Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*

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To isolate and study genes controlling floral development, we have carried out a large-scale transposon-mutagenesis experiment in *Antirrhinum majus*. Ten independent floral homeotic mutations were obtained that could be divided into three classes, depending on whether they affect (1) the identity of organs within the same whorl; (2) the identity and sometimes also the number of whorls; and (3) the fate of the axillary meristem that normally gives rise to the flower. The classes of floral phenotypes suggest a model for the genetic control of primordium fate in which class 2 genes are proposed to act in overlapping pairs of adjacent whorls so that their combinations at different positions along the radius of the flower can specify the fate and number of whorls. These could interact with class 1 genes, which vary in their action along the vertical axis of the flower to generate bilateral symmetry. Both of these classes may be ultimately regulated by class 3 genes required for flower initiation. The similarity between some of the homeotic phenotypes with those of other species suggests that the mechanisms controlling whorl identity and number have been highly conserved in plant evolution. Many of the mutations obtained show somatic and germinal instability characteristic of transposon insertions, allowing the cell-autonomy of floral homeotic genes to be tested for the first time. In addition, we show that the *deficiens* (*def*) gene (class 2) acts throughout organ development, but its action may be different at various developmental stages, accounting for the intermediate phenotypes conferred by certain *def* alleles. Expression of *def* early in development is not necessary for its later expression, indicating that other genes act throughout the development of specific organs to maintain *def* expression. Direct evidence that the mutations obtained were caused by transposons came from molecular analysis of leaf or flower pigmentation mutants, indicating that isolation of the homeotic genes should now be possible.

[*Key Words*: *Antirrhinum majus*; homeotic genes; *def*; transposons]

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The flower is one of the most intensively studied organ systems in plants. Many mutations affecting the developmental fate of its organ primordia have been documented in diverse species, although there have been few attempts to relate these in a systematic way to the mechanism of floral development (Masters 1869; Worsdell 1915; Meyer 1966; Meyerowitz et al. 1989). These mutations are homeotic in the classical sense as they result in "the assumption by one member of a meristic series, of the form or characters proper to other members of the series" (Bateson 1894). Indeed, when Bateson coined the term, homeosis, he referred specifically to the early work on plant teratologies by von Goethe (1790) and Masters (1869). In comparison with well-studied cases of homeosis in some other systems, most notably *Drosophila*, homeosis in plants shows some distinguishing features. One important difference is that plant organs are often produced sequentially so that distinct organ types may arise at different times in development. Homeotic mutations can therefore change both the times and locations at which particular organs develop. Consequently, as well as being homeotic, the mutations can be considered to be heterochronic as they may result in precocious or related development similar to certain mutations in *Caenorhabditis* (Ambros and Horvitz 1984). In contrast, different segments in *Drosophila* arise almost synchronously so that homeosis is generally considered separately from heterochrony.

We used transposon-mutagenesis to generate floral homeotic mutations with a view to studying and isolating the genes involved. One advantage of this approach is that transposon integration can be used to tag genes, and transposon excision can be used to prove that the correct gene has been isolated (Shepherd 1987; Wienand and Saedler 1987). In addition, imprecise excision can generate alleles with altered gene expression, thus providing useful material for studying gene function and regulation (Sommer et al. 1988; Almeida et al. 1989). Finally, somatic excision can indicate if the af-

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fected gene acts cell autonomously and can also be exploited to "rescue" sterile or lethal mutations.

Antirrhinum majus provides an ideal experimental system for this approach. Three different transposons (Tam*1*, Tam*2*, and Tam*3*), representing two distinct transposon families, have been isolated and characterized from this species (Bonas et al. 1984; Sommer et al. 1985; Upadhaya et al. 1985; Krebbers et al. 1987), and transposon-tagging has been used successfully for gene isolation (Martin et al. 1985). Several genes encoding flower pigment biosynthetic enzymes have been isolated and provide good markers for monitoring transposition and for trapping new transposons (Coen et al. 1986; Sommer and Saedler 1986). *Antirrhinum* has large flowers that are easy to score phenotypically and are convenient to emasculate and cross. Consequently, it has a good genetic map with many well-characterized mutations affecting flower development (Stubbe et al. 1966). Finally, sterile mutants can be readily propagated vegetatively through cuttings.

To isolate and subsequently analyze genes by transposon-tagging, a known active transposon needs to be inserted into a gene of interest. One approach to this problem would be to set up a large-scale direct self-pollination program by using lines carrying active transposons. This approach should allow the isolation of homozygous recessive mutations in known isogenic backgrounds in the M_2 generation. It should also allow transposons to be trapped in genes that have already been cloned, thus allowing isolation and characterization of any new transposons active in the stock. Any novel transposons trapped by this method could then be used as molecular tags for gene isolation and may also lead to a greater understanding of transposon behavior and evolution. Here, we describe floral homeotic mutations obtained from such an experiment; direct evidence that these and other mutations were caused by transposon insertion will be presented elsewhere (E. Coen, S. Doyle, and R. Carpenter, in press; D. Luo, E. Coen, S. Doyle, and R. Carpenter, unpubl.).

Flowers of *Antirrhinum majus* are borne in a spiral up the stem in a racemose inflorescence. Each flower grows in the axil of a bract and is zygomorphic: It can be divided into two halves by a single longitudinal plane. In transverse plane, the flower may be considered as comprising four concentric rings or whorls, each containing several organ members (Fig. 1). The members will be referred to as upper or lower, depending on their positions relative to the bract, which is considered to be the lowest organ. In addition, members lying in the plane of symmetry will be referred to as middle members. The first or outermost whorl comprises five sepals, the lowest two being alternate to the bract. The corolla occupies the second whorl and consists of five petals; these are united for part of their length to form a tube that terminates in five lobes. The two upper lobes have a shape distinct from the lower three. Five stamen primordia are initiated alternately with the petals and constitute the third whorl. The uppermost middle stamen primoridum fails to develop fully and yields an aborted

