The war of the whorls: genetic interactions controlling flower development

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The analysis of mutations affecting flower structure has led to the identification of some of the genes that direct flower development. Cloning of these genes has allowed the formulation of molecular models of how floral meristem and organ identity may be specified, and has shown that the distantly related flowering plants *Arabidopsis thaliana* and *Antirrhinum majus* use homologous mechanisms in floral pattern formation.

MUCH of the development of flowering plants depends on meristems, the groups of dividing cells that are the source of new plant structures. On the flanks of apical meristems, found at the apex of each growing stem, additional collections of cells are set aside in defined sequences and patterns. These become either new meristems, or primordia for organs such as leaves or petals. The time and place of the formation of new meristems and organs, and their type, determine the growth and form of the plant.

In this review we discuss our current knowledge of the genetic control of meristem behaviour in flower development. In particular, we concentrate on recent advances made in the study of the meristems of two distantly related species, *Arabidopsis thaliana* (a small weed in the family *Brassicaceae*) and *Antirrhinum majus* (common snapdragon, in the *Scrophulariaceae*). In each species new mutations and newly cloned genes have revealed some of the processes that regulate meristem activity.

Arabidopsis and *Antirrhinum* development

After germination of *Arabidopsis* seed, the vegetative apical meristem produces leaves in a spiral arrangement, separated by short lengths of stem (internodes). This gives the basal part of the plant the form of a rosette. After the vegetative phase, the apical meristem reorganizes into an inflorescence meristem, which initially produces (in the same spiral pattern) a few small (cauline) leaves which will become separated by long internodes. After this transient phase, the inflorescence meristem produces floral meristems, still in a spiral pattern. Each floral meristem develops into a single flower by making concentric arrangements of the four types of floral organs in the order: sepals, petals, stamens, carpels. The result is a plant with a basal leaf rosette and an inflorescence (Fig. 1*a*).

Antirrhinum plants develop somewhat differently. The initial vegetative meristem produces a shoot with pairs of opposite leaves, with each pair at right angles to the previous one, and separated by long internodes. After the vegetative phase, this meristem converts into an inflorescence meristem, which produces much smaller leaves (bracts) in a spiral arrangement. The bracts are separated by short internodes, and each has a floral meristem in its axil. The floral meristems give rise to a similar series of floral organs as do the corresponding meristems of *Arabidopsis* (Fig. 1*a*).

The wild-type flowers of both *Arabidopsis* and *Antirrhinum* (Fig. 1*b*, *c*) consist of four whorls, or concentric regions, each occupied by organs of different types. The outer whorl (whorl 1) contains sepals; whorl 2, petals; whorl 3, stamens; and whorl 4, which occupies the centre of the flower, carpels. We will describe the identity of organs starting from the outermost whorl, so that wild-type flowers are described as sepal, petal, stamen and carpel. *Arabidopsis* flowers have four sepals, four petals, six stamens and an ovary of two united carpels. *Antirrhinum* flowers have five sepals, five petals, four stamens (initially there are five stamen primordia but one is aborted early in development) and two united carpels (Fig. 1*b*). The flowers differ in symmetry: *Antirrhinum* flowers have only one plane of mirror-image symmetry (zygomorphic) whereas *Arabidopsis* flowers have two (Fig. 1*c*).

Homeotic changes in floral organs

Studies on the genetic control of meristem behaviour have concentrated on two classes of genes: those that control the identity of meristems, and those that determine the identity of organs. The genes in both classes can be considered homeotic, as their mutant phenotypes are the appearance of normal types of meristems or organs in positions typically occupied by other types. We begin by describing a series of homeotic mutations that affect the identity of floral organs.

Mutations are known in both species which affect the identity of organs occupying particular whorls[1–12]. Most of these mutations alter the identities of organs in two adjacent whorls (Fig. 2, Table 1). One class of mutants affects whorls 1 and 2, giving carpels instead of sepals in whorl 1 and stamens in place of petals in whorl 2. The overall phenotype is thus carpel, stamen, stamen, carpel (for example, *apetala2* and *ovulata* mutations have this effect). A second class of mutations affects whorls 2 and 3, and gives sepals instead of petals in whorl 2 and carpels instead of stamens in whorl 3, giving the phenotype sepal, sepal, carpel, carpel (*pistillata*, *apetala3*, *deficiens*, *globosa* and *sepaloidea* mutations are in this class). Mutations in these first two classes can, depending on the locus or the particular allele, reduce organ number, in addition to affecting organ identity. A third and final class affects whorls 3 and 4 and gives petals instead of stamens in whorl 3, and sepals or variable structures in whorl 4 (*agamous* and *plena* mutations are in this class). Mutations in the third class also give extra whorls of petals or sepals inside whorl 4.

The existence of similar classes of mutations in the taxonomically distant genera *Antirrhinum* and *Arabidopsis* suggests that the mechanisms controlling floral organ identity have been highly conserved in evolution. Indeed, preliminary data show that *AP3* is homologous to *DEF* (T. Jack, L. Brockman and E.M.M., unpublished results), and *AG* to *PLE* (D. Bradley, R. Carpenter and E.S.C., unpublished results), at the level of DNA sequence as well as at the level of mutant phenotype. But there are some differences in the phenotypes of the homologous mutations in the two species, such as the appearance of sepals in the fourth whorl of *Arabidopsis ag* mutants, whereas the corresponding organs in *Antirrhinum ple* mutants are of variable type, often showing carpelloid features.

