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Pathways for inflorescence and floral induction in *Antirrhinum*

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SUMMARY

The presentation of flowers on a modified stem, the inflorescence, requires the integration of several aspects of meristem behaviour. In *Antirrhinum*, the inflorescence can be distinguished by its flowers, hairy stem, modified leaves, short internodes and spiral phyllotaxy. We show, by a combination of physiological, genetical and morphological analysis, that the various aspects of the inflorescence are controlled by three pathways. The first pathway, depends on expression of the *floricaula* gene, and is rapidly and discretely induced by exposure to long daylength. Activation of this pathway occurs in very young axillary meristems, resulting in a floral identity. In addition, the length of subtending leaves and hairiness of the stem are partially modified. The second pathway affects leaf size, internode length, and stem hairiness, but does not confer floral meristem identity. This pathway is induced by long daylength, but not as rapidly or discretely as the *floricaula*-dependent pathway. The third pathway controls the switch in phyllotaxy from decussate to spiral and is activated independently of daylength. The coordination of these three programmes ensures that apical and axillary meristem behaviour is integrated.

Key words: flower induction, inflorescence development, *floricaula*, meristem, daylength

INTRODUCTION

Flowering can be influenced by many different environmental conditions, including daylength and temperature (Bernier, 1988). Although the common effect of induction is the production of flowers, a variety of other responses usually occur to present the flowers on a modified stem, the inflorescence. The traits most commonly associated with the inflorescence are the modification of leaves and a change in internode length compared to that of the vegetative stem. Most physiological studies have concentrated on the induction of flowering and less attention has been paid to induction of the associated inflorescence traits.

Analyses of mutants altered in flowering indicate that the production of flowers and inflorescence traits can be separated. In the *floricaula* (*flo*) mutant of *Antirrhinum* and its counterpart, *leafy* (*lfy*) in *Arabidopsis*, flowers are replaced by shoots (Carpenter and Coen, 1990; Coen et al., 1990; Schultz and Haughn, 1991; Weigel et al., 1992; Huala and Sussex, 1992). These mutants, however, still go through a series of morphological changes characteristic of a wild-type inflorescence. In *Antirrhinum*, the inflorescences of wild-type plants and *flo* mutants have smaller leaves (usually called bracts) than in the vegetative region, separated by shorter internodes and arranged in a spiral (Coen et al., 1990). In *Arabidopsis*, the inflorescence of the *lfy* mutant shows lengthening of internodes (bolting) similar to wild type and a switch from producing rosette to cauline leaves (Schultz and Haughn, 1991; Weigel et al., 1992). The *squamosa* (*squa*) mutant of *Antirrhinum* and its homologue *apetala1* (*ap1*) in *Arabidopsis* are similarly affected in the switch from inflorescence to floral meristems (Huijser et al., 1992; Mandel et al., 1992).

In wild-type plants, inflorescence and floral meristems develop together under normal growth conditions and there are two models to explain this synchrony. The first involves a linear pathway from inflorescence to floral development, such that the two meristem types are activated in tandem. According to this view, the *flo* and *lfy* mutations only affect the second step of this pathway so that inflorescence shoots replace floral meristems in axillary positions. Similar interpretations have been made of phase change mutants, which emphasise the most common sequence of plant development as vegetative to inflorescence to floral (Poethig, 1990; Huala and Sussex, 1993; Schultz and Haughn, 1993; Lawson and Poethig, 1995). In tobacco and pea it has also been shown that excised apices are committed to become inflorescences in culture before axillary meristems become floral (Singer and McDaniel, 1986; Ferguson et al., 1991; Irish and Nelson, 1991).

The second model involves parallel pathways to the induction of inflorescence or floral meristems. In *Arabidopsis*, the first floral meristems are usually seen prior to internode elongation or visible differentiation of coflorescences (Bowman et al., 1993; Hempel and Feldman, 1994). Both these features are taken to be properties of the inflorescence, suggesting that floral meristems may be initiated before inflorescence traits. Unfortunately, however, the earlier appearance of one programme does not distinguish between independent induction of floral and inflorescence meristems or simply a difference in the time taken for their initiation and appearance of traits.