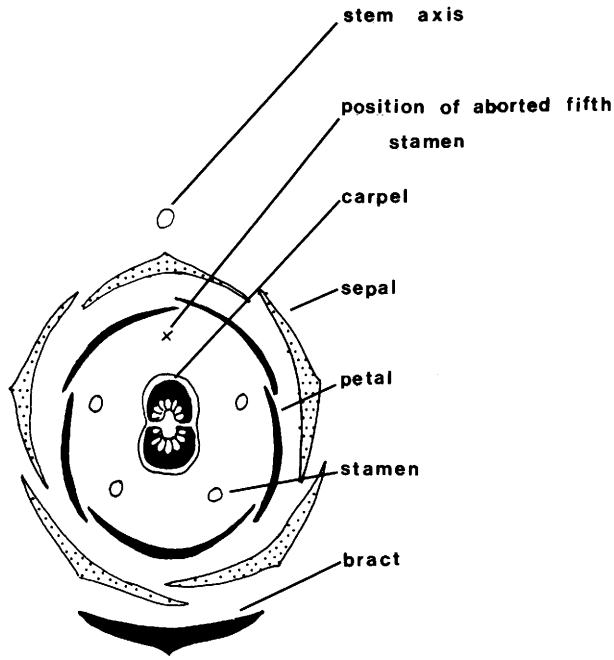

Figure 1. Floral diagram of a wild-type *Antirrhinum* flower.

or rudimentary structure. Consequently, the adult flower has only four stamens, the upper two being shorter than the lower pair. The central, fourth whorl is occupied by two united carpels forming a gynoecium with a bilocular ovary. For brevity, the identity of whorls will be indicated in sequence starting from the first whorl; thus, in the wild-type flower, the order is sepal (whorl 1), petal (whorl 2), stamen (whorl 3), and carpel (whorl 4). The different whorls appear sequentially, with the outer whorl (1) developing first and the central whorl (4) last (Awasthi et al. 1984).

Results

Transposon mutagenesis strategy

Several plants from lines carrying highly active transposons were grown at 15°C, the temperature at which greatest transposition of the Tam elements occurs (Harrison and Fincham 1964; Harrison and Carpenter 1973; Carpenter et al. 1987). These plants were self-pollinated and gave rise to 13,000 M_1 progeny. Taking a few selfed seeds from each of these plants gave an M_2 generation of 40,000 plants (see Materials and methods for details). Several flower homeotic and pigment mutations were obtained and selected for detailed analysis. Analysis of the pigment mutations will be presented in a separate paper (D. Luo, E. Coen, S. Doyle, and R. Carpenter, unpubl.). Here, we describe the genetic analysis of the homeotic mutations (Table 1).

Homeotic mutations

cycloidea-*608 and* cycloidea-*609* Two independent mutations were obtained that gave flowers with a more radially symmetrical appearance than wild type. Several

Table 1. *Mutations of* Antirrhinum majus *obtained by transposon tagging*

Mutant	Stock number	Description	Progenitor
Wild-type morphology	98,75	sepal, petal, stamen, carpel	
Homeotic mutant			
cycloidea	608	semi-peloric flowers	75
	609	trumpet-shaped flowers	75
deficiens	621	sepal, sepal, carpel	522
sepaloidea	620	sepal, sepal, carpel	T144
	619	sepal, split petals, stamen, carpel	T144
ovulata	627	carpel, stamen, stamen, carpel	605
pleniflora	624	sepal, petal, petal, variable, petal, etc.	98
	625		
	626		
floricaula	613	indeterminate shoots	98

The identity of the whorls is indicated in sequence starting from the first or outermost whorl.

different *cycloidea* (*cyc*) mutations affecting radial symmetry have been described previously (Darwin 1868; Stubbe 1966), and for ease of comparison, the most extreme of these, *cyc*-25, will be considered first. This allele gives a peloric phenotype: Flowers are radially symmetrical, and all members of whorls two and three resemble the lowest members of the corresponding whorl in wild-type flowers. For example, the uppermost middle stamen is not aborted but grows as a typical lower stamen. The number of members in the three outer whorls of the flower varies between 5 and 6.

One of the new mutations obtained gave semipeloric flowers with six sepals and petals and five or six stamens. The three lower petal lobes resembled the middle lowest lobe of wild type. Two of the upper lobes were hybrid in form, the lower half of the lobe resembling a lower wild-type lobe and the upper half resembling an upper wild-type lobe. The uppermost lobe was small and tended to fold forward, giving the flower a more rounded shape than normal. Crossing the mutant to lines carrying various *cyc* alleles gave mutant F_1 phenotypes (Fig. 2), whereas backcrossing to the wild-type progenitor gave wild-type flowers. Thus, the mutation was a recessive *cyc* allele, subsequently referred to