Molecular cloning of organ identity genes

Two of the organ identity genes have been cloned and extensively characterized[13,14]. Others have been cloned more recently, with

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TABLE 1 Phenotype of some organ identity mutants in *Antirrhinum* and *Arabidopsis*

Genotype*	Phenotype				Region affected†
	Whorl 1	Whorl 2	Whorl 3	Whorl 4	
Wild type	Sepal	Petal	Stamen	Carpel	
ovu, ap2	Carpel	Stamen	Stamen	Carpel	A
def, glo, sep, pi, ap3	Sepal	Sepal	Carpel	Carpel	B
plena, ag	Sepal	Petal	Petal	Variable‡	C

* The Arabidopsis mutations are *apetala2* (*ap2*), *pistillata* (*pi*), *apetala3* (*ap3*) and *agamous* (*ag*). The *Antirrhinum* mutations are *ovulata* (*ovu*), *deficiens* (*def*), *sepaloidea* (*sep*), *globosa* (*glo*) and *plena* (*ple*). Several mutations at a locus called *pleniflora* have recently been described in *Antirrhinum*[8]; complementation tests have shown that these are allelic to the classic *plena* mutation[22] (R. Carpenter and E.S.C., unpublished results).

† See Fig. 3.

‡ In *Antirrhinum*, whorl 4 can be petaloid, sepaloid, carpelloid or a mixture of these; in *Arabidopsis* this whorl contains sepals. In both species additional petaloid and sepaloid whorls are produced interior to whorl 4.

initial characterization still in progress (ref. 9; T. Jack, L. Brockman and E.M.M., manuscript in preparation; D. Bradley, R. Carpenter and E.S.C., unpublished results; R. Simon, R. Carpenter, S. Doyle and E.S.C., unpublished results). The first homeotic organ identity genes to be cloned were *DEFICIENS* of *Antirrhinum*, with a class two phenotype of sepal, sepal, carpel, carpel[13], and *AGAMOUS* of *Arabidopsis*, with the class three phenotype sepal, petal, petal, sepal, followed by additional whorls of sepals and petals[14]. Remarkably, the proteins encoded by each gene show extensive similarity. Each contains a region of 56 amino acids, of which 40 are identical in the two proteins. There is thus 71% amino-acid identity between these parts of the proteins. The highly conserved region in these genes is very probably involved in DNA binding; it shows considerable similarity to regions in the vertebrate serum response factor genes and to the yeast *MCM1* gene. Serum response factors are DNA-binding transcriptional regulators of the c-*fos* oncogene in humans, and of actin genes in Xenopus[15,16]. The yeast *MCM1* gene encodes PRTF, a DNA-binding transcriptional regulator of mating type-specific genes[17,18]. The functional motif shared by all these genes has recently been named the MADS box (*MCM1-AGAMOUS-DEFICIENS-SRF*) and it has been shown that additional plant homeotic genes also contain a MADS box[9,19]. Thus, some of the homeotic genes of plants, like those of insects, are members of a family of DNA-binding proteins, each member serving different functions in the organism.

There is an additional region in the centre of both the AG and DEF proteins that shows structural similarity: in each protein, this region (called the K-box because of its similarity to the region of keratin responsible for coiled-coil formation) could form two alpha helices, highly charged on one face, and mostly uncharged on the other[19]. The function of this region is unknown, though one could imagine a role in protein-protein interactions.

Model of control of organ identity

We define three regions of the floral meristem, each coincident with the domain of action of one of the three classes of floral homeotic genes. Region A comprises whorls 1 and 2; region B, 2 and 3; and region C, 3 and 4 (Fig. 3). The action of several genes in these overlapping regions could give each whorl a unique combination of functions[5]. For example, if genes acting in regions A, B and C are required for three regulatory functions a, b and c, respectively, then the combination of functions in the four whorls of wild-type would be: a, ab, bc, c (Fig. 4). In principle, this might provide sufficient information to specify the identity of organs in each whorl. That is, sepals form if a alone is expressed, a and b together direct petal development, b and c together specify stamens, and c expressed alone determines carpel formation.

The homeotic mutations described above each eliminate one of the postulated functions: the *ap2* and *ovu* mutations disrupt function a, *ap3*, *pi*, *def*, *glo* and *sep* mutations prevent function b, and mutations in *ag* and *ple* block function c. An important constraint on such a combinatorial model is that it needs to account for the mutant phenotypes observed when the genes necessary for the a, b or c functions are mutated. By this criterion, the simple model that the domains of a, b and c function are established independently of each other does not fit the data. For example, if a is required for sepal and petal development in region A, how can these organ types develop outside this region in certain mutants, as for example in the *ag* and *ple* mutants, which lack the c function, and have petals in whorl 3?