Experiments were designed to test which of these two

models is appropriate to *Antirrhinum*. The activation of *flo* was studied by growing plants under different light regimes and analysing *flo* transcript levels. The expression patterns were related to inflorescence and floral development by detailed morphological analysis. Based on these results we propose three pathways are involved. The first is *flo*-dependent and commits axillary meristems to become floral and the shoot apex to make partial inflorescence traits. This is supported by analysis of *flo* expression, which is both inducible by long daylength and confined to those meristems committed to become flowers. The second pathway is independent of *flo* and promotes full inflorescence traits. Both pathways are normally activated at about the same time if plants are grown continuously under long days (LD) or short days (SD). However, the *flo*-independent pathway requires more LD for commitment and this allows separation of the two pathways under particular growth regimes. The third pathway promotes the switch from decussate to spiral phyllotaxy and is independent of daylength. The final presentation of flowers on the inflorescence depends on the relative timing of these three programmes.

MATERIALS AND METHODS

Antirrhinum stocks and growth conditions

The wild-type *A. majus* lines JI.98 (*nivea*-^{recurrens} 98) and JI.2 (*pallida*-^{recurrens} 2) were bred at the John Innes Institute as described by Harrison and Carpenter (1979) and Carpenter et al. (1987) respectively. The stable *floricaula* mutant JI.640 (*flo*-640) was maintained as a heterozygous stock in the progenitor line JI.98 and has been described (Carpenter and Coen, 1995; Hantke et al., 1995).

Conditions for growth and maintenance of plants were as described by Carpenter et al. (1987).

Long days (LD) involved 16 hours of lights and 8 hours of dark each day, while short days (SD) were 8 hours light/16 hours dark. Continuous LD or SD refers to growth under those light conditions, uninterrupted by pulsing or moving to a different daylength condition. Plants were germinated and grown under 3 sets of basic environmental conditions:

(A) LD, 20°C, at about 140 μE/m^2/second (high pressure sodium lamps)

(B) SD, 20°C, 80 uE/m^2/s (HQI metal halide lamps)

(C) SD, 20°C, at about 230 μE/m^2/second (HQI metal halide lamps)

For Figs 1A, 4A and 4B, wild-type JI.98 or *flo*-640 were germinated and grown under light conditions A or B, to monitor growth in continuous LD or SD. Figs 2, 4C and 5 followed the growth and commitment of plants grown for 28-34 LD and used wild-type JI.98 germinated and grown under conditions A before transfer to conditions B. The induction of *flo* expression seen in Fig. 3 used wild-type JI.98 germinated and grown under conditions C before transfer to conditions A. Figs 1C, 7 and 8 show the phenotypes of wild-type JI.2 germinated and grown for 43 days under conditions C (but at 25ºC) before pulsing in conditions C. After pulsing, plants were transferred to conditions B and left to flower. Fig. 9 shows the results from a family segregating for wild type and *flo*-640 that were treated the same as the plants used for the experiments displayed in Figs 1C, 7 and 8, but all environments were at 20°C.

SEM, RNA in situ hybridization and RACE-PCR

Scanning electron microscopy was performed on replicas of inflorescences, flowers, abnormal floral structures or stems, as described by Williams and Green (1989). The methods for digoxigenin-labelling of RNA probes, tissue preparation and in situ hybridization were as described by Bradley et al. (1993) and Coen et al. (1990). Apices (the region above a common leaf number), stems (internode regions) or vegetative, decussate leaves from 5-10 plants were pooled and RNA extracted as described by Coen and Carpenter (1988). RACE-PCR was as described by Frohman et al. (1988). Limiting PCR conditions involved stopping the reactions while most were still in the exponential phase of product amplification. Gene-specific oligonucleotide primers were as follows:

flo-specific oligo - 5′ GTGGATCCTGAAGACGACGAAAATATCGTAAGCG – consisting of a *Bam*HI restriction site coupled to 25 bases corresponding to nucleotides 710-734 of the *flo* cDNA (Coen et al., 1990).

squa-specific oligo - 5′ ACTGATTCTTGCATGGACAGGA – corresponding to nucleotides 338-359 of the *squa* cDNA (Huijser et al., 1992).