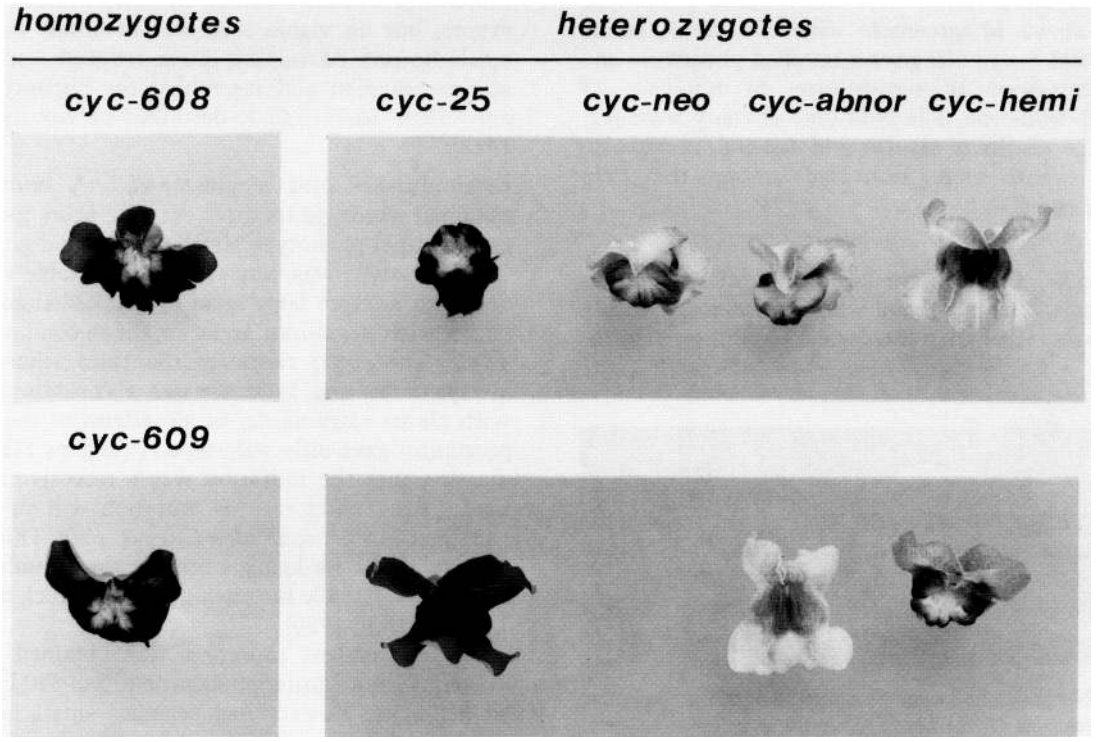

Figure 2. The two complementation groups of *cyc* mutations. (*Left*) Flowers of the homozygous *cyc*-608 and *cyc*-609 lines; (*right*) F_1 heterozygotes obtained when homozygous *cyc*-608 and *cyc*-609 were crossed with the known homozygous *cyc* mutations, *cyc*-25, cyc^{neo}, cyc^{abnor}, and cyc^{hemi}, described previously.

as *cycloidea*-608 (*cyc*-608). Interestingly, the cross with *cycloidea*^{hemiradialis} (*cyc*^{hemi}) gave an almost wild-type F_1 phenotype, with flowers showing a small notch at the joint between the upper and lower lobes, resulting in a partially open corolla (Fig. 3). This phenotype is less severe than that of either parent, indicating that *cyc*-608 and *cyc*^{hemi} show partial complementation. A similar phenomenon has also been observed for the cross of *cycloidea*^{neohemiradialis} (*cyc*^{neo}) with *cycloidea*^{radialis} (*cyc*^{rad}) (von Kuckuck 1936). From 1487 progeny of *cyc*-608, 0.34% had a revertant wild-type phenotype, and two showed a fully peloric phenotype similar to *cyc*-25, indicating germinal instability.

A second, independent mutation gave a more symmetrical, trumpet-like flower than wild type. The two upper lobes retained much of their wild-type appearance, but the three lower lobes resembled the middle lobe of wild type, turning over and down toward the corolla tube, to give an overall flattened appearance to the flowers. Crossing the mutant to lines carrying various *cyc* alleles gave mutant F_1 phenotypes, whereas backcrossing to wild type gave wild-type flowers. Thus, the mutation was a recessive *cyc* allele, subsequently referred to as *cycloidea*-609 (*cyc*-609). The F_1 phenotype of the cross with *cyc*^{hemi} gave a trumpet phenotype, but all other crosses gave the almost wild-type notched phenotype described above (Fig. 3). This is the converse of the results obtained with *cyc*-608 and indicates that there are two groups of *cyc* alleles: (1) *cyc*-608, *cyc*-25, *cycloidea*^{abnormis} (*cyc*^{abnor}), *cyc*^{neo}, and (2) *cyc*-609, *cyc*^{hemi}. Crosses between alleles from the two groups showed partial complementation with the notched phenotype described above. In agreement with this, the F_1 of the cross *cyc*-608 × *cyc*-609 gave a notched phenotype and the F_2 segregated 28 semipeloric; 61 notched; 25 trumpet; 2 wild-type. The wild-type progeny were presumably the results of reversion of *cyc*-608, because no reversion events were recovered among the 1578 progeny of *cyc*-609.

deficiens-*621* This mutant had the normal outer ring of five sepals, but the second whorl contained five individual sepals rather than the corolla of wild type. In the third whorl there were five united carpels, the top of which formed a flattened hollow ring of pollen-receptive stigmatic tissue. The number and position of the carpel primordia were identical to those of the wild-type stamens, indicating a homeotic conversion of stamen to carpel in the mutant. In the most extreme mutant phenotypes, the fourth whorl did not usually develop organs, but in less extreme cases it developed as wild-type carpels (Fig. 4a–c). Therefore, the first three whorls of the flower had been altered to give sepal, sepal, carpel, instead of the normal sepal, petal, stamen. Mutations giving a similar phenotype have been described previously at three different loci, *def*, *globosa* (*glo*), and *viridiflora* (*vir*) (Stubbe 1966). Crosses with plants carrying *def* or *glo* alleles showed that the mutation was a recessive allele of the *def* locus, subsequently referred to as *def*-621 (Table 2).

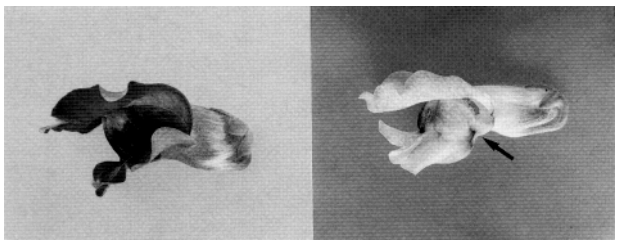

Figure 3. Notched phenotype produced in some of the F_1 *cyc* heterozygotes. (*Left*) Homozygous wild type with typical "closed" corolla; (*right*) a notch (arrow) between the upper and lower lobes, resulting in the partially open corolla as seen, e.g., in the F_1 of *cyc*-608 × *cyc*^{hemi}. The difference in the color is due to the presence of the *Eluta* gene in the *cyc*^{hemi} parent.