To account for this effect, it has been proposed that a and c might influence each other's expression[8,10,12]. Strong evidence for an interaction between a and c has come from the study of double and triple mutants (Fig. 5). Triple mutants lacking a, b and c have been constructed in *Arabidopsis* and give flowers consisting solely of organs resembling cauline leaves[10,12] (Fig. 5*d*). These leaves can be considered as a type of developmental 'ground state'. Starting from this state we now consider the phenotypes observed when the various functions are added back (Fig. 4). Addition of a alone to the ground state results in a flower with only sepals, as observed in bc double mutants in both *Arabidopsis* and *Antirrhinum* (refs 5, 10; R. Carpenter and E.S.C., unpublished results). Addition of c to the ground state gives only carpels, as seen in a b double mutants (refs 5,10; R. Carpenter and E.S.C., unpublished results). But addition of both a and c results in the phenotype sepal, sepal, carpel, carpel (that is, b mutants); showing that the actions of a and c are now restricted to the respective regions A and C. Thus, the a and c functions seem to be antagonistic, and establish mutually exclusive domains of action.

One notable difference between the a function mutations in the two species is that they are recessive in *Arabidopsis*, and semidominant in *Antirrhinum*. This might indicate that in *Antirrhinum* the mutations inhibit a function rather than cause loss of this function. One possibility is that the dominant a mutations are in fact gain-of-function alleles of c, and that the gene providing the a function is not yet genetically identified in *Antirrhinum*.

The domain of b function is established independently of the a and c functions. This is demonstrated by the phenotype of *Arabidopsis* flowers with only the b activity (that is, a c double mutants), which have leaflike ground state organs in whorls 1 and 4, and organs intermediate between petals and stamens in whorls 2 and 3 (refs 5, 10; Fig. 5*b*).

The a-b-c model for specification of organ identity predicts that the activity of the b genes is in region B, and that of the c genes in region C. This spatial regulation of gene activity seems, in this case, to be at the RNA level, as the *Antirrhinum DEF* and *Arabidopsis AP3* gene RNAs are found by *in situ* hybridization to be predominantly in petals and stamens and their primordia (region B), and the *Arabidopsis AG* gene RNA predominantly in stamens and ovary and their primordia

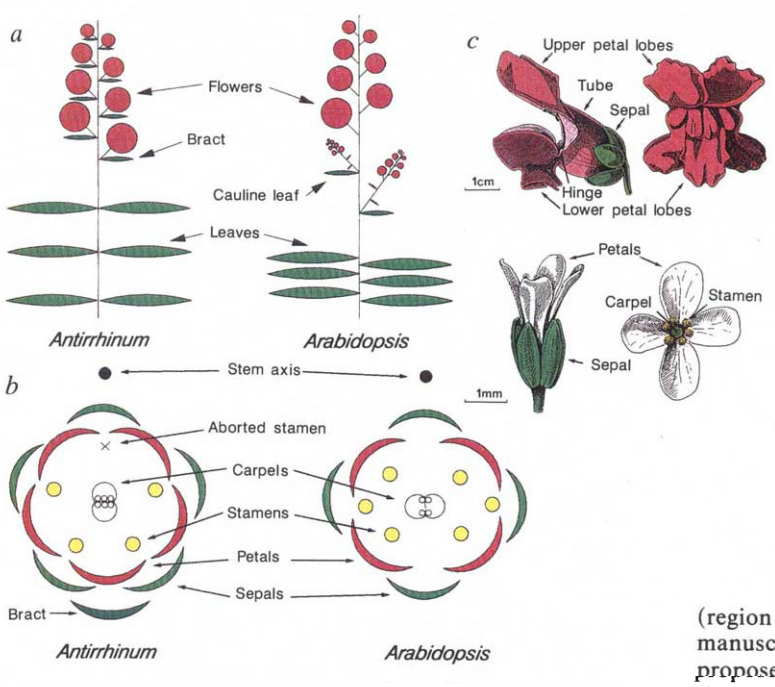

FIG. 1 *a*, Schematic diagrams of wild-type *Antirrhinum majus* and *Arabidopsis thaliana* plants. Lateral shoots arising in the axils of leaves are omitted for clarity. *b*, Floral diagrams of wild-type *Antirrhinum* and *Arabidopsis*. The small circles in the ovaries represent ovules. *c*, Flowers of *Antirrhinum* (above) and *Arabidopsis* (below). Flowers are shown in side view (left) or face view (right). The *Antirrhinum* flower shown in side view is opened slightly to illustrate the hinge between upper and lower petals. The face view of *Arabidopsis* is at a 45° orientation relative to the floral diagram in *b*. In linear dimension, *Arabidopsis* flowers are close to 10 times smaller than those of *Antirrhinum*, and in mass they are more than 1,000 times smaller. Nonetheless, the basic processes of development, and their results, are similar. *Antirrhinum* drawings adapted from ref. 27; *Arabidopsis* drawings by K. Roberts.

