

Isolation and characterization of novel mutants of *Arabidopsis thaliana* defective in flower development

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Summary

We have isolated a number of mutants of *Arabidopsis thaliana*, a member of the mustard family, that have defects in flower development and morphogenesis. Of these, five mutants have been extensively characterized. Two mutants (Fl-40, Fl-48) lacking petals show homeotic conversion of sepals to carpels. One mutant (Fl-54) displays highly variable phenotypes, including several types of homeotic variations, loss or distorted positions of the floral organs as well as abnormal structures on the inflorescence. Two other mutants (Fl-82, Fl-89) show aberrant structures in the pistils.

Genetic analyses have revealed that these mutations are single and recessive, except for one mutant whose mutational loci still remain to be determined. These mutants may prove useful for the analysis of the genetic control of flower development and morphogenesis in the higher plant.

Key words: *Arabidopsis thaliana*, flower development and morphogenesis, flower mutants, homeotic mutation, deletion of floral organs, genetic analysis.

Introduction

The flower is a reproductive organ in angiosperms, consisting of gynoecium (pistil), androecium (stamen) and other floral organs (petal, sepal). These floral organs are arranged in whorls or concentric circles. Thus far, the developmental and morphogenetic processes of flowers have been studied mainly using organographic and embryological approaches and very little study has been undertaken genetically. The difficulties encountered in the genetic approach for studying development and morphogenesis in higher plants are primarily due to their long life cycles, the large genomes and the requirement for large growing facilities such as farms and greenhouses.

Arabidopsis thaliana (L.) Heynh. is a small crucifer, which has various properties that make it a convenient plant for molecular genetic research (for review see Rédei, 1975; Meyerowitz & Pruitt, 1985; Estelle & Somerville, 1986; Meyerowitz, 1987). It has a generation time of 6–8 weeks and a genome size (7×10^7 base pairs per haploid) of about a hundredth of the size of most higher plants. The plant is small (20–30 cm in height) and easy to grow in laboratories.

The procedures for mutagenesis, Ti plasmid-mediated transformation and plant regeneration are being studied. Using this flowering plant, we have attempted to take a genetic approach to understand the process of flower development and morphogenesis. The very first and most fundamental step in the study is to isolate and characterize mutants that have defects in flower development and morphogenesis. It is expected that analyses of such mutants will reveal the genes responsible for those mutations and will eventually lead to an understanding of the molecular mechanisms involved in the developmental processes.

The flower morphology of *Arabidopsis* has been extensively studied (Müller, 1961) and shares a common structure with that of Brassicaceae (Cruciferae), the mustard family. Several mutants of *Arabidopsis* having an altered flower morphology have already been reported (McKelvie, 1962; Koornneef *et al.* 1980, 1983; Pruitt *et al.* 1987; Haughn & Somerville, 1988). Here we report the isolation of novel mutants defective in flower development and morphogenesis, and also the results of detailed examinations of the floral structures and genetic analyses of several mutants.

Materials and methods

Plant lines

An *Arabidopsis thaliana* wild-type strain, Landsberg (*erecta*), and mutant lines, M7 (*apetala-1, clavata-1*) and M10 (*apetala-2, eceriferum-2*), were obtained from Arabidopsis Information Service (Dr A. R. Kranz, Botanisches Institute, J. W. Goethe-Universität, Frankfurt am Main, FRG). Another mutant line, *pistillata*, was a gift from Dr E. M. Meyerowitz (Caltech). These mutations have been mapped (Koorneef, 1987).

Cultivation of plants

Plants were grown according to the procedures of Dr C. R. Somerville (personal communication) under continuous illumination (24L) at 22°C under the standard growth conditions. Some plants were grown under short-day growth conditions at 22°C, where 8 h illumination and 16 h darkness (8L:16D) alternate. Under the short-day growth conditions, plants become bigger than those grown under the standard conditions, but flowering delays by about 3–5 weeks.

Mutagenesis and mutant screening

The wild-type seeds were mutagenized by soaking them in 0.3% EMS (ethyl-methane-sulphonate) solution for 16–24 h according to the procedure of Dr C. R. Somerville (personal communication). The mutagenized seeds (M1 seeds) were sown, and the self-fertilized seeds (M2 seeds) were obtained. Mutants exhibiting abnormal floral structures were isolated from the M2 plants.

Phenotype analyses

To characterize floral structure, more than 20 flowers were carefully examined under a binocular microscope at low magnification. In order to examine morphological variations, the flowers were selected from the lower and upper parts of more than five inflorescences of different plants.

Thin sections of flowers were prepared as follows: samples of the flowers were fixed by dipping overnight in Bouin's fixative (75:25:5 (v) mixture of saturated picric acid, formalin and acetic acid), solidified in paraffin after gradual dehydration, thin sectioned (5–10 µm thickness) and stained with 0.5% (w/v) Azure-B solution in 0.1% sodium acetate buffer, pH 4.6.

Genetic analyses

Mutant plants were crossed with each other or with wild type by pollinating young unfertilized pistils by hand. When F₁ progeny generated from a cross between a mutant (homozygote) and a wild type showed the normal (wild) phenotype, the mutation was determined to be recessive. When the mutant was used as the male parent (pollen) in a cross, and when the mutation was inherited to the progeny, the mutation was judged to reside in the nuclear genome. When the mutation followed the laws of Mendelian segregation, namely plants with the recessive mutant phenotype and plants with the normal phenotype were segregated in a ratio of 1 to 3 in the F₂ plants generated from self-fertilization of the F₁ plants, it was concluded that the mutation was single.

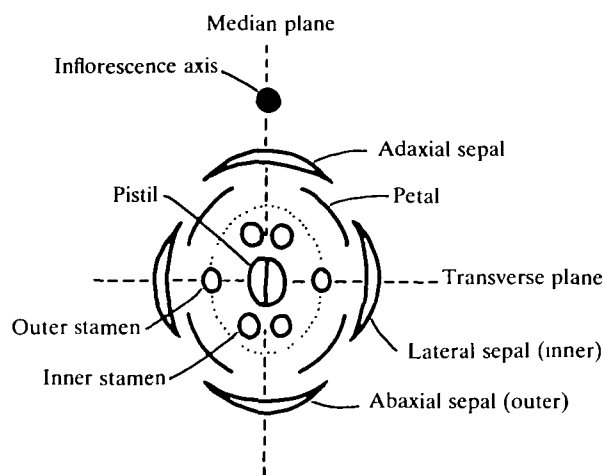


Fig. 1. Diagram of *Arabidopsis thaliana* wild-type flower.

Results

Anatomy of the flower of wild type *Arabidopsis thaliana*

The flowers of *Arabidopsis thaliana*, like those of other members of the mustard family, consist of four sepals surrounding and alternating with four white petals. Within the whorl of petals are six stamens (two short outer stamens and four long inner stamens) and a pistil having two carpels. Thus, these floral organs are arranged in concentric circles as illustrated diagrammatically in Fig. 1. It is worth noting in this connection that although there has been an alternative view that the pistils of Brassicaceae originate from four carpels (Lawrence, 1951), we follow the two-carpel hypothesis in this report for characterization of the pistil structure.

