Development 121, 2143-2154 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

phantastica: a gene required for dorsoventrality of leaves in *Antirrhinum majus*

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SUMMARY

To understand better the mechanisms that lead to dorsoventrality in the lateral organs of plants, mutants at the *phantastica* (*phan*) locus of *Antirrhinum majus* have been identified and characterised. The leaves, bracts and petal lobes of *phan* mutants show varying degrees of reduction in dorsal tissues, indicating that *phan* is required for establishing dorsal cell identity. Each *phan* mutant produces a variety of different leaf morphologies, but has a characteristic and relatively constant floral phenotype. In several different forms of *phan* mutant leaves and petal lobes, novel boundaries between dorsal and ventral cell types form ectopic axes of growth, suggesting that *phan*-dependent dorsal cell identity is required for lateral growth of the wild-type leaf and petal lobe. Comparisons between the development of wild-type and mutant petals or leaves reveal that *phan* acts early in development of these lateral organs. The possible role of the *phan* gene in evolution of different leaf forms is discussed.

Key words: *Antirrhinum majus*, leaf development, dorsoventrality, transposon mutagenesis, *phantastica*

INTRODUCTION

The leaves of most plants have two striking morphological features: they are produced laterally from the stem axis, and they are considerably broader and longer than thick. Their flattened shape, which presents a large area to incident light, involves relatively little tissue and can be considered an adaptation to their photosynthetic role. In dicotyledonous plants with entire leaves, the characteristic leaf shape results from two major shifts in the pattern of growth early in development (Avery, 1933; Dubuc-Lebreux and Sattler, 1980; Foster, 1936; Jeune, 1981; Poethig and Sussex, 1985a,b). The first occurs in a group of initial cells on the flanks of the vegetative meristem (Fig. 1A) which divide and expand to form a leaf primordium with a novel axis of growth away from that of the stem (Fig. 1B). This axis, the proximodistal axis of the leaf, is represented in the mature organ by a line along the midrib. The second occurs in cells towards the dorsal (adaxial) side of the leaf primordium which continue to contribute to proximodistal growth, but also undergo a shift in polarity of division to form the leaf laminae laterally (Fig. 1C). The more ventral part of the primordium shows little or no lateral growth and forms the ventral midrib of the mature leaf (Fig. 1D). The difference in the pattern of division between dorsal and ventral cells of the leaf primordium suggests that dorsoventrality is defined early in organ development. Further dorsoventral differences within the leaf become apparent later, as specialised cell types differentiate in layers perpendicular to the dorsoventral axis.

Other lateral organs of the plant, including bracts and floral organs are considered at least in some parts homologous to leaves (discussed by Hagemann, 1984). Their initiation from inflorescence or floral meristems is similar to the production of leaf primordia at the vegetative apex, and may also be followed by lateral proliferation which leads to flattening (e.g. Green and Linstead, 1990; Tepfer, 1953). They also show dorsoventral differences in cell type. Dorsoventrality is most obvious in bracts, sepals and petals, where a flattened shape is associated with roles in protecting the developing flower or attracting pollinators, but it is also apparent in the pollen sacs of stamens and the the ovary wall of the gynoecium (Goebel, 1905). The mechanisms that define dorsoventrality may therefore be similar in all lateral organs of the plant.

At the developmental stage when dorsoventrality becomes apparent, leaf primordia are small and consist of relatively few cells. They are therefore not ideally suited to surgical studies or to biochemical analysis. As a result, relatively little is known of the mechanisms that determine the form of lateral organs, including their dorsoventrality. An alternative method of study is to characterise mutants in which early development has been disrupted. This has been successfully applied to studies of determination in meristems and floral organ primordia of maize (DeLong et al., 1993; Smith et al., 1992; Vollbrecht et al., 1991), *Antirrhinum* (reviewed by Coen and Carpenter, 1993) and *Arabidopsis* (reviewed by Weigel and Meyerowitz, 1994). One major advantage of these species is that they allow genes which have been identified by mutation to be isolated. In the case of *Antirrhinum* and maize, this approach has exploited transposon-induced mutations, which provide the basis for gene isolation by transposon tagging (Shepherd, 1988). Subsequent detection of transcripts by in situ hybridisation has

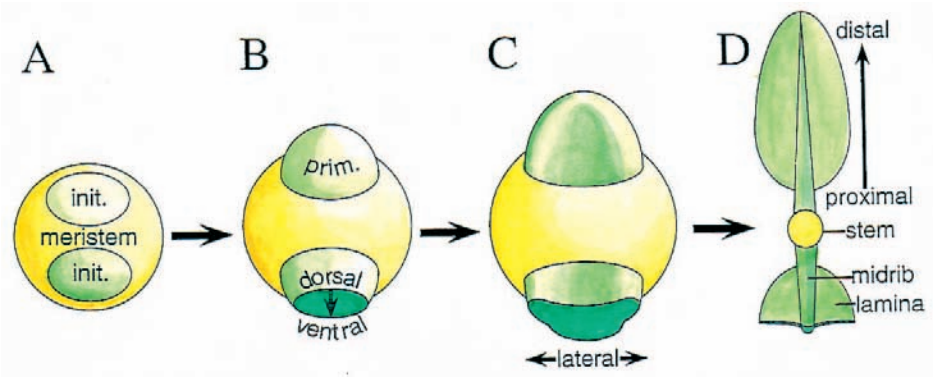


Fig. 1. Leaf initiation. Successive stages in leaf initiation at the vegetative apex are represented as viewed from above. (A) Two groups of leaf initials (init.) destined to form leaf primordia are shown within the flanks of the vegetative meristem. (B) Each groups of initials subsequently forms a primordium (prim.) with an axis of growth away from that of the apical meristem. (C) Lateral proliferation in the dorsal part of the primordium forms the laminae, and the leaf therefore shows dorsoventral asymmetry which becomes more pronounced in mature leaves (D).

revealed that many of the isolated genes show patterned expression in meristems and organ primordia consistent with their roles in determining the developmental fates of these tissues.

Here we describe the variable effects of four mutations at the *phantastica* (*phan*) locus of *Antirrhinum majus*. Mutant phenotypes suggest that *phan* expression is involved in all aspects of dorsoventrality in leaves, bracts and petals: from specifying the position of laminal initiation early in organ development, to determination of dorsal cell types at a later stage. They also suggest that subtle changes in the level or pattern of *phan* activity can give rise to a variety of organ morphologies. At least one of the *phan* mutant alleles shows the genetic instability characteristic of transposon-induced mutations, which should allow its isolation by transposon-tagging.

MATERIALS AND METHODS

Origin of *phan* mutants

The wild-type line, JI.75, was produced at the John Innes Institute, Norwich, UK (Harrison and Carpenter, 1979). The mutants *phan*^{ambigua} (*phan*-250G) and *phan*^{antiqua} (*phan*-249G), originally isolated by Baur (1926) were obtained from Zentralinstitut für Genetik und Kulturflanzenforschung, Gatersleben, Germany, and inbred as homozygous stocks for at least a further three generations. The *phan*-607 mutant arose amongst families produced by self-pollination of line JI.75, in a programme designed to identify transposon-induced mutations (Carpenter and Coen, 1990), and was kindly provided by R. Carpenter and E. S. Coen, John Innes Centre, Norwich, UK. It was shown to carry a single recessive mutation which was unable to complement the *phan*-249G and *phan*-250G mutant alleles. A fourth mutant allele, *phan*-552, was obtained in a screen for new mutations at the locus, when plants of the mutant line, *phan*-249G, were crossed as female parent to the transposon-rich, wild-type line, JI.75. The resulting F_1 seeds were germinated on moist vermiculite, and seedlings examined for alterations in the morphology of cotyledons and the first pair of leaves. A single *phan* mutant seedling was identified amongst approx. 18,000 wild-type siblings. Because this mutant showed the floral pigmentation phenotype expected of an F_1 plant, it appeared not to be the result of accidental self-pollination of the mutant parent. Therefore, the simplest explanation for the leaf phenotype of the new mutant was that this plant carried a newly mutated allele, *phan*-552, heterozygous with the *phan*-249G allele. Alterations to petal morphology in the new mutant were less severe that those in its mutant parent, *phan*-249G (see Results), suggesting that the *phan*-552 allele was dominant to *phan*-249G and responsible for the new phenotype. This was supported by analysis of plants produced by self-pollination of the new mutant, approximately one quarter of which resembled line 249G and were assumed to be *phan*-249G/*phan*-249G homozygotes, while the remainder resembled their parent and were assumed to consist of both heterozygotes and *phan*-552/*phan*-552 homozygotes. In order to obtain plants that were homozygous for the *phan*-552 allele, the new mutant was backcrossed to its wild-type parent, JI.75, to give F_1 progeny carrying either the *phan*-249G or *phan*-552 allele. One quarter of the progeny of each F_1 plant consisted of either severe *phan* mutants, assumed to be homozygous for the *phan*-249G allele, or plants that resembled the new *phan* mutant in morphology, suggesting that they were homozygous for the *phan*-552 allele. Plants assumed to be *phan*-552/*phan*-552 homozygotes were subsequently maintained by inbreeding.