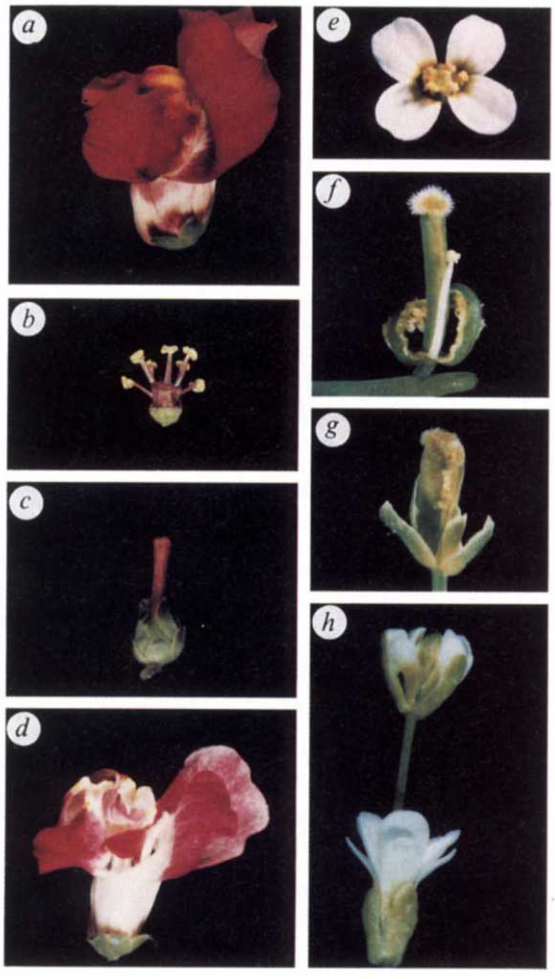

FIG. 2 Photographs of the parallel series of homeotic mutants of *Antirrhinum* (left) and *Arabidopsis* (right). *a* and *e*, Wild-type. *b* and *f*, The class one mutants *ovulata* and *apetala2* (the depicted alleles are *ovu-621* and *ap2-2*). *c* and *g*, The class two mutants *deficiens-621* and *pistillata-1*. *d* and *h*, The class three mutants *plena-624* and *agamous-2*. The *agamous* flower (*h*) shows clearly the development of the flower whorls interior to whorl 4, owing to the elongation of the pedicel of the first inner flower.

(region C) (refs 9, 13, 14, 20; T. Jack, L. Brockman and E.M.M., manuscript in preparation). This pattern is consistent with the proposed model, and also provides the opportunity to test a key element in the model, the a–c antagonistic interaction. In *Arabidopsis*, if the spatial control of *AG* expression is at the RNA level, then the domain of expression of *AG* RNA should expand to all four whorls in an a mutant (*apetala2*) background (Fig. 4). This has recently been shown to be true[20]. The molecular evidence thus accords well with the predictions of the genetic model for organ identity.

The a–b–c model raises the question of how the organ identity genes are themselves regulated as, for example, the b function genes act only in region B, regardless of the activity of the other homeotic genes. There is an *Arabidopsis* gene (*SUPERMAN*) whose mutant phenotype is consistent with a role in regulation of the spatial pattern of the b function genes[10,12]. Recessive mutations in it reduce or eliminate the fourth whorl carpels, and increase the number of stamens, which may occupy the central region of the flower (Fig. 6*a*). The wild-type gene thus appears to repress the b function in the fourth whorl. Additional gene functions must also be necessary for proper expression of the organ homeotic genes.

Genetic control of whorl and organ number

Mutations in genes needed for the c function generally give an increase in whorl number and an indeterminate growth pattern. For the *ag* mutants in *Arabidopsis*, the mutant phenotype is sepal, petal, petal, sepal, petal, petal, sepal and so on. This is sometimes described as 'flowers within flowers' because the sepals in whorls 4, 7 and so on can also be considered as the first whorl of sepals of an enclosed flower[10]. The *ple* mutants in *Antirrhinum* are similar to *ag* mutants, but give variable organs in whorl 4. These findings indicate that the c organ identity genes have two roles: the control of organ fate, and activation of a determinacy function to prevent indeterminate floral meristem growth.

Some of the other organ identity mutants also affect organ or whorl number. In one case this seems to be a result of the ectopic action of the determinacy function of the c genes. Extreme mutants of *AP2*, a gene required for the a function in *Arabidopsis*, give fewer organs than wild type in whorls 1, 2 and 3. As determinacy can be caused by the c function in whorl 4, and as a mutants have ectopic activity of the c function in outer whorls, this could explain the reduced organ numbers. Evidence favouring this interpretation comes from counting organ number in *Arabidopsis* mutants lacking a or lacking both a and c. The first three whorls have, in wild-type flowers, a total of 14 organs. An

extreme mutant lacking a (*ap2-2*) can have as few as two organs in these whorls. The removal of c function from such flowers, by making the doubly mutant a c (*ap2-2 ag*) strain, restores most of the missing organs[10].

It should be noted that the gene required for a function is not necessary for proper organ identity in the third whorl, but that it is necessary (at least in *Arabidopsis*) for proper organ number in that whorl. The mechanisms of organ identity specification and establishment of organ number are thus to some degree separate, even though some of the same gene products are involved. Another indication that establishment of organ number and organ identity may be separate processes is the existence of mutations that change organ number without affecting organ identity; for example *clavata1* of *Arabidopsis*, which causes an increase in the numbers of organs in whorls 2, 3 and 4 (ref. 2).