The *def*-621 allele was somatically very unstable. Clonal islands of pigmented tissue, sometimes comprising only four cells, were observed on the second whorl of sepals. Petal epidermal cells have a shape distinct from those of the sepal. Scanning electron micrographs showed that the clonal patches were composed of petal-like cells, with a sharp boundary separating them from the surrounding sepal cells (Fig. 5). To determine whether the subepidermal layers were also altered, small clonal patches were examined in transverse section. The cell layers beneath the epidermal patches were green, resembling mesophyll cells of the sepal rather than the unpigmented subepidermal petal cells. Occasionally, entire wild-type flowers were produced on the mutant, presumably caused by early somatic reversion events, but no viable seed was obtained. In addition, small flowers containing much reduced stamens were sometimes seen and resembled the phenotype of the *def*^{nicotinoides} (*def*^{nic}) allele described previously (Hertwig 1926).

sepaloidea-*619 and* sepaloidea-*620* A mutation was obtained which, in its most extreme form, gave a sepal, sepal, carpel phenotype similar to *def* and *glo* mutants. The phenotype was, however, very variable with a tendency to produce large areas of petaloid tissue, usually edged with sepal-like areas in the second whorl (Fig. 4d–f). The stylar tissue of the third whorl was not always united and, in some cases, was petaloid. Crossing with plants carrying *def* or *glo* alleles or the wild-type progenitor gave only wild-type F_1 progeny (Table 2), indicating that the mutation was a recessive allele of a new locus. Therefore, this mutation will subsequently be referred to as *sepaloidea*-620 (*sep*-620). This may correspond to the *vir* locus, a mutant that is unfortunately no longer available for testing (von Kuckuck and Schick 1930; Bergfeld 1956).

An independent mutation was obtained that gave flowers with a similar phenotype to *sep*-620 but less extreme. Some flowers had separate small green-edged petals in the second whorl and vestigial anthers in the third whorl clustered tightly around a gynoecium with a short style. The second whorl of other flowers contained large upper petals streaked with green tissue and small

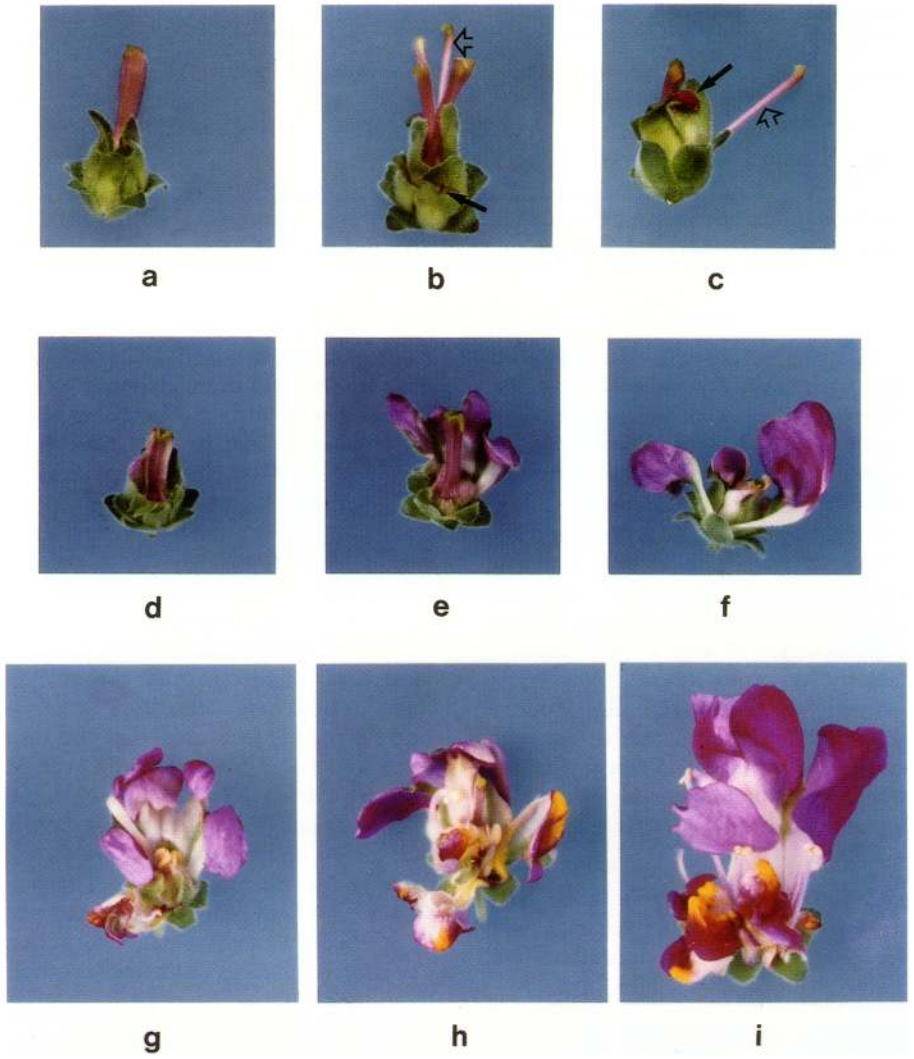

Figure 4. Diversity of flowers produced by *def*-621, *sep*-620, and *sep*-619, showing a series of progressively less severe phenotypes. (*a–c*) The *def*-621 flowers showing the second whorl of sepals with clonal patches of red petal tissue (solid arrows) and the united five carpels of the third whorl. Open arrows on flowers *b* and *c* show the central carpels of the fourth whorl. (*d–f*) *sep*-620 flowers with the extreme form (*d*) being similar to that of *def*-621. (*g–i*) Flowers from the *sep*-619 mutation, with the least phenotype on the right (*i*).

separate lower petals; the other whorls of the flower appeared normal (Fig. 4g–i). Two capsules of viable seed were obtained, and all progeny had a mutant phenotype. Crossing to plants carrying *def* or *glo* gave wild-type progeny, whereas mutant progeny resulted with *sep*, indicating that the mutation was at the *sep* locus. This mutation will be referred to as *sepaloidea*-619 (*sep*-619; Table 2). The various flowers produced by the *sep*-620 and *sep*-619 mutations can be arranged in a series of progressively less severe phenotypes (Fig. 4).