Because the *phan*-552 allele arose in line JI.75, which carries active transposons, it was potentially transposon-induced. This was further supported by the observation that the allele was germinally unstable: 10% of the progeny resulting from self-pollination of the *phan*-552/*phan*-249G mutant had a wild-type *Phan*$^{+}$ phenotype. In contrast, *phan*-607, which was also obtained from line JI.75, and *phan*-249G and *phan*-250G produced only *phan* mutant progeny on self-pollination, suggesting that the mutant alleles in these lines did not carry active transposons.

Plant culture

Plants used for morphological analysis were grown at 20°C in a 16 hour light - 8 hour dark cycle, with illumination of 50 μmol m^{-2} s^{-1} from metal halide lamps. Those for genetic analysis were grown as described by Carpenter et al. (1987).

Microscopy

Wax embedded leaf material for histological sectioning was fixed and dehydrated using the method described by Jackson et al. (1994). 5-10 μm sections were stained with toluidine blue using the method of Sakai (1973), and the wax removed with Histoclear (CellPath plc, Hemel Hempstead, UK) before mounting. Alternatively, tissue was prepared as described by Roland and Vian (1991), embedded in Agar 100 epoxy resin (Agar Scientific Ltd., Stansted, UK) and 1 μm sections stained with toluidine blue. Scanning electron microscopy of apices was carried out on resin replicas made using a modification of the method described by Green and Linstead (1990). Specimens of plant material were coated in Extrude vinylsiloxane dental impression medium (Kerr UK Ltd., Peterborough, UK), which polymerised to form moulds. After about 30 minutes, the moulds were removed and infiltrated with Agar 100 epoxy embedding resin for 15 minutes at 70°C under vacuum. The vacuum was then released, the moulds drained of excess resin, and the remaining resin allowed to polymerise at 65°C for 16-24 hours. Resin replicas were gold coated before examination at ambient temperature in a scanning electron microscope.

RESULTS

Dorsoventrality in the wild-type *Antirrhinum* leaf

The wild-type *Antirrhinum* leaf shows dorsoventral asymmetry

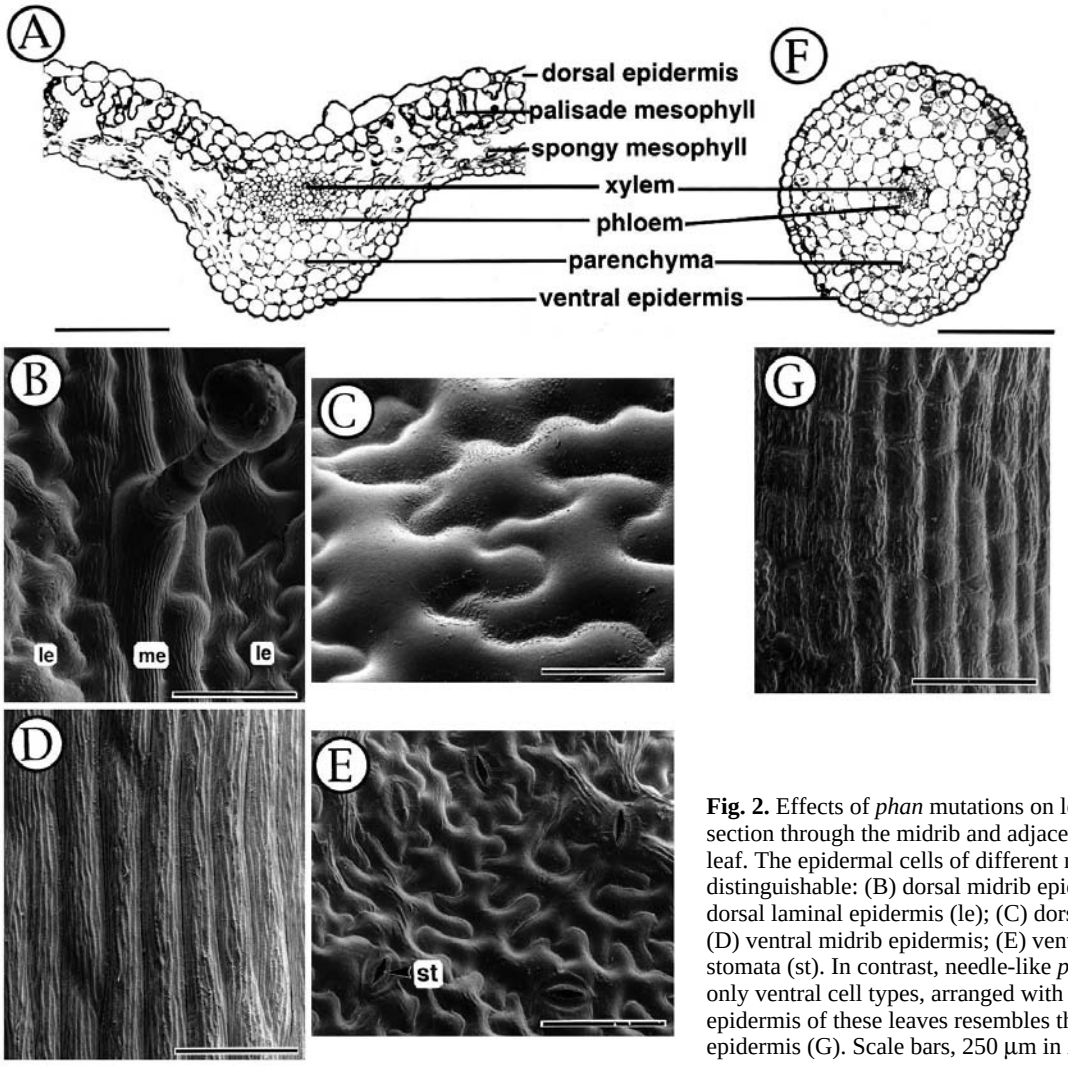


Fig. 2. Effects of *phan* mutations on leaf anatomy. (A) Transverse section through the midrib and adjacent laminal regions of a wild-type leaf. The epidermal cells of different regions are morphologically distinguishable: (B) dorsal midrib epidermis (me) and neighbouring dorsal laminal epidermis (le); (C) dorsal laminal epidermis; (D) ventral midrib epidermis; (E) ventral laminal epidermis, including stomata (st). In contrast, needle-like *phan* mutant leaves consist of only ventral cell types, arranged with radial symmetry (F). The epidermis of these leaves resembles that of wild-type ventral epidermis (G). Scale bars, 250 μm in A and F; 50 μm in B-E and G.

in two respects. First, the laminae, which make the leaf considerably wider than it is thick, arise towards the dorsal side of the midrib (Fig. 2A). Therefore the dorsal and ventral parts of the leaf are not mirror images of each other and the mature leaf possesses only one plane of symmetry, oriented vertically and running along the midrib. Secondly, both the laminae and midrib show a distinct arrangement of tissue layers. In regions of the lamina between veins, four distinct cell types are recognisable along the dorsoventral axis: dorsal epidermis, palisade mesophyll, spongy mesophyll and ventral epidermis. The ventral epidermis, shown in Fig. 2E, consists of cells which are smaller and more complex in outline than those of the dorsal epidermis (Fig. 2C) and is further characterised by the presence of stomata, which are absent from the dorsal surface of the leaf. Dorsoventral differences are also apparent in major veins, including the midrib. Here the vascular tissue consists of an arc of xylem on the dorsal side of an arc of phloem which together are surrounded by parenchyma (Fig. 2A). The epidermal cells which make up the ventral surface of the midrib are elongated along the proximodistal axis and are therefore distinct from the epidermal cells of the lamina (compare Fig. 2D,E). They can also be distinguished from the cells at the dorsal surface of the midrib, which are shorter and include hair cells (Fig. 2B). The petiole shows the same dorsoventral differences as the leaf, although it contains a much lower proportion of laminal tissue (data not shown).