RESULTS

In long days, the *flo* gene is first expressed when floral meristems arise

Wild-type *A. majus* is a non-obligative (or facultative) LD plant (Maginnes and Langhans, 1961). Relative to LD grown plants, plants under SD make about 50 more leaves before flowering, are 3-5 times higher and flower many weeks later (Fig. 1A). Plants were followed by scanning electron microscopy (SEM) to determine precisely the number, rate and appearance of nodes developing under continuous LD (Fig. 2A-D). Plants were harvested after 28, 32, 36 or 40 LD. Under SEM it was convenient to number starting from the apex, so that node 0 was the smallest, visible leaf primordium and the nodes below being numbered sequentially (Fig. 2B). The position of nodes relative to the base of the plant was also determined by counting the number of leaves on the plant when the apex was dissected. Leaf numbers were assigned starting at the base of the plant. To simplify the terminology, bracts (leaf-like structures subtending flowers) were considered as leaves in this analysis. Plants left in continuous LD developed flowers at about leaf number 20 (Fig. 1A). In plants grown for 28 LD, node 0 corresponded to about leaf number 18 which would remain vegetative. By 32 LD, nodes 0-6 (±2) corresponded to leaf numbers 26-20 (±2) which would subtend flowers in the adult plant (Fig. 1B). However, these nodes were indistinguishable morphologically from vegetative nodes at this stage. Floral meristems could be discerned morphologically in SEM on 40 LD grown plants, when the five-fold symmetry of a sepal whorl developed, quite different from the spiral of vegetative meristems (compare node 13 after 36 LD and 40 LD growth, Fig. 2C,D). The rate of node initiation was estimated to be about 2 nodes/day between 28 and 32 LD based on the average number of nodes seen in SEM at each time point when comparing synchronised plants. Therefore, the first floral meristem in continuous LD corresponded to node 0 after about 29-30 LD, when 20 leaves had been initiated.

The meristem identity genes *flo* and *squa* are expressed in wild-type *A. majus* in young floral meristems and their subtending leaf primordia (Coen et al., 1990). To determine how their expression patterns related to floral induction, RNA in situ hybridisations were carried out on plants harvested after 28, 32, 36 or 40 LD growth (Fig. 2E-H; *squa* data not shown). Both *flo* and *squa* were not expressed after 28 LD but were clearly expressed after 32 LD in nodes 0-6 (±2), correspond-