ovulata-627 Unlike other mutations described so far, this mutant was detected in the M_1 generation, indicating that it was dominant or semidominant to wild type. Several different M_1 plants, all derived from the same parent, showed a range of related phenotypes. The most severe of these gave five carpeloid members in the first whorl; the uppermost was separate and did not contain ovules, whereas the lower four formed two structures, each comprising two ovule-bearing loculi terminating in separate style-like structures. The second whorl contained separate strap-like petals, the lower three occasionally exhibiting anther-like structures; the third and fourth whorls were normal (Fig. 6). One of these severe mutants was self-pollinated and gave an M_2 of 3 wild type, 12 parental phenotypes, and 6 mutants with an extreme phenotype. The first whorl of this extreme phenotype comprised five carpels, the uppermost lacking ovules and the lower four being united to give an ovary with four ovule-bearing loculi terminating in a united band of stylar tissue. The second whorl comprised three lower stamens, whereas the upper two

Table 2. *Results of allelism tests for* def-*621,* sep-*620, and* sep-*619*

	Def/def^{gli}	*def*^{chlor}	*Glo/glo*	*sep*-619
def-621	wild type and mutant	mutant	wild type	wild type
sep-620	wild type	wild type	wild type	mutant
sep-619	–	–	wild type	mutant
Def/def^{gli}	–	–	–	wild type

Phenotypes observed in F_1 progeny when known *def* and *glo* mutations were intercrossed with *def*-621, *sep*-620, and *sep*-619. Mutant progeny indicate allelism between parental plants. Female parent is indicated in the left column and male parent along the top row.

members remained as small vestigial or aborted structures; the third and fourth whorls were wild type (Fig. 6A,B). The new phenotype may be summarized as carpel, stamen, stamen, carpel. The near 1 : 2 : 1 segregation of the M_2 progeny suggested that the mutation, called *ovulata*-627 (*ovu*-627), was semidominant, the parental phenotype being that of the heterozygote and the extreme phenotype being the *ovu* homozygote. This was confirmed because progeny from the M_2 heterozygotes segregated 1 : 2 : 1, the M_2 wild types bred true, and all progeny from backcrosses of the M_2 *ovu* homozygotes to wild type had the heterozygous phenotype.

Two of the less severe M_1 plants were also studied. One of these had elongated pointed sepals in the first whorl and large split petals in the second whorl. Somatically, the other was highly unstable and sometimes showed the full range of *ovu* phenotypes on a single raceme.

pleniflora-*624,* pleniflora-*625, and* pleniflora-*626* Several independent mutations were isolated, which gave a very similar phenotype. The first and second whorls were similar to wild type, whereas the third whorl contained petaloid members that were united for most of their length with the petals of the second whorl. The fourth whorl consisted of two members with variable structures, which showed sepaloid, carpeloid, and petaloid features. These structures were in the same relative position as the wild-type carpels. Within the fourth whorl were up to five extra whorls of petaloid members.

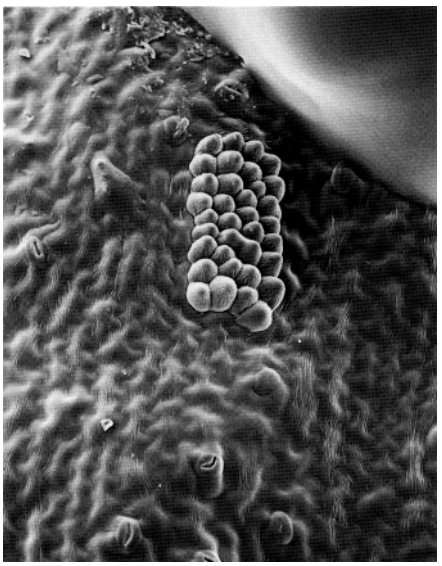

Figure 5. Scanning electron micrograph of part of the second whorl of sepals in the *def*-621 mutant, showing an island of 35 petal cells with a sharp boundary separating them from the surrounding sepal tissue. This indicates somatic excision of a transposon from the *def* gene leading to restoration of gene function.

Two of the mutations were unstable somatically and gave occasional wild-type flowers on otherwise mutant spikes. Seeds from these gave both wild-type and mutant progeny. The wild-type progeny were self-pollinated and, in each case, segregated for mutants and wild types. These results indicated that the mutations were recessive and could revert to wild type in both somatic and germinal tissue. The two unstable mutations have been called *pleniflora*-624 and *plenifora-625 (pleni*-624 and *pleni*-625, respectively) and a third phenotype, which has not so far produced seed, was called *plenifora*-626 (*pleni*-626) and propagated by vegetative cuttings. It has not yet been established whether these three mutations belong to the same or different complementation groups.

floricaula-*613* In this mutant, the switch from vegetative growth to that of the reproductive spike was as normal. In the axil of each bract, however, a secondary shoot formed instead of a pedicel and flower. This shoot, in turn, produced bracts within the axils of which further bract-bearing shoots were produced. This process could continue indefinitely to form an indeterminate shoot that had lost its ability to flower (Fig. 7).

It was necessary to propagate this mutation vegetatively, and a number of cuttings were grown at 15°C. One of these produced occasional flowers that were either self-pollinated or backcrossed to the wild-type progenitor. The progeny from the backcross were all wild-type, and when self-pollinated, gave wild-type and mutant progeny in approximately a 3 : 1 ratio (39 : 15). This indicated that the mutation was recessive, and it was called *floricaula*-613 (*flo*-613). From the occasional flowers produced by these cuttings, five capsules of viable selfed seed were obtained and the progeny from four of these capsules were all mutant. However, one of the capsules gave progeny that segregated to give 4 mutant and 18 wild-type plants, indicating that germinal reversion to the wild-type allele had occurred. The progeny from the wild types showed that some were heterozygous and others were homozygous for the wild-type allele.