Effects of *phantastica* mutations on leaf anatomy

The four *phantastica* (*phan*) mutants show similar vegetative phenotypes, although leaf morphology varies with the developmental stage of each plant. Leaves produced at, and above, the fifth node of *phan* mutants are typically needle-like and show no evidence of dorsoventrality (Figs 3B,D, 4A) They lack laminae and all cell-types associated with the dorsal region of the wild-type leaf. Internally, they consist entirely of ventral tissue types: parenchyma, phloem and xylem, which are arranged in concentric cylinders (Fig. 2F). They are therefore radially symmetrical in transverse section. The epidermis of these needles, shown in Fig. 2G, resembles that of the ventral midrib of a wild-type leaf, except that it also shows some laminal characters, in that the cells are shorter than those of the ventral midrib and occasional stomata are present, distributed evenly over the leaf surface. The phenotype of needle-like leaves suggests that *phan* is necessary for a dorsalising function which determines all aspects of dorsoventrality in leaves, including initiation of the laminae and development of dorsal cell types.

In contrast, the cotyledons and first three pairs of leaves of

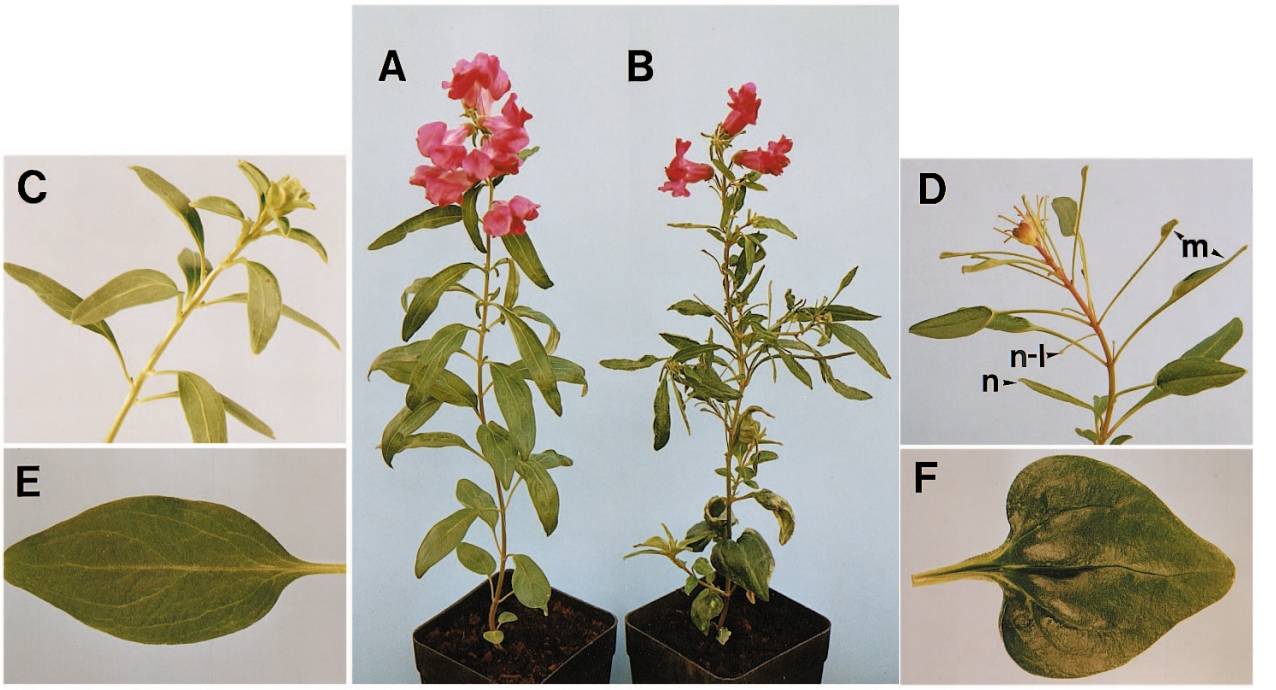


Fig. 3. Effects of *phan* mutations on morphology. (A) A wild-type plant and (B) the *phan*-607 mutant. (C) The vegetative shoot of a wild-type plant, and (D) the corresponding region of a *phan* mutant with narrow (n), needle-like (n-l) and mosaic (m) leaves. (E) A leaf from the second node of a wild-type plant, and (F) a broad, heart-shaped leaf typical of the equivalent node of a *phan* mutant.

phan mutants are broader and more heart-shaped than the corresponding leaves of wild-type plants (Fig. 3F), containing more cells in transverse section (data not shown). They are further characterised by patches of ventral epidermal tissue, which include stomata, on their dorsal surfaces (Fig. 5A,C). The boundary between these regions of ectopic ventral epidermis and the surrounding dorsal epidermis forms a ridge in an axis perpendicular to the leaf surface (Fig. 5B). In section, these ridges resemble the edges of a wild-type leaf, containing palisade mesophyll on the side covered by dorsal epidermis, and spongy mesophyll covered by ventral epidermis on the other (compare the ridges in Fig. 5D with the leaf margins in Fig. 6A). Within the ridges, the leaf lamina consists of ventral epidermal tissue on both upper and lower surfaces, with only spongy mesophyll between. Larger patches of ventral epidermis tend to be elongated in an axis parallel to the leaf venation, while smaller patches are more isodiametric. Patches are also larger and more frequent in the proximal part of the leaf where their size, shape and distribution resemble those of clones showing altered levels of chlorophyll in both *Antirrhinum* (Hudson et al., 1993) and tobacco (Poethig and Sussex, 1985b). This suggests that each may consist of clonally related cells, initiated relatively late in leaf development.

The transition between early heart-shaped leaves and later needle-like leaves of *phan* mutants is rarely abrupt. Leaves at intermediate nodes are typically narrower than those of wild-type or are mosaics of needle-like and laminal tissues (Fig 3D). Narrower leaves contain fewer cells in transverse section than wild-type and their laminae arise from more dorsal positions on the midrib (Fig. 6A). The most common form of mosaic leaf is one in which the proximal region is needle-like and the distal region laminal (Fig. 3D), although leaves with needle-like tissue distal to laminal tissue also occur. In both cases, the lamina forms an additional dorsal axis at the junction with needle-like tissue (Fig. 6B). The morphology of the boundary between ventralised (needle-like) tissue and dorsal (laminal) tissue therefore resembles that found at the boundary of patches of ventral epidermal cells in earlier, broad leaves of *phan* mutants.

Effects of *phan* mutations on leaf morphogenesis

The morphogenesis of needle-like leaves was compared with that of wild-type. Leaf primordia first become visible as bulges on the flanks of the apical meristem. At this early stage, mutant and wild-type primordia are indistinguishable (compare Fig. 7A,C). The proximal region of each primordium, the leaf buttress, extends laterally around the circumference of the apex, and therefore appears flattened. The distal region is also flattened, though less so. The dorsal flanks of the wild-type primordium in this region grow both distally and laterally to accentuate the flattened shape soon after initiation (Fig. 7A,B). In contrast, growth in the equivalent part of the *phan* mutant primordium is limited to the distal axis, and this produces a needle-like leaf (Fig. 7C). Only the very proximal part of the mutant leaf, produced from the leaf buttress, retains any flattening (Fig. 4A,D). Early developmental stages of mosaic leaves were also observed. In these, initiation of the laminae occurred at a more dorsal position on the primordium than in wild-type (Fig. 7D).

