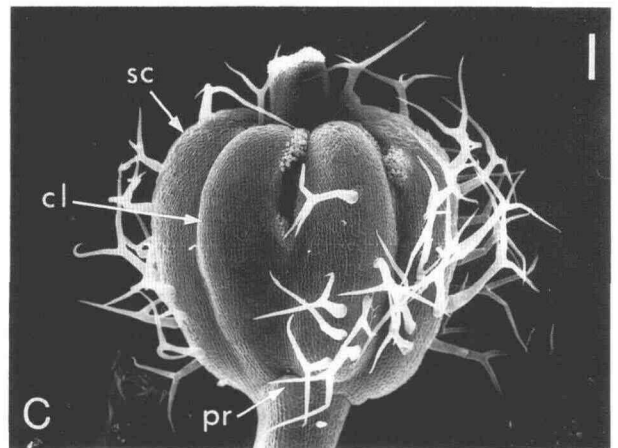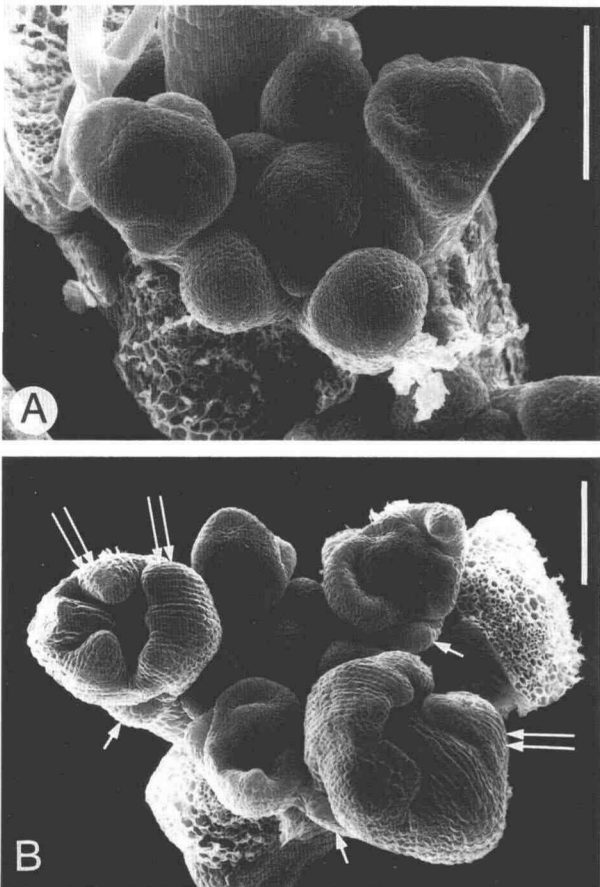Bar = 0.1 mm.

Figure 10. Early Ontogeny of Ap2-6 Flowers.

(A) An immature Ap2-6 inflorescence.
(B) Later stages of development of Ap2-6 flowers. Note small outgrowths in the perianth (arrow) in place of lateral sepals that failed to elongate. The organs in positions of petals (double arrows) are fused at the base or along their entire length with organs in the position of median sepals.
(C) A young flower bud showing lateral sepal primordium (pr) and carpel-like organs of the petal whorl (cl) fused to median sepal-carpels (sc).
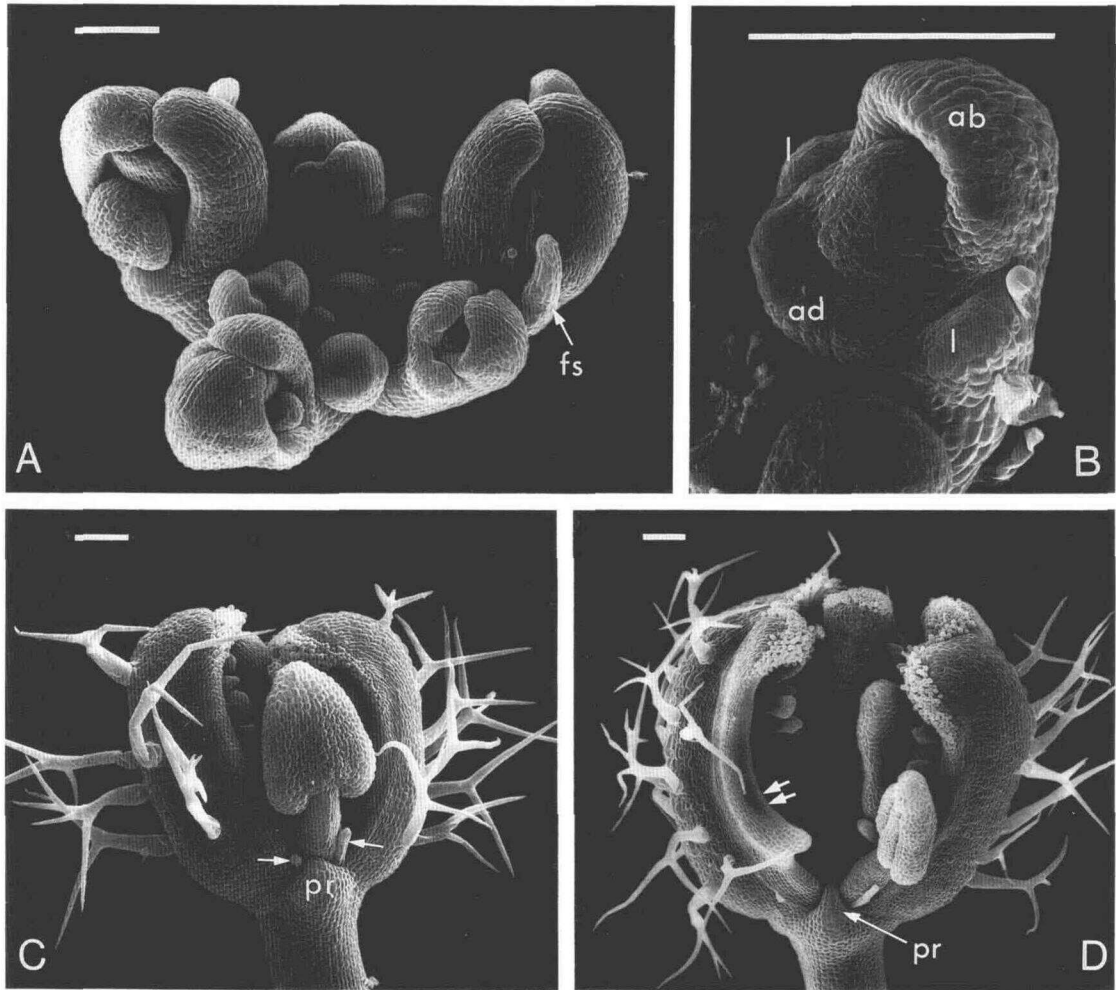Bar = 0.1 mm.