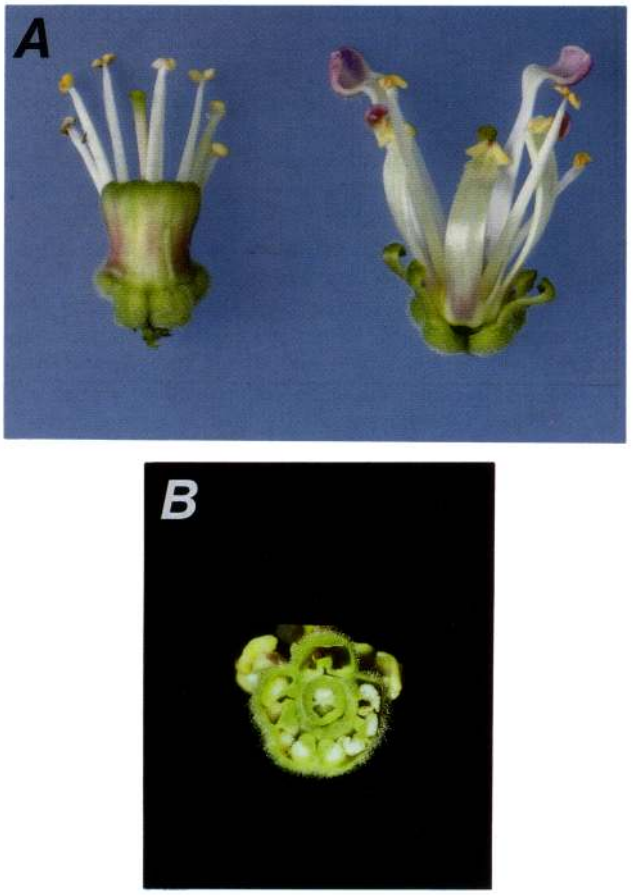

Figure 6. (*A*) Flowers of the mutant *ovu*-627. (*Left*) Homozygote showing the united carpels growing in place of sepals and three stamens in place of the three lower petals, giving a total of seven stamens. (*Right*) Heterozygous form of *ovu*-627, giving a mutant phenotype that is less extreme than that of the homozygote. (*B*) Transverse section through a homozygous *ovu*-627 showing that ovules are arranged within the outer lower four carpels, whereas the upper appears empty. Ovules are produced within the normal bilocular central ovary.

Discussion

Ten floral homeotic mutations were obtained, which can be divided into three classes. The first class affects the identity of members within the same whorl and includes the *cyc* alleles that confer varying degrees of radial symmetry to the flower. Two independent mutations giving this phenotype were obtained, and genetic analysis of these allowed two groups of *cyc* alleles to be defined. Extreme alleles of one group confer a peloric phenotype in which all members of whorls two and three resemble the middle lowest member of the corresponding whorls in the wild-type flower. The second group also gives flowers that are more symmetrical than wild type but retain a degree of zygomorphy. Crosses between plants carrying alleles from the different groups give F_1s with an almost wild-type phenotype but with two small notches on the lower flower lip, showing that alleles from the two groups complement each other partially. This suggests that *cyc* may be a complex locus composed of two interacting functional components. Alternatively, it is possible that there are two distinct *cyc* genes that are linked on the chromosome and whose products interact.

The second class of homeotic mutations affects the identity and sometimes, in addition, the number of whorls. Mutations at two loci, *def* and *sep* were obtained which, in the most extreme form, resulted in sepals growing in place of petals and carpels instead of stamens to give the phenotype sepal, sepal, carpel. The development of the fourth whorl did not usually occur in the extreme phenotype but comprised two united carpels in less severe forms. A third unlinked locus, *glo*, has also been described, which gives this phenotype (von Kuckuck and Schick 1930). The phenotype carpel, stamen, stamen, carpel is conferred by the most extreme allele obtained at the *ovu* locus. Unlike the other homeotic mutations obtained, *ovu* alleles are semidominant to wild type.

These two homeotic phenotypes suggest a simple model for the determination of whorl identity involving the combinatorial interaction of two functions, *a* and *b*. The *a* function is expressed in the outer two whorls of wild type, whereas the *b* function is expressed in the middle two whorls so that expression of *a* alone gives sepals, of *a* and *b* gives petals, of *b* alone gives stamens, and of neither gives carpels (Fig. 8). The *ovu* phenotype is consistent with the absence of the function *a*. The semidominance of *ovu* alleles suggests that they may be preventing the expression or action of the *a* function

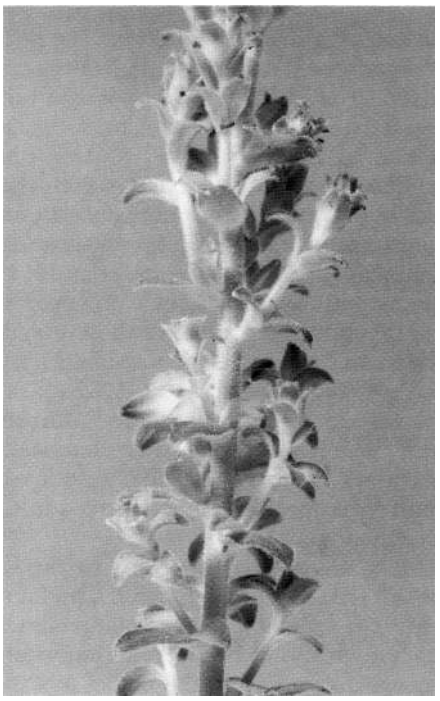

Figure 7. The *flo*-613 mutation showing a raceme with a shoot of indeterminate growth, instead of a flower, being produced within the axil of each bract.

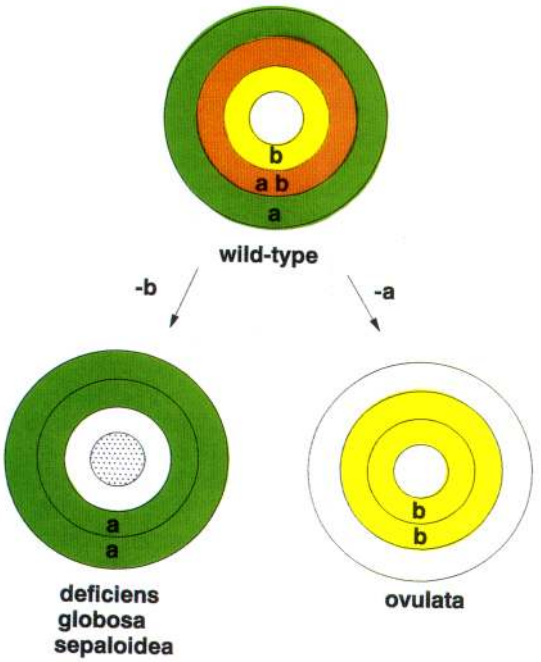

Figure 8. Model for the determination of whorl identity. In the wild type, the first whorl of sepals (*green*) expresses the *a* function; the second whorl of petals (*red*) expresses *a* and *b*; the third whorl of stamens (*yellow*) express *b* alone; the fourth whorl of carpels (*white*) expresses neither function. Mutant phenotypes are indicated below. The *def*, *glo*, and *sep* mutants are explained by loss of the *b* function. The stippled area indicates that the fourth whorl does not usually develop in the extreme phenotypes. In less extreme cases it gives carpels. The *ovu* phenotype is explained by absence of the *a* function.