Development of the early, broader leaves of *phan* mutants was also characterised. The apical meristem at this stage of development is larger than that of more mature plants. Mature mutant leaves are broader than those of wild-type, containing more cells in transverse section, and mutant primordia appear

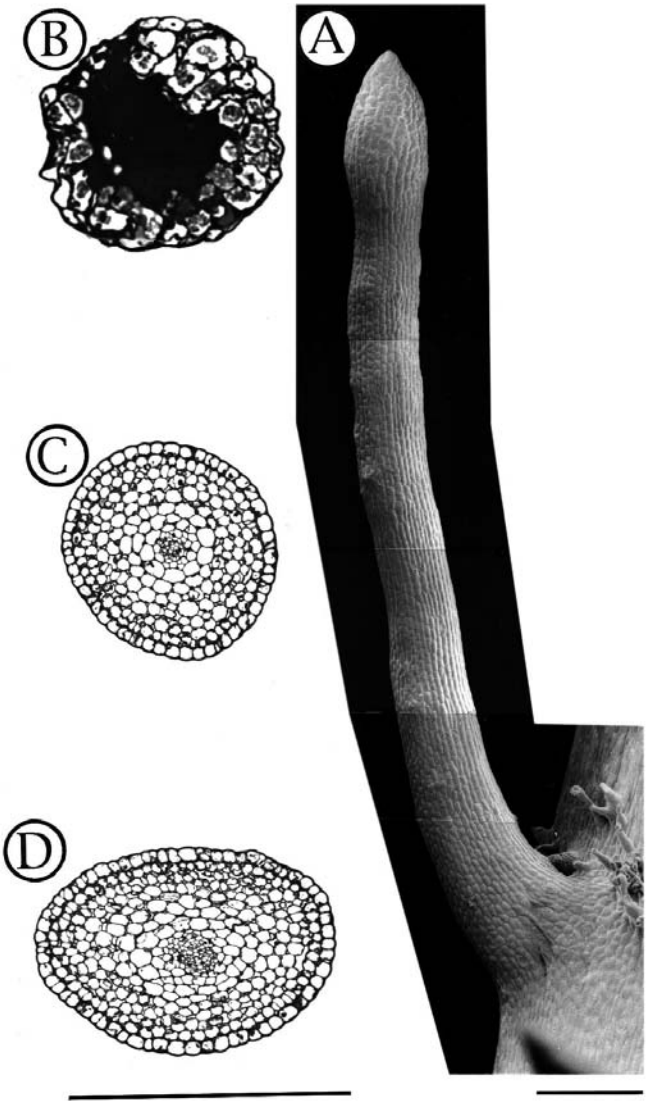


Fig. 4. A needle-like *phan* mutant leaf. (A) A mature needle-like leaf from a *phan* mutant, and transverse sections cut from the distal (B), middle (C) and proximal (D) parts of the same leaf. Only the most proximal region, derived from the leaf buttress, shows dorsoventral flattening, although it consists of only ventral cell types and has radially symmetrical vascular tissue. The darkly staining cells, of unknown function, in the distal region are also found in the tips of wild-type leaves. Scale bars, 250 μm.

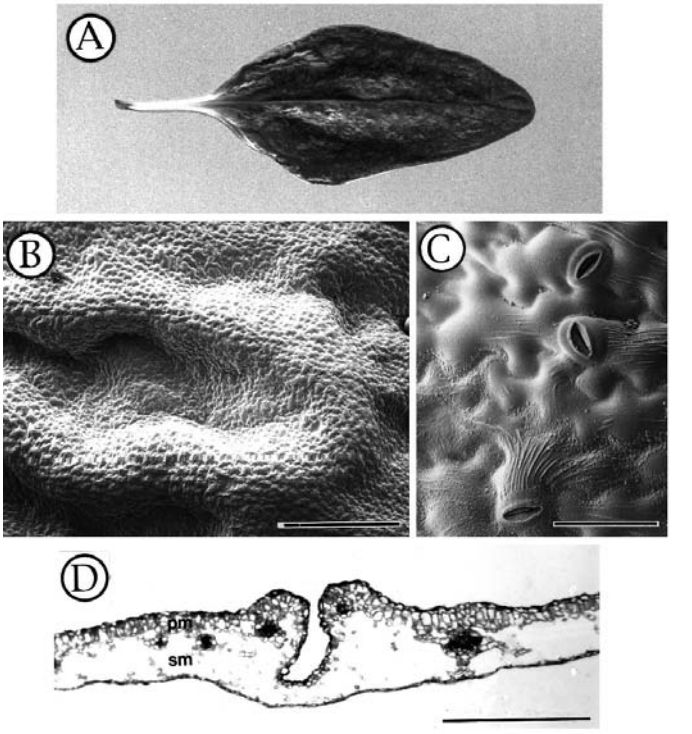


Fig. 5. Ectopic patches of ventral cell types in *phan* mutant leaves. (A) A leaf from the third node of a *phan* mutant plant. Patches of ventral epidermal tissue appear lighter than the surrounding dorsal epidermis and the leaf surface is uneven due to the presence of frequent ectopic laminal ridges. (B) Scanning electron micrograph of an ectopic patch of ventral tissue and the surrounding ectopic ridge. (C) Numerous stomata, characteristic of ventral epidermis, are present within the patches. (D) A transverse section through a patch of ventral tissue. The region within the ridges lacks palisade mesophyll cells (pm) but retains cells of the spongy mesophyll (sm). Scale bars, 500 μm in A, B and D; 50 μm in C.

slightly broader than those of wild-type soon after initiation (Fig. 7E,F). The initiation of ectopic laminal tissue, which surrounds patches of ventral cells in early mutant leaves, was not observed, suggesting that it occurs later in leaf development.

Effects of *phan* mutations on corolla morphology

Although the four *phan* mutants are virtually identical in vegetative development, each shows a characteristic floral morphology which does not vary between individual flowers of the same mutant line. Only development of the corolla is affected, and the remaining organs (sepals, stamens and carpels) are indistinguishable from those of wild-type flowers.

The wild-type corolla consists of five petals: two upper, two lateral and one lower. All are united over the proximal part of their length to form the corolla tube, at the distal end of which, the upper pair are separated from the lower three in the hinge region. The lower three petals remain further united forming the corolla face, before separating into distinct petal lobes (Fig. 8A). In analogy to the leaf, the outer (abaxial) surface of the corolla can be considered ventral, and the inner (adaxial) surface dorsal. As in leaves, the epidermal cells which form the two surfaces show different morphologies. In the petal lobes and corolla face, cells of the dorsal epidermis are characterised by conical projections, while ventral epidermal cells form a smooth surface, although this is punctuated by hairs (Noda et al., 1994).

Of the four mutations, *phan*-250G has the most severe effect on corolla morphology. The petal lobes are reduced to needles consisting of cell types characteristic of the ventral part of a wild-type petal (Fig. 8B) This ventralised morphology can therefore be considered homologous to that of needle-like leaves. Although the upper and lowest petal lobes are each reduced to a single needle, each lateral petal lobe is represented by a pair of needles.