Figure 11. Early Ontogeny of Ap2-7 Flowers.

(A) An immature Ap2-7 inflorescence. Lateral sepal primordia elongate only in early Ap2-7 flowers, whereas late flowers have filamentous sepals (fs), or sepal primordia do not elongate after initiation.
(B) A developing flower bud showing normal initiation of sepal primordia. Adaxial (ad), abaxial (ab), and lateral (l) sepals.
(C) and (D) Lateral view of young flower buds showing sepal (pr) and petal (arrows) primordia that failed to develop to maturity and a developing stamen-like organ fused to carpelloid organs of the perianth (double arrow).
Bar = 0.1 mm.

more, in wild-type flowers, the two median sepals (abaxial and adaxial) differ from the lateral sepals in their time of initiation, size, shape, and position on the receptacle. These data suggest that, in the *A. thaliana* calyx, the median and lateral sepals are not equivalent, favoring the "two whorl" hypothesis.

Structure of the Gynoecium

Two major and conflicting hypotheses have been proposed with regard to gynoecial morphology in the *Brassicaceae*. Payer (1857) described the gynoecium as consisting of two lateral carpels fused in the median plane (Sattler, 1973). The fused margins of these carpels bear the placentae with one row of ovules each. His view is based on developmental analysis, and supported by work of Eichler (1865) and Eggers (1935). An alternate hypothesis, introduced by Saunders (1925, 1929) and Eames and Wilson (1928, 1930), claims that the crucifer gynoecium is made of four carpels: two sterile carpels forming the ovary and two fertile ones, consisting of placental tissue, one-half of the septum, and two rows of ovules.

Based on our analysis of Ap2 mutants, we favor the "two carpel" hypothesis for two reasons. First, the unfused
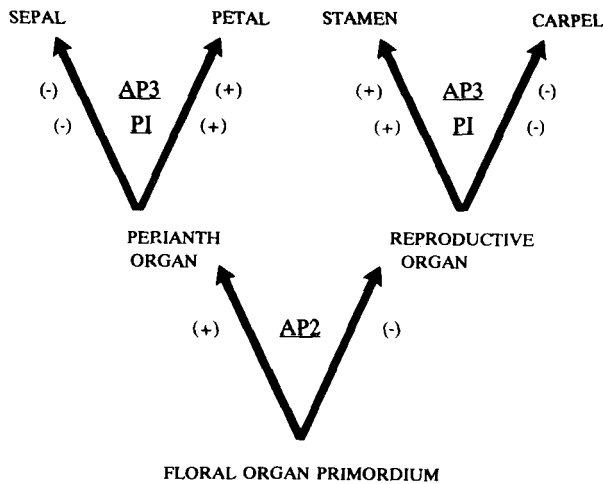
Figure 12. Determination of Floral Organ Identity in *A. thaliana*.