The floral organs are unambiguously distinguishable from each other on the basis of their shape, colour and size. In addition, some specific surface structures could be used as the markers to identify the organs. For example, the trichomes are found on the outer surface of the sepals, but not on other floral organs. The branched trichomes are seen on both sides of the leaves. Each floral organ also shows specific inner structures, such as the patterns of vascular bundles, specific cells and tissues. The structural patterns of the flowers including the numbers and shapes of the floral organs are generally stable and not affected by growth conditions with the exception of the number of stamens. We have occasionally observed flowers with 4 or 5 stamens (13 out of 44 flowers examined). The floral structures of wild-type *Arabidopsis thaliana* described above have been used as the standards for screening and characterization of mutants. Using the mutagenesis procedure described in Materials and Methods, we have obtained a number of mutants among the M2 progeny

Table 1. Average number of floral organs in flowers of the mutant strains of *Arabidopsis thaliana*

Strains	Illumination condition*	Number of flowers examined	Pistil (carpels/pistil)	Stamen	Petal	Sepal (curved sepal†)	Remarks
wild type	24L	22	1.0 (2.0)	5.7	4.0	4.0 (0)	normal structure
	8L:16D	22	1.0 (2.0)	5.5	4.0	4.0 (0)	
Fl-40	24L	22	1.0 (2.1)	1.6	0	3.3 (2.2)	homeotic conversion of sepals to carpels, loss of petals
	8L:16D	22	1.4‡ (2.1)	3.7	0	4.0 (1.7)	
Fl-48	24L	26	1.0 (2.1)	0.8	0	3.1 (2.1)	homeotic conversion of sepals to carpels, loss of petals
	8L:16D	21	1.1‡ (2.0)	3.0	0	3.5 (2.8)	
<i>ap-2</i>	24L	20	1.0 (2.0)	5.3	3.8	3.9 (0)	homeotic conversions of petals to stamens and of sepals to leaves
	8L:16D	22	1.1‡ (2.0)	4.8	3.6	3.7 (0)	
Fl-54	24L	21	1.0 (2.0)	4.7	1.8	3.8 (0)	various homeotic conversions, abnormal inflorescence
	8L:16D	21	1.0 (2.0)	5.0	2.4	3.3 (0)	
Fl-82	24L	20	1.0 (3.6)	6.2	4.2	4.2 (0)	large pistils composed of many carpels
	8L:16D	20	1.1 (3.3)	6.4	4.1	4.1 (0)	
Fl-89	24L	20	1.0 (2.0)	5.4	3.4	4.0 (0)	pistils with two stigmas
	8L:16D	—	—	—	—	—	

*24L, grown under continuous lighting. 8L:16D, grown under 8 h lighting and 16 h dark.

† Curved sepal is the converted, ovule-bearing sepal. See text.

‡ Axillary buds and pistils were counted.

that have defects in floral structures. Among the mutants, five were chosen and extensively investigated. The characteristic morphological features and the results of the genetic analyses of these mutants are summarized in Tables 1 and 2, respectively. Detailed descriptions of the mutants are given subsequently.

Flower structures of the homeotic mutants, Fl-40 and Fl-48

The flowers of a mutant strain, Fl-40, have unusual structures quite different from those of the wild type (Fig. 2A–C). There are substantial phenotypic variations among the flowers even in one plant. The typical phenotypes of this mutant flower are illustrated in Fig. 3A–F. Petals are completely absent and there are fewer stamens and sepals (Table 1). In some flowers, neither the normal stamens nor the normal sepal can be found (Fig. 3A), whereas some other flowers contain one or two stamens (Figs 2B, 3B,C). There are some flowers that have a pair of seemingly normal sepals in the transverse position (Fig. 3C). It appears that only the pistil maintains its normal

structure in this mutant, although the carpels are not fused in some flowers (Figs 2C, 3D,E).

The most remarkable feature of this mutant is that all the flowers have two curved, thick, green structures on the outermost circle at the median position with respect to the inflorescence axis. At the top of these structures, there is a dense growth of white unicellular hairs. The hairs look similar to the stigmatic papillae of the normal pistils. On the inner surface of the structures, small light-green particles are attached with a short stalk along the margins of the structures (Fig. 2B). The size (100–150 µm in diameter and 50–90 µm in width) and the shape of the particles are comparable to those of the ovules in the ovary. Microscopic analyses of the thin sections (Fig. 2G,H) of the particles show that the pattern of cells in the particles is identical to that of the ovules in the ovary of the wild type, namely multiple cell layers surround an embryo sac, which is located in the centre, and the micropyle, which is at one end of the particle (Fig. 2I). The particles are attached to the branches of the marginal vascular bundles that run along the margins of the structures.

These morphological analyses of this mutant show that the novel structures carrying the ovule-like particles are structurally equivalent to, or possibly the same as, carpels. The pattern of the vascular bundles and that of particle attachment are consistent with those of the theoretical primitive carpels proposed by Eames (1931) (Fig. 3G). These observations led us to conclude that the outer pair of sepals is converted homeotically into a pair of carpels. The converted sepals, however, differ from the normal carpels in the following two aspects; (1) the mutant sepals have trichomes on the outer surface like the normal sepals (Fig. 2B), whereas the normal carpels never bear trichomes, (2) the anther-like structures containing the pollen are often attached to the side edge of the converted sepals (Fig. 2B).

All the structural features of Fl-40 described above are also observed in another independently isolated mutant, Fl-48 (Fig. 2D). However, the variant flowers without stamens appear more frequently in Fl-48 than in the former mutant (Table 1). In this connection, the fertilization frequency of Fl-48 is lower than that of Fl-40.