Whereas petals of the *phan*-250G mutant are reduced to needles, those of the *phan*-249G and *phan*-552 mutants consist of reduced petal lobes with needles arising from their ventral

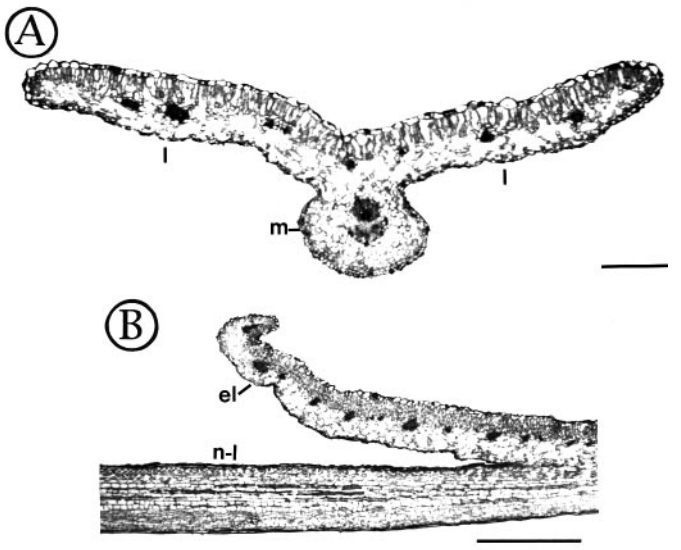


Fig. 6. Anatomy of *phan* mutant leaves with laminae. (A) Transverse section of a narrow *phan* mutant leaf. The laminae (l) are produced at a more dorsal position on the midrib (m) than in wild-type (compare with Fig. 2A). (B) A longitudinal section through a mosaic *phan* mutant leaf at the boundary between laminal and needle-like tissue. In this region, the lamina forms an ectopic dorsal axis (el), distinct from that of the needle-like tissue (n-l). The distal part of the leaf is to the left. Scale bars, 250 μm in A and 500 μm in B.

surfaces (Fig. 8C,D). Each needle is the most distal part of a ridge of ventral tissue which extends between the points where the petal lobe joins its neighbours (Fig. 8E).

To determine the relationship between the needles and petal lobes in these mutants, floral development of the mutant *phan*-249G was compared with that of wild type. In wild-type flowers, five petal primordia, initiated on the flanks of the floral meristem, give rise to the petal lobes (Fig. 9A-E). A more proximal group of cells, which encircles the floral meristem in a continuous band, divides to displace the lobes distally and form the corolla tube. Only the ventral surface of the corolla is visible until the petal lobes expand and reflex to reveal their dorsal surfaces.

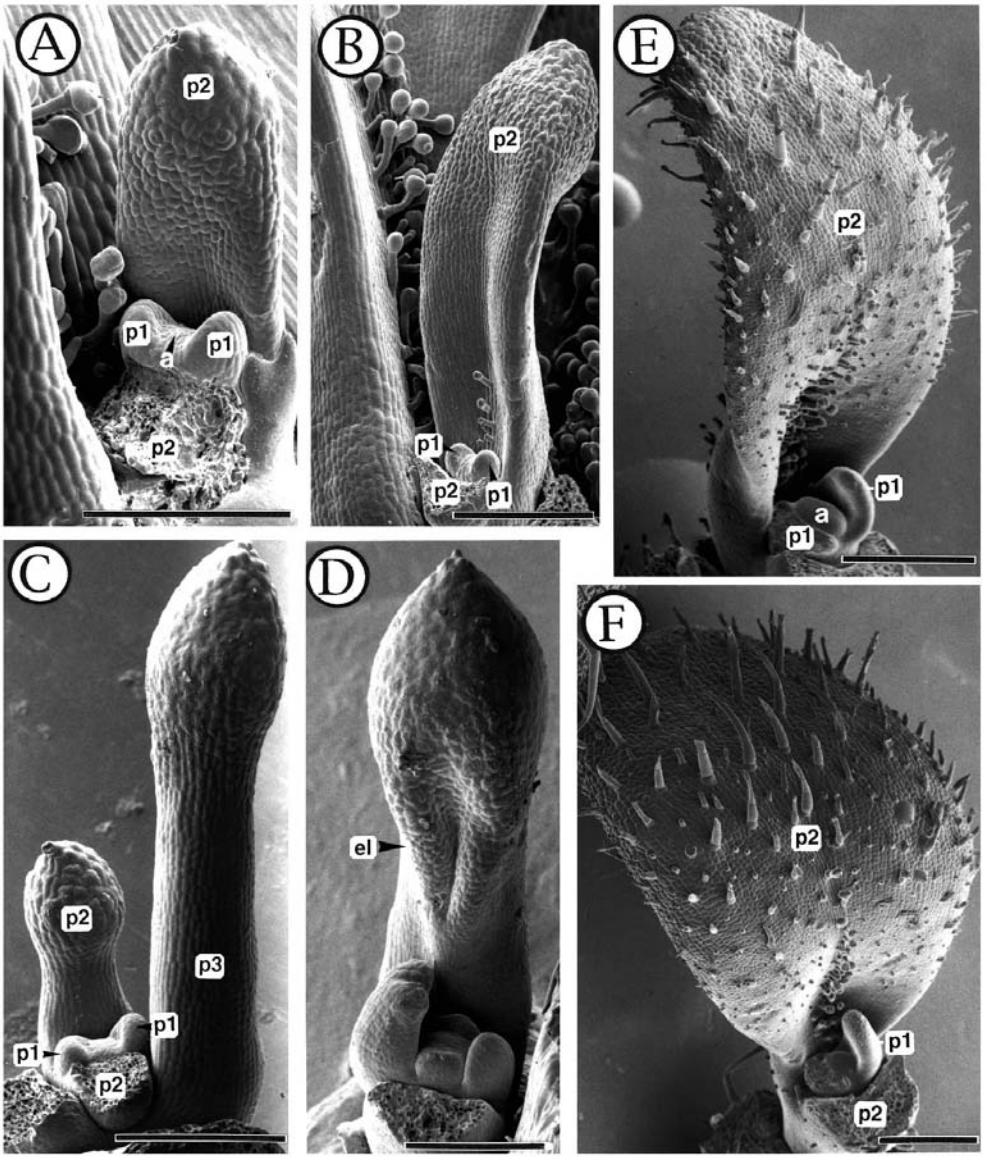


Fig. 7. Morphogenesis of wild-type and *phan* mutant leaves. (A) A wild-type apex late in vegetative development. Two newly initiated leaf primordia (p1) flank the apical meristem (a). One of the older pair of leaves (p2) has been removed. At this stage, p2 shows obvious dorsoventral flattening. Subsequent growth rapidly extends the leaf axis and also increases flattening to the stage shown in B. The equivalent region of a *phan* mutant shoot is shown in C. Newly initiated primordia (p1) are indistinguishable from those of wild-type, but fail to undergo lateral expansion and therefore extend into needle-like leaves (p2 and p3). (D) Formation of a mosaic leaf. The oldest leaf shown is needle-like in its proximal region but forming an ectopic lamina (el) at its distal end. The lamina is at a more dorsal position than in wild-type (compare with B) and extends across the primordial axis. (E) A wild-type apex early in development. The the first true leaf of the plant is the largest shown (p2). The equivalent *phan* mutant leaf (F) is slightly broader than wild-type, but has yet to show ectopic laminal tissue characteristic of early mutant leaves. All scale bars, 250 μm.