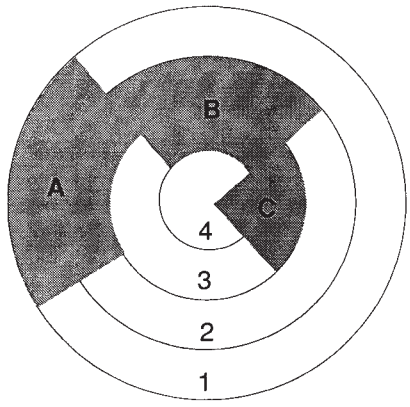

FIG. 3 Schematic diagram of the four whorl regions of a floral primordium (viewed from above, as in Fig. 1*b*), and the three regions, A, B and C, which are the domains of action of the three classes of organ homeotic genes.

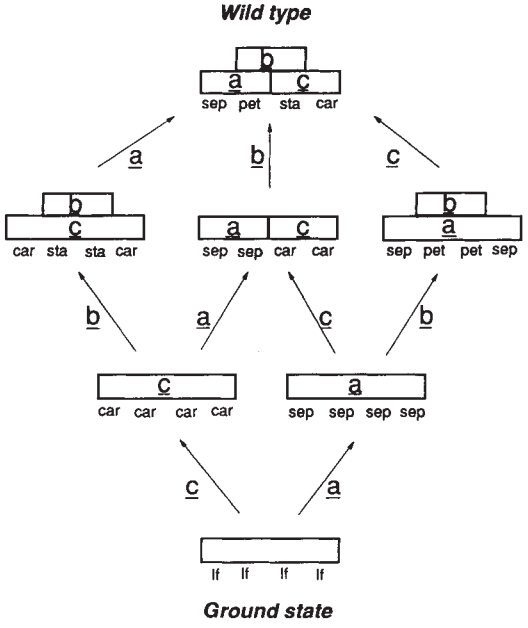

FIG. 4 Model illustrating the combinatorial interaction of the a, b and c functions in wild-type and various single, double and triple mutant combinations. The phenotype of the triple mutant is shown (and labelled ground state) at the bottom. The phenotypes observed when different combinations of functions are 'added back' to the ground state are shown above. lf, Leaf with some carpelloid features; car, carpel; sep, sepal; pet, petal; sta, stamen.

Genetic control of differences in whorls

In *Arabidopsis*, all organs in the same whorl have similar morphologies. In *Antirrhinum* and other species whose flowers are zygomorphic, one or more whorls contain organs with distinct morphologies. For example, in whorl 2 the upper two petal lobes of *Antirrhinum* have a distinct shape from the lower three, and in whorl 3 the uppermost stamen is aborted early in development (Fig. 1). Many mutations have been described in zygomorphic species that can reduce or eliminate the differences between organs in a whorl and thus render the flower more or less radially symmetrical[6,11]. These represent a separate class of organ homeotic genes from those discussed above, because they change organs to a type normally found in the same whorl, and not in a different one. In these mutants all of the organs in a whorl resemble one particular organ (generally the upper or lower organ) of the corresponding wild-type whorl.

The most intensively studied example of this phenomenon is in *Antirrhinum*. Several different mutations of the *cycloidea* (*cyc*) gene have been described which give flowers with a more symmetrical appearance than wild type[8,21,22] (Fig. 7). Extreme *cyc* mutations give radially symmetrical flowers with all petals resembling the lowest petal of wild type. Unlike wild type in which the uppermost stamen is aborted, all five stamen primordia develop fully in these mutants to give mature organs of similar length to the lower stamens of wild type. Thus, all organs in whorls 2 and 3 resemble the lowest organs of the corresponding whorl in the wild-type flower. In addition to this phenotype, there are also many *cyc* alleles which confer intermediate phenotypes, ranging from almost symmetrical flowers to nearly wild type.

As described earlier, many mutations give organs with identities inappropriate to the whorl in which they develop, so it is possible to ask if the action of CYC on particular organs depends on the whorl which they occupy. For example, the uppermost organ in whorl 3 of wild-type *Antirrhinum* is aborted as a result of CYC action because in extreme *cyc* mutants all five stamen primordia develop fully in whorl 3 (Fig. 1). If stamens grow in whorl 2, as in *ovu* mutants, the upper two organs of this whorl are also vestigial or aborted. The two upper organs are therefore aborted whether stamens grow in whorls 2 or 3. Furthermore, in *cyc ovu* double mutants, all stamen primordia develop fully in whorls 2 and 3 to give a flower with 10 stamens (R. Carpenter and E.S.C., unpublished results). This suggests that CYC interacts with primordia in a similar way, irrespective of the whorl that they occupy, and therefore that the fate of a primordium depends on an interaction between functions determining whorl identity and those determining the differences between upper and lower organs. These observations have suggested a polar-coordinate model for the control of primordium fate in each whorl[8] (Fig. 8).