Genetic analyses of the Fl-40 and Fl-48 mutants

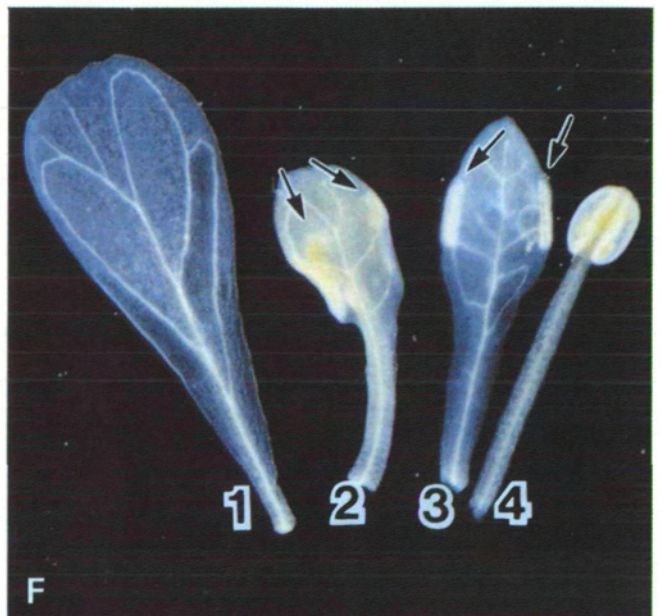
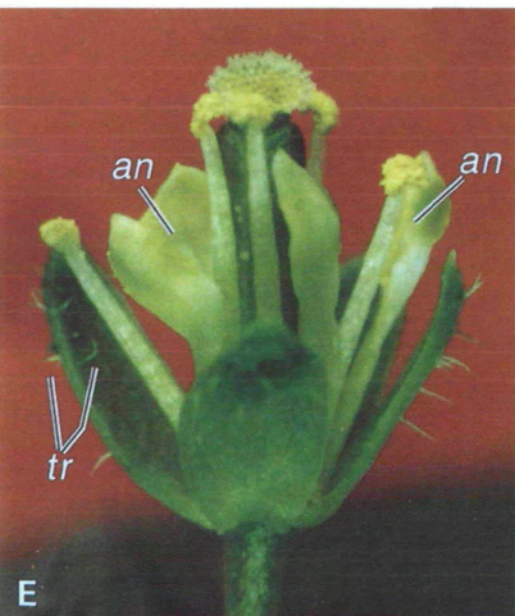
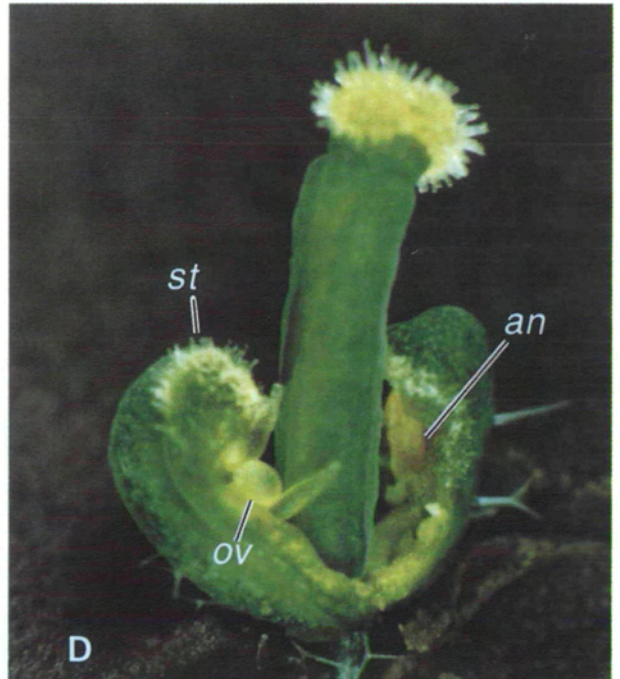
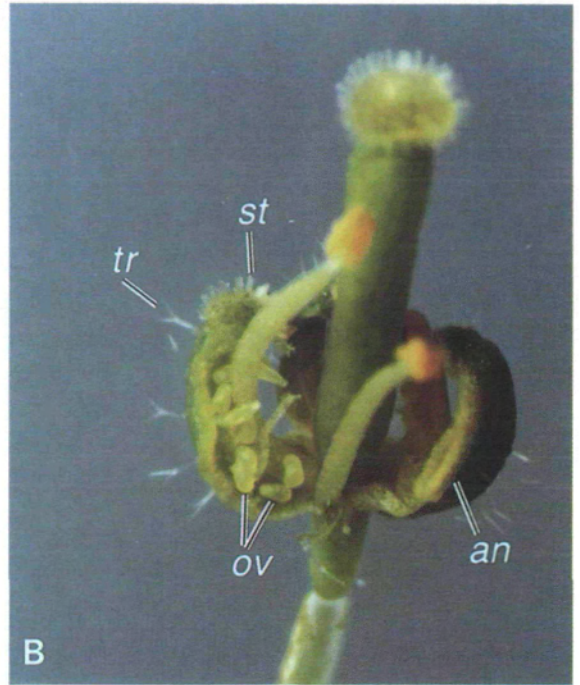
The homozygous lines of Fl-40 and Fl-48 are each examined genetically by making crosses with several strains. The results are summarized in Table 2. Since each of the Fl-40 and Fl-48 mutations followed the laws of Mendelian segregation in the F₂ generation, they represent single mutations on the nuclear genome and are recessive with regard to the wild-type allele. When Fl-40 and Fl-48 were crossed with each other, the flowers of the F₁ progeny were indistinguishable from those of both parental strains, indicating that the mutations of Fl-40 and Fl-48 are allelic and codominant.