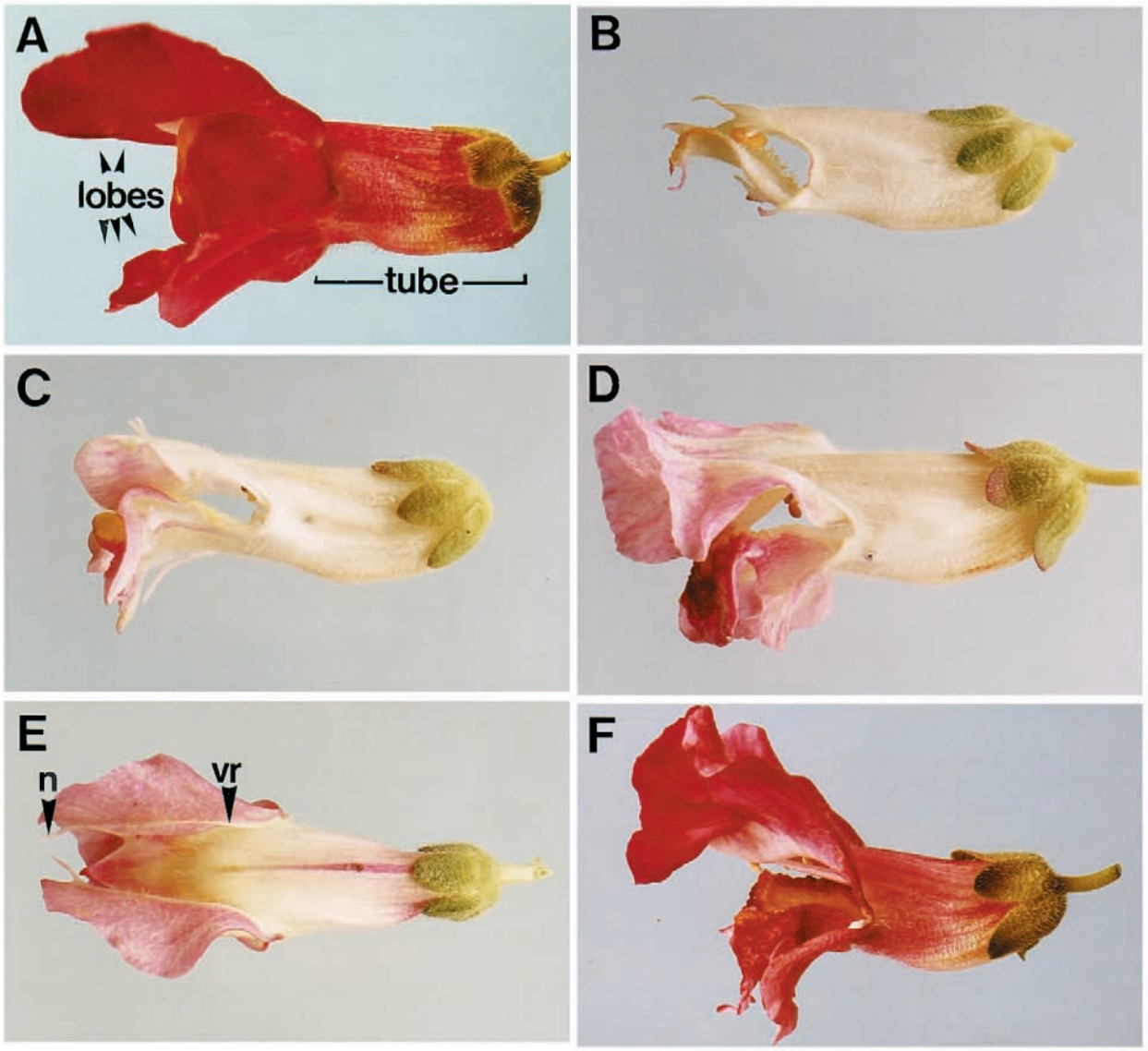
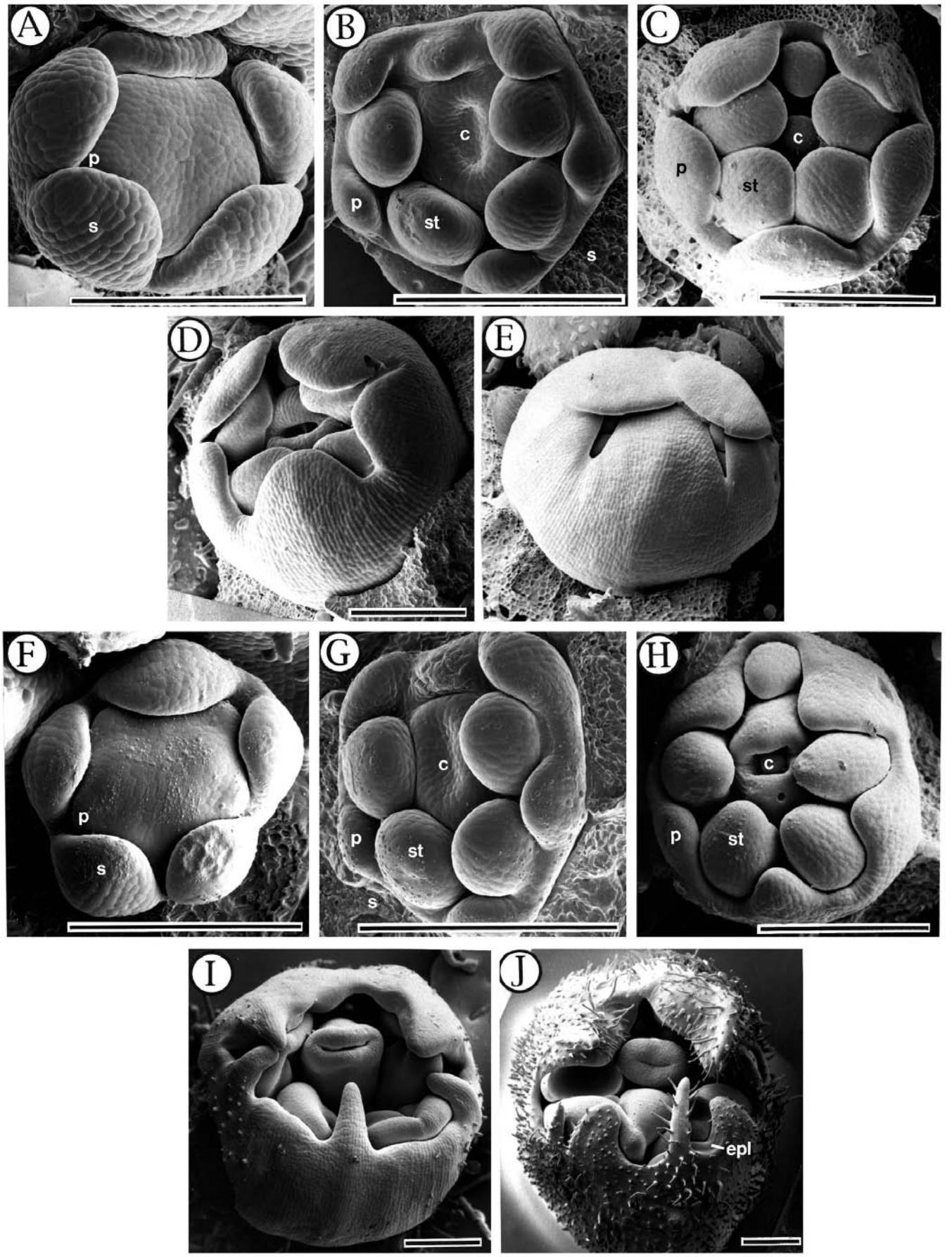


Fig. 8. Effects of *phan* mutations on flower morphology. The wild-type flower (A) consists of five petals which are united proximally to form the corolla tube, but separate distally into five petal lobes. (B) A flower of the *phan*-250G mutant in which petal lobes have been reduced to needles. Progressively lesser reductions in lobe tissue are shown by the *phan*-249G (C) and *phan*-552 (D) mutants. (E) The needles in these mutants (n) form the most distal part of a ridge of ventral tissue running across the ventral surface of the corolla. This ventral ridge (vr) can be seen as lighter coloured tissue. (F) A flower of the *phan*-607 mutant. Differences in corolla pigmentation result from different combinations of alleles affecting anthocyanin synthesis in the inbred lines.

At initiation, the petal primordia of the *phan*-249G resemble those of wild-type flowers (Fig. 9F,G). However, their distal regions fail to grow laterally and extend only distally to form needles (Fig. 9H-J). A novel axis of growth is initiated on the dorsal slope of each primordium, and this produces a reduced petal lobe (Fig. 9J). The ridge of ventral tissue associated with the needles represents the lateral edges of the early petal primordia. The lobes remain relatively inconspicuous until the flower opens, when they expand and reflex to displace the needles ventrally. The morphology of these petals can therefore be considered homologous to that of mosaic mutant leaves with needle-like tissue distal to laminal tissue. In both cases, the most distal part of the primordium develops without dorsoventrality, to form needle-like tissue, while a novel dorsal axis of growth is initiated at the boundary between dorsal and ventral tissue types.

The effect of the *phan*-607 mutation on corolla morphology is similar, but less severe than those of the other mutations. The petal lobes are larger and needles are rarely formed (Fig. 8F). The ridge of ventral tissue may remain united with the petal lobes as far as their distal edges. Because the lobes undergo more lateral expansion than the ridge, they become folded. A further characteristic of all mutants, except *phan*-250G, is that patches of ventral epidermis are seen on the dorsal surfaces of their petal lobes and corolla face. As is the case with patches of ventral epidermis in heart-shaped mutant leaves, the boundary between dorsal and ventral epidermis forms a ridge (Fig. 10A). This ridge has a similar morphology to the edge of a wild-type petal (Fig. 10B), and can therefore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to the edge of a wild-type petal (Fig. 10B), and can therefore be considered an additional petal axis.