Homeotic changes in floral meristems

Some mutations affecting earlier regulatory steps than those under the control of the organ homeotic genes might be expected to prevent or alter the formation of floral meristems. Mutants of this type, in which inflorescence meristems (or structures intermediate between inflorescence meristems and floral meristems) appear in the positions normally occupied by floral meristems, are *floricaula* (*flo*) and *squamosa* (*squa*) in *Antirrhinum*[8,9] and *leafy* (*lfy*) and *apetala1* (*ap1*) in *Arabidopsis*[3,23]. In *flo* mutants of *Antirrhinum* vegetative growth and the transition to the inflorescence meristem is similar to wild type. Instead of floral meristems being produced in the axils of bracts, however, the meristems in these positions are of the inflorescence type (Fig. 9). Thus, indeterminate shoots are produced in the bract axils, and each of these shoots can in turn produce further shoots in the axils of their bracts. *Arabidopsis lfy* and *ap1* and *Antirrhinum squa* mutants have a similar phenotype, though they show only partial conversion of floral meristems to inflorescence meristems (Fig. 6*b*).

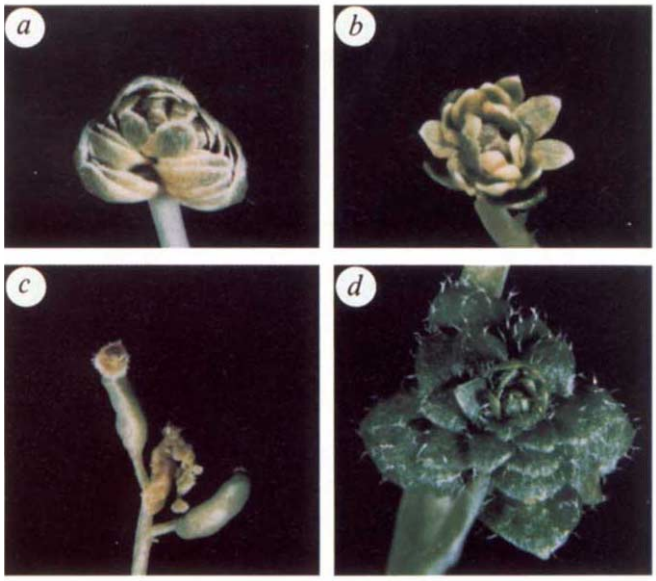

FIG. 5 Double and triple mutant strains of *Arabidopsis*. *a*, Flowers lacking both the b and c functions (genotype *ap3-1 ag-1*), and as a consequence having sepals as the only floral organs. *b*, Flowers lacking both the a and c functions (genotype *ap2-1 ag-1*). This causes the organs of whorls 1 and 4 to be leaflike, whereas the organs of whorls 2 and 3 are intermediate between petals and stamens. *c*, Flowers lacking the a and b functions (genotype *ap2-2 ap3-1*). All organs are carpels, and as the 'strong' *ap2-2* allele of *apetala2* was used, many organ positions are unoccupied. *d*, Flowers lacking all three organ homeotic gene functions, and containing only leaflike ('developmental ground state') organs in the positions normally occupied by floral organs (genotype *ap2-1 ap3-1 ag-1*). The leaflike organs have some carpelloid features such as stigmatic tissue at the tips.

The *FLO* gene of *Antirrhinum* has been cloned and characterized[24]. It produces a transcript which has the potential to encode a protein of 396 amino acids. The protein has a proline-rich amino terminus and an acidic region, both features of transcriptional activators[25,26]. But the sequence shows no extensive similarity with the organ specification genes or other sequences available in data banks, so that a role other than transcriptional regulation is not excluded. *In situ* hybridization shows that *FLO* is expressed very early in wild-type inflorescences. Earliest expression is in bract primordia and is followed by expression in sepal, petal and carpel primordia, but no expression is seen in stamen primordia. Expression in each organ is transient and is not observed in later stages of development. Taken together, these results suggest that *FLO* protein not only acts as a switch between inflorescence and floral meristems, but also may be involved in directing or maintaining specific patterns of gene expression in the early floral meristem. One can speculate that the expression of *FLO* in certain primordia may activate genes, such as organ identity genes, required for their normal development. Similarly, the absence of *FLO* from whorl 3 may be necessary for normal stamen development[24]. If true, an additional piece of the floral development puzzle will begin to fall into place: the spatial patterns of expression of the organ homeotic genes are the key to organ identity in developing flowers, but these genes seem to be regulated by an earlier-acting group of genes, some of which have meristem-conversion phenotypes.

The pattern of *FLO* expression also illustrates that early and late genes need not interact in a simple hierarchical fashion. For example, the absence of *FLO* RNA in whorl 3 might be explained if the combination of organ identity gene functions in this whorl (b and c) inhibited *FLO* expression[24]. This hypothesis has been recently confirmed by the observation that *FLO* RNA is present in whorl 3 of mutants lacking b or c (S. Hantke, R. Carpenter and E.S.C., unpublished results). Thus,