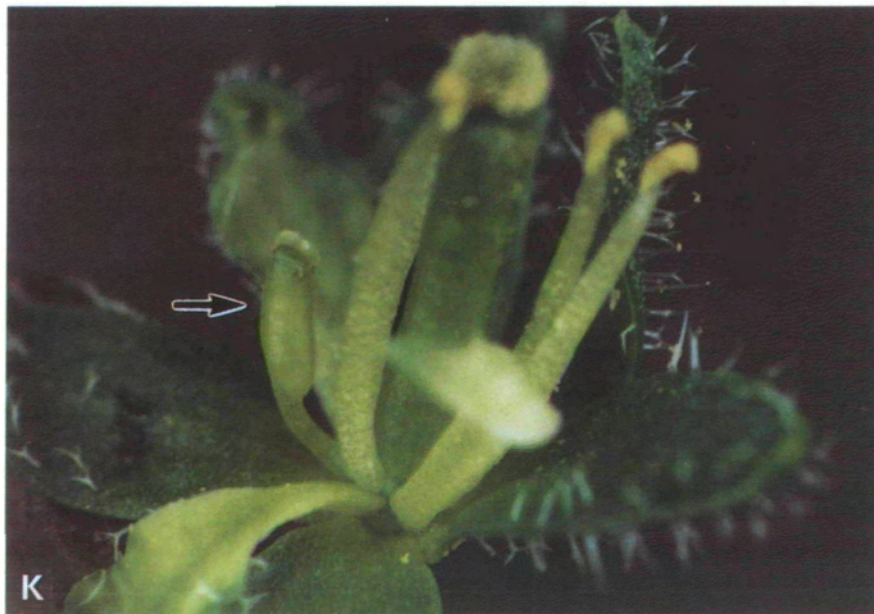
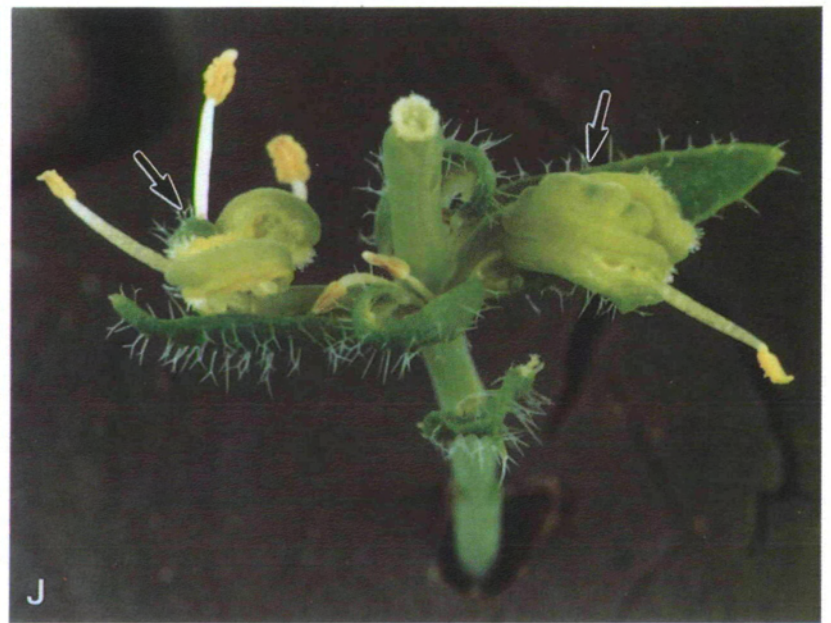
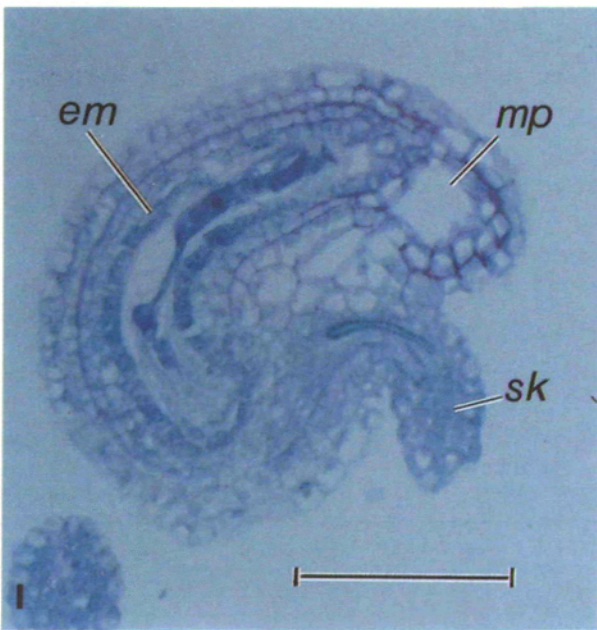
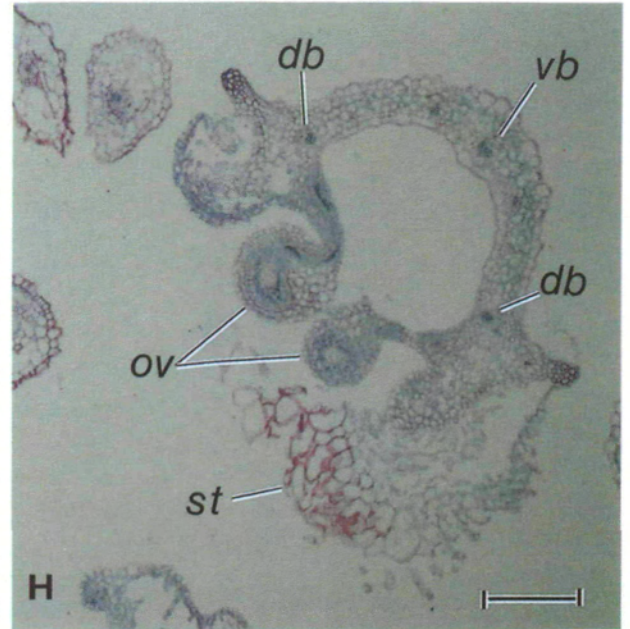
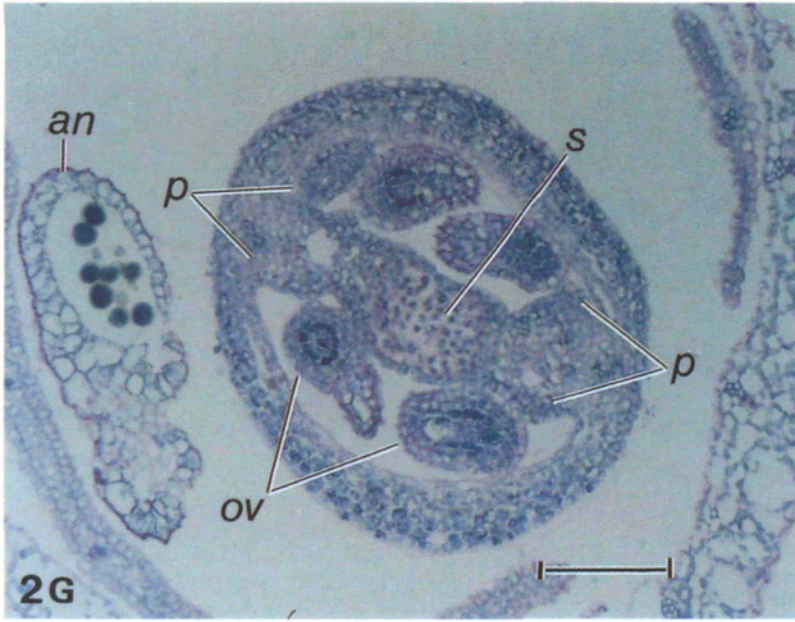
The two mutants were crossed further with several mutant lines known to have abnormal floral structures. The Fl-40 and Fl-48 mutants are non-allelic with *apetala-1* (*ap-1*), *clavata-1* (*clv-1*) and *pistillata* (*pi*). However, the mutations of Fl-40 and Fl-48 are allelic with the *apetala-2* (*ap-2*) mutation, which is known to cause homeotic conversions of petals into stamens and sepals into leaves or bracts as described below. The heterozygous plants from the reciprocal crosses between the Fl-40 and *ap-2* strains and between the Fl-48 and *ap-2* strains always show the phenotypes of the typical *ap-2* flowers. In the F₂ generation, the hidden phenotypes of Fl-40 or Fl-48 were segregated in a ratio of 3:1. Thus, the *ap-2* mutation is dominant over the Fl-40 and Fl-48 mutations.