rather than causing a loss of function. The *def*, *glo*, and *sep* phenotypes would result from loss of the *b* function. The absence of the fourth whorl in some of the *def* mutants suggests that the *b* function may also affect the determination of whorl number. Mutant phenotypes have been described in *Arabidopsis*, which show many similarities to those of *Antirrhinum* (*def*, *sep*, and *glo* are similar to *pistillata* and *apetala*-3; *ovu* is similar to *apetala*-2; Komaki et al. 1988; Bowman et al. 1989). For *Arabidopsis*, models have also been proposed involving concentric functions, and some of these may be analogous to the *a* and *b* functions proposed here (Haughn and Sommerville 1988; Bowman et al. 1989). The remarkable similarities between mutants in *Antirrhinum* and *Arabidopsis*, which belong to two taxonomically distant plant subclasses, suggest that the genetic control of whorl identity has been highly conserved in the evolution of dicotyledonous plants.

The *pleni* mutants give flowers with the first three whorls of the type sepal, petal, petal. The fourth whorl comprises two variable carpeloid/petaloid/sepaloid structures and within these a proliferation of petaloid whorls occurs. According to the model presented here, the wild-type *pleni* product might be required for a third function (*c*), which inhibits expression of the *a* function in the third whorl, so that the mutant expresses both *a* and *b* to give petal. In addition, it would act in the third or fourth whorls to delimit whorl number such that the mutant reinitiates both the production of whorls and the expression of the *a* and *b* functions, giving them a petaloid character. In this respect *pleni* mutants may be similar to heterochronic mutants in *Caenorhabditis elegans*, which repeat early lineages at later stages of development (Ambros and Horvitz 1984). The *pleni* phenotype is similar to other mutations described in *Antirrhinum* (Bergfeld 1956) and also to the *agamous* phenotype in *Arabidopsis*, although, in this case, sepals grow in the fourth whorl (Bowman et al. 1989). It is important to note that the spatial pattern of the expression of the *a*, *b*, and *c* functions may also correspond to a temporal sequence as the different whorls 1–4 arise sequentially.

Genes affecting both identity and number of homologous parts have also been described in *Drosophila* (see Ingham 1988). All of the *Drosophila* mutants give fewer rather than more homologous members compared to wild type. This may reflect a fundamental difference in the mechanism by which the repetition of parts occurs in these two systems. In *Drosophila*, segmentation proceeds from an undivided structure, the egg, to a subdivided one, so that mutants failing to subdivide correctly give fewer segments. In plants, whorl primordia are produced by sequential growth rather than by subdivision, giving the potential for indeterminant growth. The flower has been considered as homologous to a shoot with an imposed determinate growth pattern so that mutations in genes required for determinancy might be expected to give a proliferation of whorls (von Goethe 1790; Worsdell 1907; Arber 1937).

Thus far, the actions of the whorl identity genes have been considered separately from the first class of genes that affect the identity of members within a whorl. However, it is likely that these two gene classes also interact in a combinatorial way. For example, only three stamens develop in the second whorl of *ovu* mutants because the upper two members are vestigial or aborted. Therefore, these two members adopt a fate similar to that of the uppermost member of the wild-type stamen whorl (Fig. 1). Abortion of this member depends on the action of the wild-type *cyc* product, because in extreme *cyc* mutants all stamens develop fully and resemble the lower stamens of wild type. This suggests that the Cyc^+ product interacts with primordia in a similar way, irrespective of the whorl in which they occur and that the fate of a primoridum therefore depends on an interaction between the functions determining whorl identity and the functions determining the differences between upper and lower members within a whorl (*cyc*). In wild-type, the fate of a primordium may therefore be determined by a polar coordinate system (Fig. 9). Expression of the whorl identity functions varies along the radius (r) of the flower. The *cyc* function varies along the vertical (y) axis of the flower, with its effect generally increasing from the lower to the upper parts of the axis. This results in bilateral symmetry, because for half of the flower, every member has a unique specification, identical with its mirror image in the other half (Fig. 9).

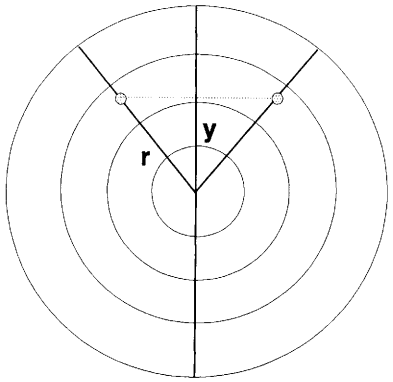

Figure 9. Polar coordinate system for specifying primordium fate. The four whorls are shown as concentric rings. Expression of the whorl identity functions varies along the radius (r) of the flower. The *cyc* function varies along the vertical (y) axis of the flower, with its effect generally increasing from the lower to the upper parts of the axis. Two members with the same combination of functions and, hence, the same developmental fate, are shown joined by a dotted horizontal line. Mutations eliminating the whorl identity functions result in some members from different whorls having similar specifications so that they adopt similar developmental fates. Mutations that abolish *cyc* function remove differential expression along the y axis such that all members of a whorl adopt a fate similar to that of the lower member of the wild-type whorl.

The third class of mutants gives indeterminant shoots in place of flowers in the axils of bracts. In the *flo* mutant, these lateral shoots bear bracts that can produce further lateral shoots in their axils, thus giving an indeterminant growth pattern. The wild-type *flo* product is therefore required for switching indeterminate shoot meristems to floral meristems and presumably activates the expression of the other classes of floral homeotic genes, either directly or indirectly. The phenotype is similar to other mutations in *Antirrhinum* (Chittenden 1928; von Kuckuck and Schick 1930; Bergfeld 1960) and to the *anantha* mutant of tomato, which also results in indeterminate branching of the inflorescence without production of flowers (Helm 1951; Paddock and Alexander 1952).