Effects of temperature on *phan* mutant phenotypes

All four mutants are more similar to wild-type when grown at higher temperatures. For example, the *phan*-607 mutant grown at 17°C produces large cotyledons with frequent patches of ventral epidermis and ectopic leaf edges on their dorsal surfaces. Subsequent leaves are almost all needle-like (Fig. 11). In marked contrast, leaves of the same line grown at 25°C are similar in outline to those of wild-type and rarely show patches of ectopic ventral epidermis. A similar response to temperature is shown by the other three *phan* mutants. One explanation for this effect is that all four mutants retain some degree of *phan* expression and that this is greater at higher temperatures. Alternatively, the effect of the *phan* mutations may be to reveal the temperature sensitivity of one or more other genes which are also involved in determining dorsoventrality.

DISCUSSION

Although each *phan* mutant shows a variety of leaf phenotypes, they suggest a relatively simple model for the determination of dorsoventrality. This involves a dorsalising function, DF, which is expressed in the most dorsal cells of the wild-type leaf primordium (Fig. 12A). Soon after primordial initiation, a plate of cells near the ventral boundary of the expression domain is induced to change division pattern and so form the laminae by lateral proliferation. DF expression persists in the dorsal part of the lamina where it is necessary to specify the identity of dorsal cell types (dorsal epidermis, palisade and spongy mesophyll).

Needle-like leaves of *phan* mutants lack laminae and dorsal cell types: a phenotype suggesting complete loss of DF (Fig. 12B). Other leaf forms produced by *phan* mutants can be explained by reductions in the domain of DF expression. Expression confined to a more dorsal region, as depicted in Fig. 12C, is predicted to have two effects. First, the laminae will be formed at a more dorsal position and a larger part of the primordium will develop with ventral identity. Secondly, because the primordium is widest at its midpoint, the shifted margin of the expression domain will contain fewer laminal initial cells and the leaf may therefore be narrower. Such narrow leaves with laminae at more dorsal positions are characteristically produced at intermediate nodes of *phan* mutants. In other primordia, the DF domain could be restricted to either a more distal or a more proximal position (Fig. 12D,E). In these cases, the region lacking expression would develop a needle-like morphology. Cells at the boundary of the domain would form laminal tissue, and because the boundary crosses the dorsal side of the primordium, the lamina would form a novel axis of growth in this region. The mosaic leaves of *phan* mutants show morphologies consistent with this prediction. Early leaves of *phan* mutants are broader than wild type and show ectopic patches of ventral cell types. This morphology could result from localised loss of DF relatively late in leaf development (Fig. 12F). Lack of DF would prevent differentiation of dorsal cell types, and the newly introduced boundary of DF expression around each patch of cells would induce formation of ectopic laminal tissue. Proliferation at the introduced boundaries might also contribute to increased lateral growth. Because patches of ventral cells are more frequent in the proximal region, this would produce a heart-shaped mutant leaf with its widest point closer to the petiole than in wild type.

One explanation for the general decrease in dorsal tissues in successive *phan* mutant leaves is that the dependence of DF on *phan* expression increases during vegetative development. Alternatively, it might reflect a gradual loss of *phan* activity remaining in mutants.

Leaf morphogenesis shows a number of similarities to development of insect wings. The wing is formed from a group of

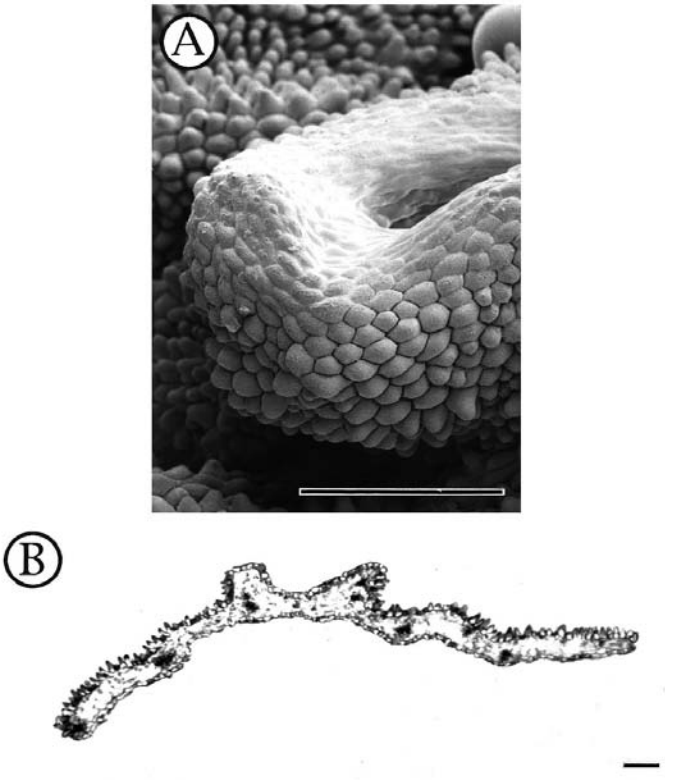

Fig. 10. Ectopic ventral tissue in *phan* mutant petals. (A) A ridge of tissue has formed at the boundary between dorsal epidermis (conical cells) and ectopic ventral epidermis (flatter cells) in a *phan*-607 mutant petal lobe. (B) Transverse section through a mutant petal lobes containing an equivalent patch of ectopic ventral epidermis. The ectopic dorsal ridges resemble the lateral margins of the petal. Scale bars, 250 μm.

Fig. 9. The effects of *phan* mutations on floral development. (A-E) Stages in the development of wild-type flowers. In (A) petal primordia (p) are visible between sepal primordia (s). Sepals have been removed in subsequent stages to reveal development of the corolla. The petal primordia grow in length and laterally to cover the developing stamens (st) and carpels (c), by the stage shown in E. In flowers of the *phan*-249G mutant, petal primordia resemble those of wild-type in early developmental stages (F-G), but subsequently show reduced lateral growth (H). They therefore form spikes at the distal end of the corolla tube (I). Ectopic petal lobes (epl) are formed on the dorsal side of these spikes later in development (J). The epidermal hairs on the corolla of the flower in J are a sepaloid character occasionally seen in both mutant and wild-type plants and therefore not characteristic of *phan* mutant flowers. All scale bars, 250 μm.

Fig. 11. Effects of temperature on the phenotype of *phan* mutants. Plants of the mutant line *phan*-607 were grown at 25°C, 20°C and 17°C. Those at higher temperatures more closely resemble wild type. All plants are the same age.

initial cells, the imaginal disc, present within the larval body. In both wings and leaves, localised cell proliferation leads to growth in a new axis, away from the insect body or stem, and lateral growth produces a dorsoventrally flattened organ (García-Bellido and Mirriam, 1971; Milner and Muir, 1987). Wings, like leaves, also show differences between dorsal and ventral cell types. In *Drosophila*, a transcription factor encoded by the *apterous* (*ap*) gene is necessary both for dorsoventrality and for production of the wing axis (Cohen et al., 1992). Expression of *ap* becomes established within a dorsal domain of the wing imaginal disc during larval development. Juxtaposition of *ap*-expressing and *ap*-nonexpressing cells leads to activation of a number of genes in domains running along the ventral boundary of the *ap* expression domain (Diaz-Benjumea and Cohen, 1993; Williams et al., 1993, 1994) As a result, cells adjacent to the boundary form the wing margin, while cells dorsal and ventral to the margin initials proliferate to form the wing blade. Expression of *ap* persists in dorsal cells of the developing wing, and is necessary for determination of dorsal cell type. The roles of *ap* in wing development therefore appear similar to those proposed for DF in the leaf. This is illustrated by the effect of removing *ap* expression from clones of dorsal initial cells. These ap^- cells develop as patches of ventral tissue surrounded by ectopic wing axes (Diaz-Benjumea and Cohen, 1993) and are therefore analogous to patches proposed to result from loss of DF in early leaves of *phan* mutants. However, *ap* and DF differ in one important respect. Wing discs that lack *ap* expression fail to grow in the proximodistal axis, indicating that *ap* is required for specification of this axis (Butterworth and King, 1965). In contrast, even an extreme needle-like leaf, which has shown no lateral growth, retains a proximodistal axis, suggesting that determination of this axis is independent of DF in leaves.