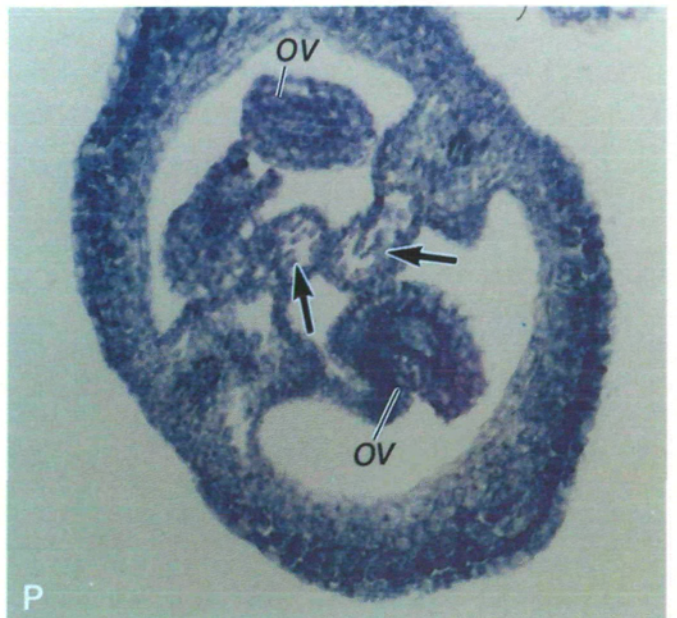
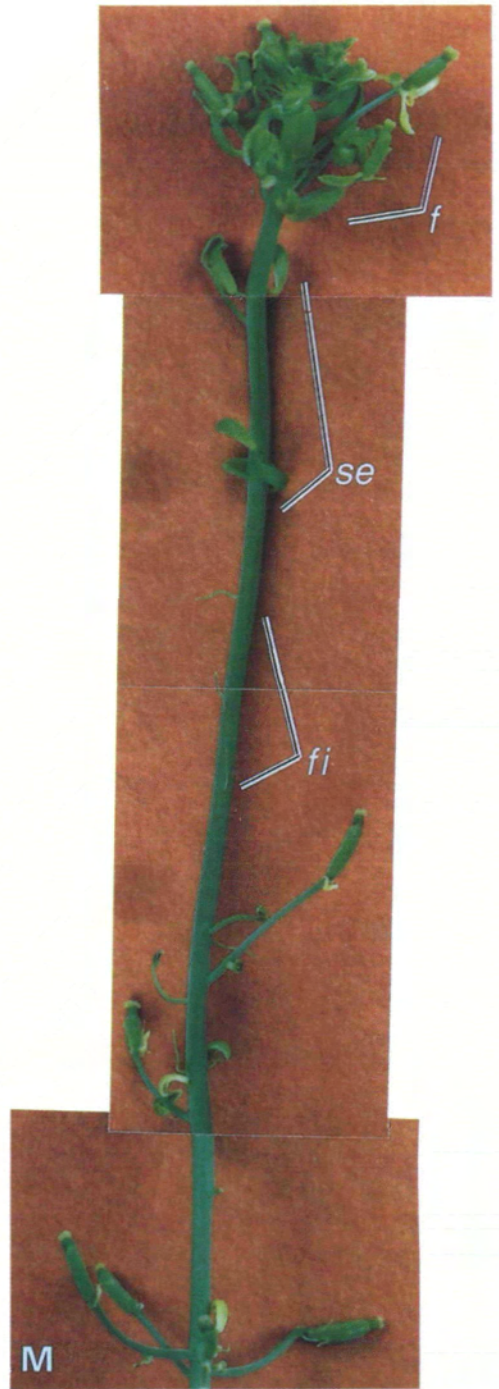
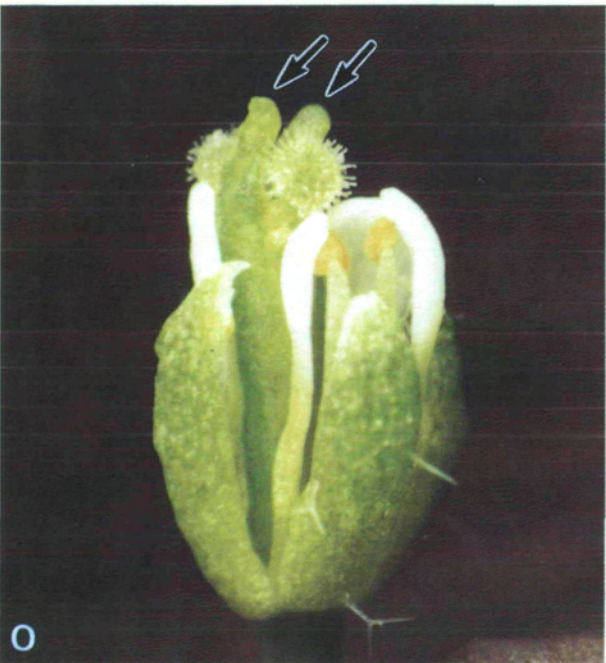
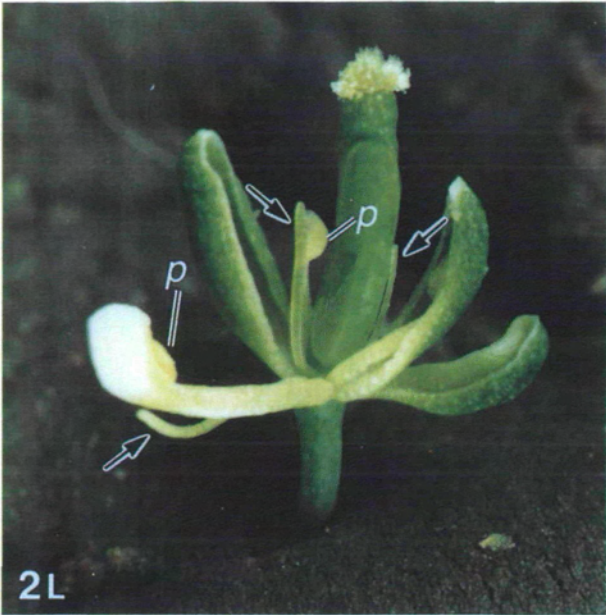
Although the mutational loci are allelic, the floral structures of Fl-40 and Fl-48 mutants are considerably different from those of the *ap-2* mutant (Figs 2E,

3H). The *ap-2* mutant was originally reported by Koornneef (1980) as a mutant having reduced petals and large sepals. Recently, the floral structure of this mutant has been studied extensively by Meyerowitz and colleagues (Pruitt *et al.* 1987, Meyerowitz, 1987) and by Haughn & Somerville (1988). They report partial or complete homeotic conversion of petals to stamens and of sepals to leaf-like structures. We have confirmed their observations. Unlike Fl-40 and Fl-48, the *ap-2* mutant always carries four sepals, four petals, and four to six stamens (Table 1). The petals of *ap-2* show intermediate structures between the petals and stamens of the wild-type strain. The petals are small and wrinkled and carry yellow swellings like pollen sacs along their margins (Fig. 2F). The pollen grains as well as the pollen mother cells are found