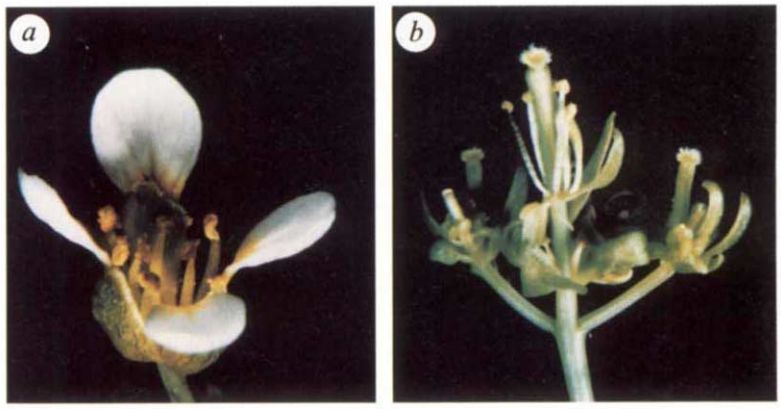

FIG. 6 Additional *Arabidopsis* mutations, thought to interact with the organ identity genes. *a*, A *superman*-1 homozygote, showing extra stamens and reduced ovary. One wild-type function of this gene seems to be preventing b function in whorl 4. *b*, An *apetala*1-1 homozygote, showing partial conversion of floral primordia to inflorescence meristems. Note that each pedicel is capped by a group of flowers, rather than a single flower.

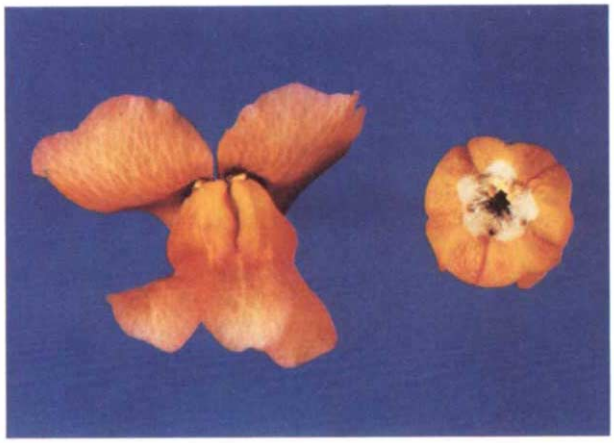

FIG. 7 Face view of wild-type (left) and extreme *cycloidea* (right) *Antirrhinum* flowers. In *cyc* mutants all petals resemble the lowest petal of wild type. Because the lowest petal-lobe of wild type tends to fold back against the tube (Fig. 1), in *cyc* mutants all of the petals are folded back. The genetic backgrounds of the lines illustrated carry mutations in two pigmentation genes, resulting in orange petals.

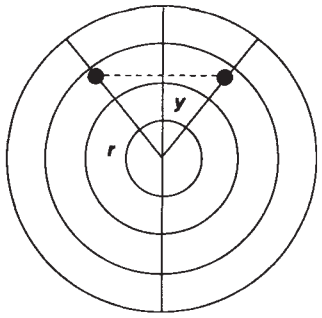

FIG. 8 Polar coordinate model for bilateral symmetry in *Antirrhinum*. The four whorls are shown as concentric rings. The vertical line (*y* axis) indicates where the plane of symmetry bisects the flower (the symmetry plane is at right angles to the plane of the page). The identity of organs at each position in the flower can be determined by direct comparison with Fig. 1*b*. Upper organs are towards the top of the *y* axis and lower organs towards the bottom. Expression of organ identity functions varies along the radius (*r*) and the *cyc* function varies along the *y* axis, increasing in activity towards the top of the flower. Two primordia with the same combination of functions and, hence, the same developmental fate, are shown joined by a dotted horizontal line (these correspond to the two upper petal primordia, see Fig. 1*b*). Mutations eliminating the organ identity functions result in some primordia in 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 primordia in a whorl adopt a fate similar to that of the lower primordia of the wild-type whorl.

even though *FLO* is activated earliest in the floral meristem as a whole, in cells giving rise to whorl 3 organs, b and c functions could be established earlier than *FLO* and therefore regulate its expression.

Timing of gene action

It is therefore possible that the time and duration of gene action, as well as its place, is of central importance to understanding the genetic interactions underlying floral development. The timing of *DEF* gene function in *Antirrhinum* and *AP2* and *AP3* gene function in *Arabidopsis* have been studied. One of the effects of *def* mutations is that sepals grow in place of petals in whorl 2, presumably because the b function is absent. The unstable allele, *def-621*, shows distinct clonal patches of petal tissue on the sepals in whorl 2 (ref. 8). These patches are separated from the surrounding sepal epidermal cells and the underlying mesophyll tissue by sharp boundaries. They can be explained by somatic excision of a transposon from the *DEF* locus, restoring gene function and hence petal morphology. Some of the patches consist of as few as four cells, presumably reflecting restoration of *DEF* function in the last few cell divisions of organ development. This indicates that DEF can act at late stages to direct cells of whorl 2 toward a petal developmental program. But the phenotypic effects of the *def* mutation can also be detected at early developmental stages, when petal and sepal primordia acquire distinct morphologies. The *DEF* product is thus active in whorl 2 throughout its development, from the early stages when petal primordia become distinct to the final cell divisions of the petal. This is consistent with the observed expression pattern of *DEF* throughout flower development[13].