Arrows indicate a hypothetical, hierarchical progression of developmental decision-making during floral organogenesis. For each decision, expression (+) or inexpression (−) of the genes shown is required to determine which of two possible fates an organ will follow.

carpelloid organs that develop in the perianth from a single primordium have both placental and ovarian tissue with single rows of ovules along each margin. Such a carpel is predicted by the two carpel hypothesis but not by the "four-carpel" hypothesis. Second, when the gynoecia of Ap2-6 and Ap2-7 flowers are improperly fused, no more than two separate parts are observed, and the split is always in a median plane with a row of ovules on each unfused margin. The two carpel hypothesis predicts such a morphology if the two lateral carpels of the developing gynoecium fail to fuse.

The Role of the *AP2* Locus

Considering the phenotypes of all *AP2* alleles, one can conclude that the *AP2* gene product: (1) functions during floral development to determine the fate of the cells in organ primordia of the calyx and the corolla, (2) does not affect the time or position of primordium initiation, (3) is essential for the formation of mature perianth organs but not reproductive organs, (4) probably acts early in organogenesis, and (5) must suppress developmental pathways leading to the formation of reproductive organs in the first and second whorls. The sepal-carpels, carpel-like, and stamen-like organs in the perianth of the mutants indicate that organs of the perianth whorls have the capacity to develop as reproductive organs, but do not in the presence of wild-type *Ap2* activity. The fact that lateral sepals do not develop beyond a primordium stage suggests that the *AP2* gene product is needed at least this

early in development. Such a conclusion is also supported by the temperature-shift experiments of Bowman et al. (1989).

Figure 12 illustrates a model for the genetic regulation of organ type in *Arabidopsis* flowers. The wild-type *AP2* gene product could be viewed as a part of a developmental regulatory mechanism that directs primordial cells to commit to a perianth, rather than a reproductive developmental pathway. Additional genes would then be required to establish the exact identity of the perianth and reproductive organs (sepal versus petal and stamen versus carpel). Both *PI* (Haughn and Somerville, 1988; Bowman et al., 1989) and *AP3* (Bowman et al., 1989) may represent such genes. This simple model (Figure 12), which has elements in common with the ones described by Haughn and Somerville (1988) and Bowman et al. (1989), predicts that loss-of-function mutations in both *AP2* and *PI* genes would result in a flower consisting of only carpels. Indeed, preliminary analysis has shown that flowers of *pi,ap2-6* and *pi,ap2-7* double mutants do have such a phenotype (L. Kunst and G. W. Haughn, unpublished results). We hope to test this model further and to reach a more complete understanding of the role of the *AP2* locus through additional genetic and molecular characterization of these developmental mutants.

METHODS

Plant Material

The mutant lines described here were isolated from an ethyl methane sulfonate-mutagenized population of *Arabidopsis thaliana* Heynh, ecotype Columbia (Haughn and Somerville, 1986). Before being used for analyses reported here, the lines were back-crossed to the wild type four times (Ap2-5), or three times (Ap2-6 and Ap2-7), and individuals with the mutant phenotype were reselected from segregating populations. Lines MSU15 (*cer2, bp, ap2-1*) used for complementation analysis, MSU8 (*an, py, gl1, cer2, ms*) used for genetic mapping, and Apetala2-1 (*ap2-1*) used for morphological examination (all in Landsberg *erecta* background) were a gift from Maarten Koornneef (Department of Genetics, Wageningen Agricultural University, The Netherlands).

Plants were grown at 22°C under continuous fluorescent illumination supplemented with incandescent light (100µE to 150 µE m^{-2} s^{-1} PAR) on Terra-lite Redi-earth prepared by W. R. Grace & Co. Canada Ltd, Ajax, Ontario, Canada).

Genetic Mapping

Recombination frequencies were determined by analyzing F_2 progeny using the method of Suiter, Wendel, and Case (1983). All values were corrected for double cross-overs 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 and Scanning Electron Microscopy

Morphological characterization of mutant phenotypes was performed on at least 20 individual flowers using the dissecting microscope. Flowers were taken from various positions within the inflorescence at different stages of inflorescence development to determine the extent of structural variation associated with each mutation.

Flower buds or young inflorescences for thin sectioning and scanning electron microscopy (SEM) were vacuum-infiltrated with 3% glutaraldehyde in 0.02 M sodium phosphate buffer (pH 7.2) and fixed overnight at 4°C. The samples were then rinsed in buffer, post-fixed in 1% OsO_4 in the same buffer for 2 hr, and dehydrated on ice in a graded acetone series. At this point the material was divided into two groups and processed separately for either light microscopy or SEM. The specimens for light microscopy were gradually infiltrated with Spurr's epoxy resin (Spurr, 1969) and flat-embedded. Sections 1 μm to 2 μm thick were made with a Reichert ultramicrotome and stained with 1% toluidine blue in 1% borate. The flowers for SEM were critical point-dried in liquid carbon dioxide, mounted on stubs, and coated with gold in an Edwards S150B sputter coater before examination in a Phillips 505 scanning electron microscope at an accelerating voltage of 30 kV.

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