Fig. 2. Flowers of *Arabidopsis thaliana* mutants. Plants were grown under continuous illumination (24L), or under short-day conditions (8L:16D, 8 h light:16 h dark). (A) Wild type flower. 24L. $\times 20$. Bar, 800 μm . (B) Fl-40 mutant flower. 24L. *an*, anther; *ov*, ovules; *st*, stigmatic papillae; *tr*, trichomes. $\times 22$. (C) Fl-40 mutant flower. 24L. Arrows indicate open carpels that correspond to a pistil. $\times 20$. (D) Fl-48 mutant flower. 24L. *an*, anther; *ov*, ovules; *st*, stigmatic papillae. This flower lacks normal stamens. $\times 27$. (E) *Apetala-2* mutant flower. 24L. *an*, anther-like structure. *tr*, trichomes on inner and outer surfaces of sepals. $\times 22$. (F) *Apetala-2* mutant petals. A petal and a stamen of wild type are also shown for comparison. Samples were fixed in Bouin's fixative dehydrated in butanol and examined under a dark-field microscope. 1, a wild type petal; 2,3, *ap-2* mutant petals; 4, a wild-type stamen. Arrows indicate immature pollen grains. (G) Transverse section of a wild-type flower. *an*, anther; *ov*, ovule; *p*, placenta; *s*, septum. Bar, 100 μm . (H) Transverse section of the converted sepal of the Fl-40 mutant. *ov*, ovules attached to the inner margins of the converted sepal; *vb*, central sepal bundle; *db*, marginal sepal bundle; *st*, stigmatic papillae. Bar, 100 μm . (I) Longitudinal section of an ovule attached to the converted sepal of the Fl-40 mutant. *em*, embryo sac; *mp*, micropyle; *sk*, stalk of ovule. Bar is 50 μm . (J) Fl-40 mutant flower. 8L:16D. Arrows indicate adventitious flowers. $\times 18$. (K) *Apetala-2* mutant flower. 8L:16D. Arrow shows a small extra pistil. $\times 30$. (L) Fl-54 mutant flower. 24L. *p*, pollen grains. Arrows show stamens without anther sacs. $\times 18$. (M) Fl-54 mutant inflorescence. 24L. *f*, flowers; *fi*, filaments; *se*, sepal-like structures with a stalk. $\times 6$. (N) Fl-82 mutant flower. 24L. $\times 18$. (O) Fl-89 mutant flower. 24L. Arrows indicate horn-like projections. $\times 22$. (P) Transverse section of a pistil of the Fl-89 mutant. *ov*, ovules. Arrows show unfused septa.







inside the tissue. On the other hand, the shape of the sepals rather resembles that of the leaves. The sepals are flat and do not cover the young buds. There are trichomes on both inner and outer surfaces of the sepals (Fig. 2E). As mentioned earlier, the normal sepals of the wild type do not have trichomes on their inner surfaces, while the leaves do carry trichomes. The number and structure of other floral organs are generally normal (Table 1). Some mutant flowers, however, have four or five stamens (Fig. 3I) more frequently than the wild type.

Although the phenotypes of Fl-40 and Fl-48 mutants and that of the *ap-2* mutant are generally quite different from each other, similar phenotypes are occasionally observed. In one specimen among the twenty *ap-2* mutant flowers examined, sepals had stigmatic papillae at their apices and a few ovule-like particles at the margins just like the converted sepals of the Fl-40 and Fl-48 mutants. Under the short-day growth conditions, some flowers of the Fl-40 and Fl-48 mutants had an extra pistil between the sepals and the stamens on a short stem developed from the floral axis (receptacle). In other Fl-40 and Fl-48 mutant flowers, two axillary flowers, each containing a pistil and stamens are formed on a short stem at a median position with respect to the inflorescence axis (Figs 2J, 3F). This phenomenon is known as ecblastesis. In these flowers, the sepals are large and flat and have trichomes on both inner and outer surfaces like the sepals of the *ap-2* mutant. Similar extra pistils are occasionally found in the flowers of *ap-2* mutant grown under the short-day conditions (Figs 2K, 3J). These results show that the phenotypes of the three homeotic mutants, Fl-40, Fl-48 and *ap-2*, become more similar to each other when grown under the short-day conditions. The reason for this is not understood.

Flower structure and genetics of the Fl-54 mutant

Another mutant, Fl-54, shows variable phenotypes in the shape, number and position of the floral organs (Figs 2L, 3K–N, Table 1). Several kinds of homeotic changes are observed in the floral organs. Of the 42 flowers examined, everyone had a somewhat different phenotype.

Most of the stamens lack anther sacs at the end of the filaments (Figs 2L, 3K). In some flowers, the top of the filaments is white and flat like a petal (Fig. 3N). In some other flowers, filaments are green, and have stigmatic papillae at the top, indicating partial conversion to carpels. Some stamens are extremely thin and are attached to the ovary wall. The number of petals is reduced to one or two (Table 1). Pollen grains are occasionally seen along the margins of the petals (Figs 2L, 3M), indicating incomplete conversion of the petals to stamens. In some other flowers,

petals are green and look like sepals. Most of the flowers have three sepals, which are slender and have no, or only a few, trichomes. In some flowers, the margins of the sepals are white, showing partial conversion to petals. It appears that the pistils are less affected by the mutation and set seeds well by means of artificial pollination.

In flowers lacking some floral organs, the remaining organs are not necessarily positioned symmetrically as shown schematically in Fig. 3K–N. In some cases, short filaments and/or small knobs are visible on the flower receptacles, at positions corresponding to the missing organs. These structures might be the floral organs whose development had been abolished in earlier stages.

The structure of the inflorescence is also abnormal in the Fl-54 mutant. It is known that the inflorescence of Brassicaceae is racemose (Cronquist, 1981) and the flowers are formed along the inflorescence axis in a spiral arrangement at more or less constant intervals. In the Fl-54 mutant, however, the flowers are clustered in relatively short segments along the inflorescence. Between the clusters of flowers are segments in which short green filaments and some sepal-like structures with a short stalk are clustered (Figs 2M, 3O). The sepal-like structures bend with respect to the inflorescence axis. The shape and orientation of the structures are similar to those of the abaxial sepal (Fig. 1). When grown under the short-day conditions, two thin filaments are generated additionally from the junction of the sepal-like structure and the stalk (data not shown).

Several genetic crosses were performed to examine the mutant gene of Fl-54. The mutation responsible for Fl-54 is nuclear, single and recessive. Allelism tests show that Fl-54 is not allelic with the *ap-1*, *ap-2*, *clv-1*, *pi*, Fl-40 or Fl-82 mutants (Table 2).

Flower structure and genetics of the Fl-82 mutant

The flowers of the Fl-82 mutant have extraordinarily large pistils (Fig. 2N), usually consisting of three or more carpels (Fig. 3P–S, Table 1). In some flowers, the carpels are not fused well (Fig. 3Q). Anthers with pollen grains are sometimes attached at the side edge of the unfused carpels. In some other flowers, there are two separate pistils (Fig. 3R). Fertilization frequency of this mutant is generally low and the pistils of unfused carpels are completely sterile. The number and shape of other floral organs are usually normal, except that some mutant flowers occasionally have five sepals, five petals and eight stamens (Fig. 3S). It is likely, therefore, that the extra carpels are not generated from other organs by homeotic conversion, but rather they are formed by multiplication of the carpel primordia.