Additional evidence for the action of genes required for the b function over an extended period has come from studies on the effects of changing environmental conditions during flower morphogenesis. One *ap3* allele in *Arabidopsis* confers a temperature-sensitive phenotype, with a restrictive temperature of 29 °C, and a permissive one of 16 °C. By shifting the growth temperature during organogenesis, it has been shown that *AP3* activity is required in whorl 2 from early periods up to relatively late stages of flower development, including the time when visible differentiation is in progress[5]. As *ap3* seems to affect the same b function as does *def*, these results are consistent with the mosaic studies.

In addition to genes acting for extended periods of development, there are other genes with a more transient action. For example, temperature-shift experiments with certain *ap2* alleles (required for the a function) indicate that *AP2* is not required at any time after an early stage of flower development[5]. In this respect, the action of class a genes might be quite different from that of class b, at least in the second whorl. It should be recognized, however, that the temperature-sensitive period of these *ap2* alleles may be a function of the particular allele, or of an underlying temperature-sensitive part of flower development, and may therefore not indicate the full period of *AP2* activity.

Conclusion

Plant form is largely a result of meristem behaviour. We have distinguished between meristems, which produce other meristems or collections of organs, and organ primordia, which develop to a single recognizable organ. We have described on this basis two classes of homeotic mutation: those which affect meristem identity, and those which affect the fate of floral organ primordia. This last class contains two types: organ identity can be inappropriate to a floral whorl, or in the case of zygomorphic flowers, inappropriate to a position in a whorl. Study of the phenotypes of the mutations, of the patterns of expression of the RNAs coded by the genes, and of the predicted amino-acid sequences of the gene products has led to specific models for the way in which these genes direct flower development. Further study of these and other mutations may in the future lead toward solutions to additional problems in flower development, such as the way in which a vegetative meristem converts to an inflorescence meristem, or the nature and control of the complex patterns of cell differentiation necessary in each organ after its identity has been specified.

Two general conclusions can be reached from the comparison of flower development genes in two distantly related species. The first is that the basic mechanisms that define organ identity in developing flowers appear to be the same in both species. Genes exist whose mutants have similar phenotypes and similar interactions, and a single model can explain the action of these genes in both species. In those cases known so far, the genes with similar phenotypes are homologous at the DNA level. This

FIG. 9 The *floricaula* homozygous *Antirrhinum* inflorescence, in which each flower is replaced by a shoot.

shows that the basic processes of floral organ specification are evolutionarily old, and that the very different forms taken by flowers of different plant families, at least among the dicotyledonous plants, result from more recent modifications of developmental processes. This recognition allows experimental approaches to morphological evolution as wild-type or altered *Antirrhinum* genes can now be introduced into *Arabidopsis* plants with mutations in the homologous gene. When *Antirrhinum* transformation procedures are discovered, the converse experiment will also be possible. Such experiments will reveal whether the evolutionary changes that lead to the very different flower forms of the two plants are due to changes in the homeotic genes themselves, changes in their regulators, or changes in the downstream genes that they regulate.

The second conclusion is that the processes of flower development that have been revealed are remarkably size-invariant. *Arabidopsis thaliana* flowers, when mature, have a mass of around 550 μg, whereas *Antirrhinum majus* flowers have a mass more than 1,000 times greater. Nonetheless, the basic processes that specify their meristem and organ types appear to be homologous. We soon may learn how the relative timing of cell division and regulatory gene expression are controlled, and how the domains of gene expression are developmentally regulated, and thus comprehend how similar structures of very different sizes may evolve from a common ancestor. □

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Tomographic imaging of subducted lithosphere below northwest Pacific island arcs

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The seismic tomography problem does not have a unique solution, and published tomographic images have been equivocal with regard to the deep structure of subducting slabs. An improved tomographic method, using a more realistic background Earth model and surface-reflected as well as direct seismic phases, shows that slabs beneath the Japan and Izu Bonin island arcs are deflected at the boundary between upper and lower mantle, whereas those beneath the northern Kuril and Mariana arcs sink into the lower mantle.

THE depth range involved in the recycling of material from the Earth's surface back into the mantle has been among the most controversial issues in geodynamics in the past two decades. Many studies have investigated the fate of subducted lithosphere near the transition between the upper and lower mantle to learn more about the amount of mass transport across this boundary[1]. Although many seismologists explain the lower-mantle heterogeneity beneath Middle America on the basis of deep subduction of the Farallon/Pacific plate[2–8], the subject has remained controversial in studies of northwest Pacific subduction zones.

In the northwest Pacific, where old lithosphere of the Pacific plate subducts below island arcs[9], Jordan and coworkers[10–13] concluded from residual sphere analyses that the subducted slab continues below the deepest earthquakes, to depths of at least 1,200 km. There is some evidence to support this interpretation[14–17]; other studies, however, have suggested that the images of subducted slabs determined by this method can be influenced by noise in the data and by effects of lower-mantle and near-receiver structure[18–22].

Variations in the propagation velocity of seismic waves owing to the presence of subducted lithosphere can be imaged by seismic tomography. The tomographic method employed in this study involves the interpretation of seismic-wave arrival-times determined from millions of seismograms in terms of the Earth's three-dimensional structure. The controversial issues concerning deep subduction have not so far been satisfactorily resolved by

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