By analogy to the role of *ap* in wing development, the domain of DF expression in leaves may correspond to cells that express *phan* and other genes required for DF activity. Differences in cell fate within the domain may then be the result of interactions between DF and other functions with adjacent or partially overlapping domains. For example, overlap between the DF domain and that of a ventralising factor might activate localised expression of genes required for production of the lamina. The existence of potential target genes has been revealed by mutations in a number of dicot species which specifically reduce laminal proliferation, without affecting dorsoventrality of the leaf (e.g. McHale, 1992).

DF expression might be established in response to a morphogen which forms a gradient in the apical meristem. Support for the role of a gradient in establishing leaf dorsoventrality has been provided by surgical experiments carried out on vegetative meristems of potato (Sussex, 1955), *Epilobium* (Snow and Snow, 1959) and *Sesamum* (Hanawa, 1961). Incisions that isolated leaf initials from the apex frequently led to the production of leaves with radial symmetry (a phenocopy of the needle-like leaves of *phan* mutants). Smaller cuts, positioned so as to only partially isolate the initials, produced leaves with narrower laminae (Sussex, 1954) similar to those proposed to result from partial loss of DF in *phan* mutants. These results suggested that a gradient with a source originating in the apex was responsible for determination of dorsoventrality in leaf initials.

Wild-type leaf primordia become distinguishable from those of *phan* mutants when they begin to show lateral proliferation soon after initiation. For DF to be effective at this stage, its expression must have been established earlier, possibly prior to leaf initiation. In the case of the *Drosophila* wing, expression of *ap* begins in the imaginal disc (Cohen et al., 1992), presumably in response to dorsoventral polarity in the larval body (reviewed by St Johnstone and Nüsslein-Volhard, 1992). It therefore not only maintains dorsoventral polarity from the larval body to the wing, but also serves to translate it into formation of a new wing axis. By analogy, DF may allow apical-basal polarity in the vegetative meristem to specify dorsoventrality in leaves and to elaborate a new lateral axis as a result. While the potential nature of apical-basal polarity in the meristem remains obscure, its existence is revealed by patterned expression of a number of genes in this region (Fleming et al., 1993; Medford et al., 1991; Smith et al., 1992).

The model proposed to explain the effects of *phan* mutations on leaf morphogenesis is equally applicable to development of petal lobes. In wild-type flowers, expression of DF is proposed to occur in dorsal cells of the petal primordia. Lateral proliferation towards the boundary of expression forms the petal lobes, and the DF later specifies the identity of dorsal cell types. In the most extreme mutant, (*phan*-250G), the petal

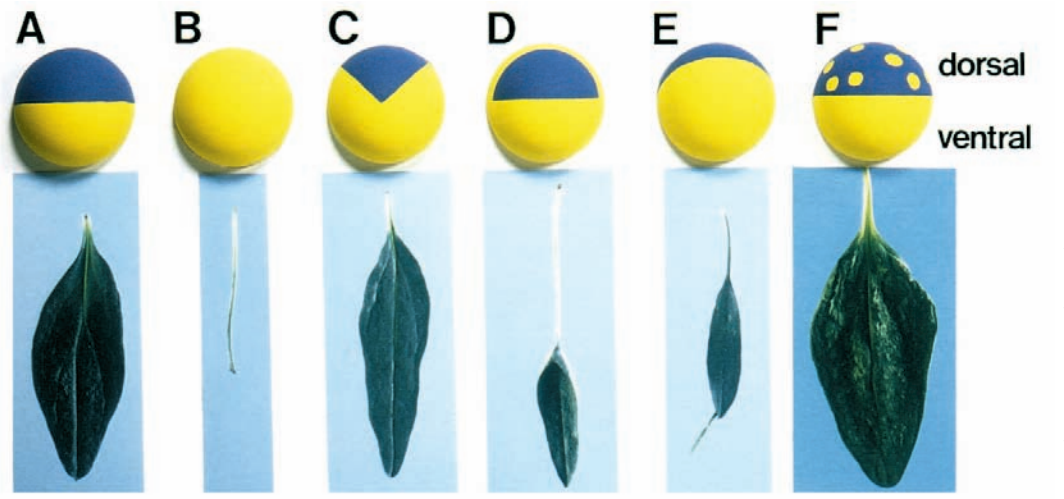

Fig. 12. A model for the action of the dorsalising function in leaf development. Early leaf primordia are depicted as yellow hemispheres viewed from a distal position. Dorsal regions of the primordia experiencing DF expression are coloured blue. Different leaf morphologies are shown below the patterns of expression which are proposed to have given rise to them. Mature leaves are viewed from above, with their distal ends towards the bottom of the page. (A) A wild-type leaf; (B) a needle-like *phan* mutant leaf; (C) a narrow leaf of a *phan* mutant; (D,E) mosaic mutant leaves; (F) an early *phan* mutant leaf showing ectopic patches of ventral cell types. See text for further explanation.

primordia develop as needles of ventral tissue. This morphology is similar to that of needle-like leaves and suggests absence of DF expression from petal primordia. The floral phenotypes of the remaining four mutants are consistent with reductions in the domain of DF expression to more dorsal positions. The regions without DF develop as needles or form part of a ridge of ventral tissue, and reduced petal lobes are formed at the shifted boundary of DF expression on their dorsal flanks: a morphology which is similar to that of the mutant leaf shown in Fig. 10E. The differences in floral phenotype between the four *phan* mutants suggest that each shows a characteristic reduction in the domain of DF expression. Those with stronger expression are predicted to produce larger petal lobes at more dorsal positions on the primordia. The final feature of *phan* mutant petal lobes is that they may show patches of ventral cell types surrounded by ectopic lobe tissue on their dorsal surfaces. This effect on petal development appears similar to that observed in early leaves of *phan* mutants, and can also be explained by loss of DF expression from groups of dorsal initial cells.

The similarity of the effects of *phan* mutations on leaves and petal lobes is consistent with the view that these are homologous structures. However, dorsoventrality in the corolla tube and in floral organs other than petals remains unaffected in the mutants. This is particularly striking in the case of sepals, which in wild-type are very similar in morphology to bracts. Whereas the bracts of *phan* mutants are commonly reduced to needle-like structures, their sepals invariably resemble those of wild-type. One explanation for this difference is that dorsoventrality in floral organs other than petal lobes may be independent of *phan* expression. Alternatively, the level of DF expression in *phan* mutants may be limiting in leaves, bracts and petal lobes, but sufficient for determination of dorsoventrality in other organs.

Leaves of *phan* mutants may resemble those characteristic of other plants. For example, needle-like mutant leaves have a similar morphology to tendrils and spines, which in many species are considered to be modified leaves or leaflets (Goebel, 1905). Likewise, peltate leaves with laminal tissue which completely surrounds the petiole, as in nasturtium (*Tropaeolum majus*), resemble those of *phan* mutants in which the proximal region lacks dorsoventrality and the distal region is laminal. The development of peltate leaves also mirrors that of a mosaic mutant leaf, in that the proximal part of the lamina which forms an axis distinct from the petiole, is formed by proliferation on the dorsal surface of the primordium after initiation (Hagemann, 1984; Troll, 1932). In *phan* mutants, these differences in leaf morphology are proposed to result from relatively small changes in the strength or position of DF expression. Equally subtle changes may therefore be responsible for evolution of a number of leaf forms.

At least one of the *phan* mutant alleles is germinally unstable and able to revert to wild type at a frequency characteristic of other transposon-induced mutations in *Antirrhinum* (Carpenter and Coen, 1990). This mutant should therefore allow the *phan* locus to be isolated by transposon tagging, and the role of the gene in determination of dorsoventrality to be examined further.

We thank Des Bradley, Rosemary Carpenter and Enrico Coen for constructive comments on the manuscript. This work was supported by BBSRC.

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(Accepted 4 April 1995)