Genetic analyses show that the Fl-82 mutation(s) is

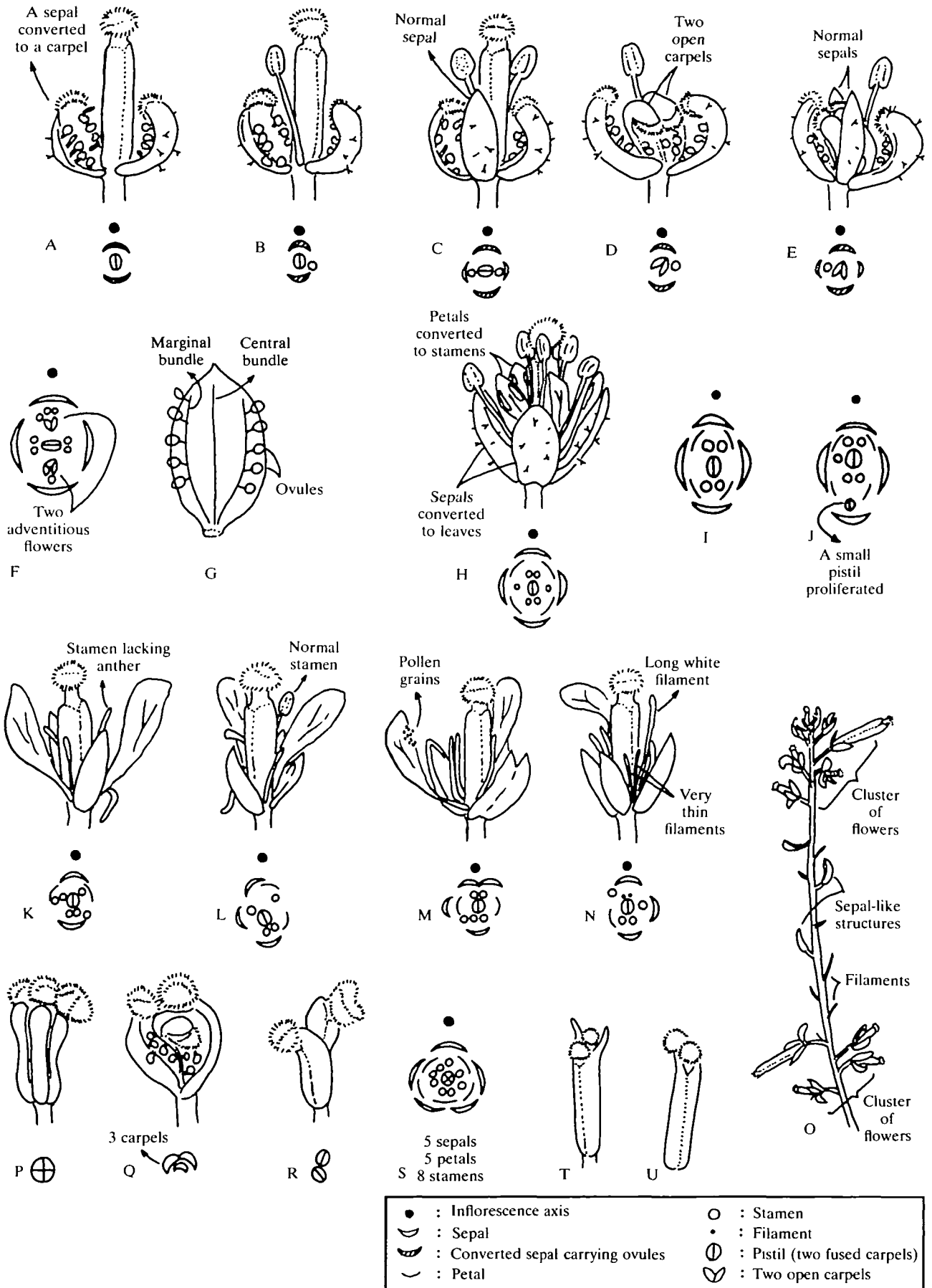


Table 2. Genetic analyses of the mutant strains of *Arabidopsis thaliana*

Strains	Nature of mutation	Number of loci	Remarks
Fl-40	nuclear recessive	single	allelic with <i>ap-2</i> and Fl-48 not allelic with <i>ap-1</i> , <i>clv-1</i> , <i>pi</i> , Fl-54, Fl-82 or Fl-89
Fl-48	nuclear recessive	single	allelic with <i>ap-2</i> and Fl-40 not allelic with <i>ap-1</i> , <i>clv-1</i> , <i>pi</i> , Fl-54, Fl-82 or Fl-89
Fl-54	nuclear recessive	single	not allelic with <i>ap-1</i> , <i>ap-2</i> , <i>clv-1</i> , <i>pi</i> , Fl-40 or Fl-82 male sterile*
Fl-82	nuclear recessive	not determined	not allelic with <i>ap-1</i> , <i>ap-2</i> , <i>clv-1</i> , <i>pi</i> , Fl-40 or Fl-54 female semi-sterile
Fl-89	nuclear recessive	single	not allelic with <i>ap-1</i> , <i>ap-2</i> , <i>clv-1</i> or Fl-40 female sterile†

* Most of the anthers do not bear enough pollen.

† Less than 1% of the pistils set seeds in artificial pollination using wild-type pollen.

recessive and nuclear (Table 2). It is not clear, however, whether the mutation is single. The heterozygotes with the *ap-1*, *ap-2*, *clv-1*, *pi*, Fl-40 or Fl-54 mutations display the normal phenotype, indicating that these mutant loci are not allelic with the Fl-82 mutation(s). The phenotype of the *clv-1* mutant is somewhat similar to that of the Fl-82 mutant. The pistil of the *clv-1* mutant consists of three or four carpels, which are always fused completely to form a

club-like pod (McKelvie, 1962; Koornneef *et al.* 1983; Pruitt *et al.* 1987; Haughn & Somerville, 1988).

Flower structure and genetics of the Fl-89 mutant

The flower of the Fl-89 mutant has a pistil with two clumps of stigmatic papillae and two horn-shaped green projections at the top (Figs 2O, 3T,U). The horns are located at the top of the carpels, whereas the clumps of stigmatic papillae reside on the seam-line of the carpels. Structural abnormalities of this mutant are also found inside the pistils. Although the carpels are always fused completely and the ovules seem to be normal, the septum tissues are not fused to form the partition (Fig. 2P). The number of the other floral organs is seemingly normal, but the sepals and the petals are slightly more slender than those of the wild type.

This mutant grows slower than the wild type. It takes about 2 months for the first flowers to appear. It remains to be clarified whether this delay is caused by the same mutation responsible for the defects in floral structure.

The results of the genetic analyses are listed in Table 2. The mutation is single, recessive and located on the nuclear genome. The allelism test shows that it is not allelic with any of the *ap-1*, *ap-2*, *clv-1* and Fl-40 mutations.