Cell-autonomy of mutants

Many of the homeotic mutants show instability in somatic or germinal tissue, suggesting that they were transposon induced. In some cases, the instability may be used to investigate the cell-autonomy of the genes affected. The *def*-621 allele shows clonal patches of petal tissue on the epidermis of the second whorl of sepals. These patches are separated by sharp boundaries both from the surrounding sepal epidermal tissue and from the underlying mesophyll tissue and can be explained by somatic excision of a transposon from the *def* locus restoring gene function. This suggests that the product of the wild-type *def* gene is not diffusible between cells and acts cell autonomously, at least in the epidermis of the second whorl. The observation of some very small patches indicates that the *def* product is active throughout development of the petal, from the early stages when petal and sepal primordia become distinct to the final cell divisions of the petal. This agrees with studies on the transcription of *def*, which show that gene expression is maintained from early to late stages of flower development (Sommer et al. 1990). The consequences of *def* expression may be different at each developmental stage, so that at early stages it may affect patterns of cell division and expansion and, hence, the form of the petal; at later stages it may determine whether cells have the petal or sepal characteristics typical of fully developed flowers. This may explain why both the form and the cell types of the second whorl organs appear to be intermediate between petal and sepal in some of the less extreme *def* alleles such as def^{nic} and $def^{chlorantha}$ (Hertwig 1926).

As the clonal patches of *def*-621 occur only in the second and not the first whorl of sepals, there must be other genes that act specifically in the second whorl throughout its development, which are necessary either for *def* expression or for the action of the *def* product. Two such genes may be *glo* and *sep*, as mutations of these give a similar phenotype of the *def* mutant. Interestingly, the *apetala*-3 mutation of *Arabidopsis*, which gives a similar phenotype to *def*, *glo*, and *sep*, is also thought to act up to a late stage of organ development based on temperature-shift experiments (Bowman et al. 1989).

A further feature revealed by the small clonal patches is that expression of *def* in whorl 2 early in development is not necessary for its later expression. This is because *def* can be expressed correctly late in development without any previous history of *def* activity in the organ. This rules out models in which *def* is switched on by a transient early signal and then *def* maintains independently its own expression by autoregulation. In this respect, small clonal wild-type patches in mutant tissue are more informative than the usual mosaic analysis of recessive mutants, carried out in other organisms such as *Drosophila* and maize, which invariably involve mutant patches in wild-type tissue and thus do not give any information about self-maintenance of gene expression.

Somatic instability was also observed for the *flo* mutant, because occasional flowers were seen in otherwise mutant inflorescences. In four cases, seed derived from these flowers gave only mutant progeny, whereas, in one case, the progeny segregated wild type to mutant in a ratio of 3 : 1. The observed case of germinal transmission of the revertant phenotype suggests that the parental flowers were the results of somatic excision of a transposon from *flo* restoring gene function. The failure of germinal transmission in most cases shows that functional gametes can be produced from homozygous mutant tissue and suggests that *flo* does not act cell autonomously. Another explanation is that some of the flowers observed are not the result of somatic transposon excision but are due to leakiness of the *flo* mutation.

The germinal and somatic instability observed for

many of the homeotic mutations suggests that they were caused by transposon insertions. In addition, molecular analysis of leaf and flower pigmentation mutants obtained from the same transposon mutagenesis experiment has shown that in most cases they were caused by transposon insertion, allowing new genes to be isolated and new transposons to be trapped (D. Luo, E. Coen, S. Doyle, and R. Carpenter, unpubl.). It should now be possible to determine which transposons are responsible for the homeotic mutations, allowing the isolation and molecular analysis of the genes involved.

Materials and methods

Antirrhinum *stocks*

JI.98 *nivea*^{recurrens} – 98 (*niv*^{rec} – 98) and JI.75 (TR-75) were bred at the John Innes Institute and are described by Harrison and Carpenter (1979) and Carpenter et al. (1987). The lines JI.522 and JI.523 were wild-type revertants of JI.98 and had been inbred as homozygous stocks for at least three generations. Families T144, T145, and T147 were heterozygous revertants of a highly unstable line carrying *niv*^{rec} – 98. The *cyc*-25 mutation was obtained from L.K. Crowe at Oxford prior to 1963 and has subsequently been maintained at our Institute. The stock JI.26 *globosa* allele arose spontaneously at John Innes in 1963 and is maintained as a heterozygous stock. All alleles carry the same number as the lines in which they are maintained. We are grateful to C. Lehmann for supplying the series of *cyc* and *deficiens* (*def*) mutations described by Stubbe (1966).

The conditions under which the plants were grown were the same as those described in Carpenter et al. (1987). Scanning electron micrographs were prepared as described by Williams and Green (1988).

Transposon mutagenesis strategy

In each case, 10 or 15 plants of lines that carry highly active transposons, (JI.75, JI.98, JI.522, and JI.523 and families of T144, T145, and T147) were grown at a constant 15°C, the temperature at which the highest frequency of transposition occurs (Harrison and Fincham 1964; Harrison and Carpenter 1973). These plants were self-pollinated, and seed capsules were collected separately. Seed from these plants gave rise to families of either 15 or 30 plants from each capsule, with a total of 13,000 M_1 plants grown in the greenhouse. As most mutations were likely to be recessive, these M_1 plants were also self-pollinated. To economize on labor, two capsules from each of the 15 or 30 plants within a family were pooled and mixed thoroughly to give a family bag of seed. In addition, two capsules from each plant derived from a particular line were collected in one bag and mixed thoroughly to form a line bag. For the M_2 generation, seed from each of the family bags was sown to give either 48 or 96 plants, depending on whether the M_1 family comprised 15 or 30 plants, to give a total M_2 generation of 40,000 plants grown in the field.

The main advantage of using family bags was that any mutation isolated could be traced back to its family, and further plants carrying the same mutation could be isolated. The line bags allowed the production of a large pool of mutagenized seed, which can be screened easily for diverse mutations, providing a long-term genetic resource.

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