Discussion

The development and morphology of the flowers of *Arabidopsis thaliana* have been studied genetically and many mutants having a distorted flower morphology have been isolated. The analyses of the mutants clearly show that the developmental process as well as the morphology of the flowers are under the control of numerous genes. When one of the genes mutates, drastic alterations may occur in the flowering process. According to a classical view, a flower is

Fig. 3. Phenotypic variations of the mutant flowers. Flower structures and its diagram are shown.

(A–F) Fl-40 and Fl-48 mutant flowers. Hatched crescent in the diagram indicates the converted sepals on the median plane. (D,E) Fl-40 and Fl-48 mutant flowers with unfused carpels; (F) a flower with two adventitious flowers.

(G) A theoretical primitive carpel (redrawn from Eames, 1931).

(H–J) *Apetala-2* mutant flowers. (H) A flower of normal arrangement. Each floral organ arises at the corresponding positions to those of wild-type flowers.

(I) A flower with four stamens; (J) A flower with a small extra pistil.

(K–N) Fl-54 mutant flowers. Number of floral organs is reduced. Stamens usually lack anthers. Position of organs is distorted in every flower. (K) Six filaments (may correspond to stamens) in the distorted positions; (L) a flower having a normal stamen; (M) a petal partially converted to a stamen; (N) a long white filament in the position of a petal. Very thin filaments present.

(O) A Fl-54 mutant inflorescence. Thin filaments and sepal-like structures appear in the positions of flowers.

(P–R) Pistils of Fl-82 mutant flowers. (P) Four fused carpels; (Q) three unfused carpels; (R) two separate pistils.

(S) A Fl-82 mutant flower with five sepals, five petals and eight stamens.

(T,U) Pistils of Fl-89 mutant flowers. (T) A pistil with two stigmas and horn-like projections; (U) a pistil with two stigmas but without clear horn-like projections.

considered to be a modified shoot and floral organs are modified leaves developed from the primordial organ (Esau, 1977). If a certain genetic regulatory system that determines the type of floral primordia is disturbed by mutation(s), homeotic conversions may occur.

Of the mutants isolated, five mutants have been extensively characterized: three of them (Fl-40, Fl-48, Fl-54) have homeotic variations accompanied by the disappearance of several floral organs, and the rest (Fl-82, Fl-89) have alterations localized in the pistils. The three homeotic mutants have variable phenotypes, while the phenotypes are not so variable in the two pistil mutants. It is likely that the difference in variability among the mutant phenotypes is due to the nature of the functions of the mutant genes. It is possible that the products of the homeotic genes may be required throughout the process of flower development. Alternatively, these genes may work only at an early stage(s) of flower development when primordial organs are formed. In any case such homeotic genes may cause more pleiotropic effects than other genes responsible for flower development. In contrast, in the pistil mutants, the mutated genes may work in cells forming the primordial pistil (pistil primordia). Since the pistil is considered to be developed later than other organs, the mutations may not affect the development of other floral organs.

In the Fl-40 and Fl-48 mutants, the positions of the floral organs affected by the mutations are mainly median to the inflorescence axis. As shown in Fig. 3A–E, the converted sepals are usually located on the median plane, whereas the sepals of the wild-type are on the transverse plane. In flowers having only one or two stamens, the remained stamens are in the transverse position. Additional pistils and axillary flowers which sometimes appear under the short-day growth conditions are located on the median plane between the sepals and the stamens (Fig. 3F). These positional preferences of the mutational defects would reflect the median distribution of the mutated gene function in flower development.

Although the mutated gene of the Fl-40 and Fl-48 strains is allelic with that of the *ap-2* mutant, phenotypes of the former mutants are considerably different from that of the latter. In general, morphological aberrations are more profound in the Fl-40 and Fl-48 mutants than in the *ap-2* mutant. In view of the fact that the *ap-2* mutation is dominant over the Fl-40 or Fl-48 mutations, we assume that the genes in the Fl-40 and Fl-48 mutants are more extensively damaged than those in the *ap-2* mutant. The nature of these mutations will be unravelled when their genes are cloned and characterized. The other independently isolated floral mutants (*flo2*, *flo3*, *flo4*) (Haughn & Somerville, 1988) appear to have phenotypes that are somewhat

similar, in some aspects, to those of the Fl-40 and Fl-48 mutants. It remains to be clarified whether these mutations are allelic with each other or not.

The floral structure of the Fl-54 mutant is highly variable. Several homeotic variations were detected in the sepals, petals and stamens. Some floral organs are missing or not fully developed. The positions of these floral organs often deviate from their standard positions. The highly pleiotropic effects of this mutation probably indicate that the mutated gene product plays a crucial role in the development of these floral organs.

Of particular interest is the fact that the mutation in the Fl-54 mutant results in the abnormal structures on the inflorescence. Clusters of thin filaments and sepal-like structures are formed on the inflorescence axis between clusters of flowers. The position of the sepal-like structures relative to the axis suggests that they correspond to the abaxial sepal which appears first in flower development of Brassicaceae (Sattler, 1973). It is likely, therefore, that the sepal-like structures represent traces of aberrantly developed flowers in which only the abaxial sepal is developed but not other floral organs. If this is true, it would imply that the Fl-54 mutation may cause two different kinds of disturbance in the normal process of flower development. One is to stop the floral bud development immediately after the appearance of abaxial sepals, and the other is that flowers are ultimately developed and pistils are formed but the flowers show homeotic changes among floral organs. These two modes of disturbance somehow appear alternately on the inflorescence axis, as the inflorescence grows. Further study is necessary to clarify the mechanism underlying this phenomenon.

Two mutants, Fl-89 and Fl-82, have structural abnormalities in the pistils. The stigma and septum of the pistils in the Fl-89 mutant are altered. According to the pistil development scheme in Brassicaceae, two septa grown from the region between the two placental bands meet in the centre and fuse with each other (Sattler, 1973). The developmental step in which the septa fuse is apparently blocked in the Fl-89 mutant. The abnormal stigma structure, the two clumps of papillae and the horn-shaped projections would be the result of incomplete septal fusion. The mutated gene of the Fl-89 mutant may be required for the development of the pistil primordium, especially in the stages of septal fusion. The organization of the pistils in the Fl-82 mutant is also incomplete. Usually the pistils consist of three or more carpels which are often not fused. Consequently, the ovules are exposed to air and the septa are not well developed. In addition to the structural abnormalities, the pistils of Fl-82 and Fl-89 are functionally defective, as judged by the low fertilization frequency of the pistil.

Although it is not known which step(s) of fertilization is blocked in each mutant, the structural defects of the pistils would be responsible for the sterility of these mutants.

Attempts to identify the genes responsible for these mutations are in progress in our laboratory. When the genes are identified, their structure and expression may be examined in order to provide vital clues for understanding the mechanism of flower development and morphogenesis in higher plants.

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