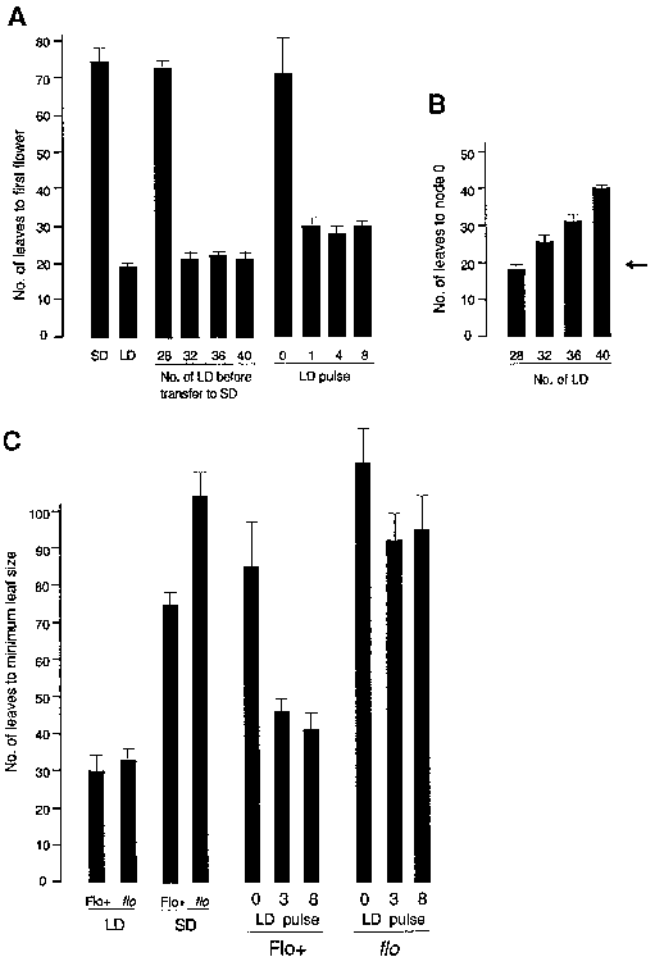

Fig. 1. Development of wild-type *Antirrhinum* and *flo* mutants under different daylength conditions. (A) Number (No.) of leaves to the first flower on adult plants grown under continuous LD or SD; or for 28, 32, 36 or 40 LD before transfer to SD; or for 43 SD and given pulses of 0, 1, 4 or 8 LD. Six plants were analysed for each time-point and similar results were found in three independent experiments. (B) Number of leaves to the youngest visible leaf primordium at the apex (node 0) on plants analysed by SEM (see Fig. 2). The arrow indicates the position of the first flower on adult plants. (C) Number of leaves to the minimum leaf size of wild-type *Antirrhinum* and *flo* mutant plants in a segregating family grown under different conditions. Twelve to fifteen plants were analysed of each phenotype.

ing to leaf numbers 20-26 (±2) which subtended flowers in the adult plant. These nodes had arisen during day 29-30, based on the rate of node initiation revealed by SEM. Apices harvested at later time-points revealed that all nodes produced by the apical meristem after 32 LD also expressed *flo* (Fig. 2G,H) and *squa* (data not shown). This showed that the induction of *flo* and *squa* correlated with those meristems giving rise to flowers.

Expression of *flo* correlates with commitment to flower development

The correlation of *flo* expression with the appearance of floral meristems was investigated further by determining whether plants were committed to flower when *flo* was expressed. Wild type plants were transferred from continuous LD to SD after 28, 32, 36 or 40 days (Fig. 1A). Transfer after 28 LD caused plants to flower at a position similar to continuous SD grown plants (about leaf number 75). After 32 LD, plants flowered at a position similar to plants grown in continuous LD (leaf number 20), indicating that they had been committed to flower by the LD treatment. Thus the time of *flo* activation, between 28 and 32 LD, correlated with the stage of commitment to flower. Although wild-type flowers usually developed on plants transferred from LD to SD, flowers of abnormal morphology were often found (in an apparently random distribution) along the flowering region of the stem. In the 32 LD grown plants, these abnormal flowers constituted 50% or more of the axillary nodes. These were clearly different from vegetative nodes, always having a whorled arrangement of organs, with sepals on the outside and variable structures within. The most aberrant flowers had only rudimentary petal-, stamen- and carpelloid-like structures while others were borne on elongated pedicels and appeared to have all organ types, arranged correctly but not expanded. The fertility of these structures was not tested. Very rarely, a vegetative shoot developed in the axil of a leaf at a node within the flowering region.

Induction of floral meristems is coupled to *flo* activation

Floral meristem identity genes *flo* and *squa* were expressed after 32 days in continuous LD, corresponding to the time when plants were induced and committed to flower. This correlation may have simply reflected the programming of these genes to appear after 32 days or when 18-20 leaves had been produced, irrespective of the daylength conditions. Alternatively, these genes could be very early markers for meristems committed to flower. To distinguish between these possibilities, plants were grown in continuous SD for various times (31-54 days or until about 19-31 leaves were visible by dissection and SEM, dependent upon the experiment) before transfer to continuous LD. Subsequently, groups of plants were harvested for RNA extraction and RT-PCR, or RNA in situ hybridisation. When plants had been grown for 39 SD (19 visible leaves, including primordia) before transfer to LD, expression of *flo* was first detected after 2 LD and its level increased thereafter (Fig. 3A). Although PCR methods are difficult to quantify, the results from a number of different experiments and the limiting conditions used suggested that greater *flo* RNA levels were present at progressive time-points in LD. Replicate experiments using similarly aged or older plants, suggested a range of 1-3 days for *flo* induction by LD. In some cases low levels of *flo* were detected in control, day 0 plants, but this level was always lower than after induction by LD and it was not ascertained whether the variability between experiments was only due to RT-PCR sensitivities, or the particular developmental stage of the plant at day 0 before induction.

RNA in situ hybridisations confirmed the PCR data, with *flo* expression just being visible between 1-4 days after induction (Fig. 3B-D). The expression was limited to the youngest emerging primordia at the apex. In apices harvested at later time-points, the lowest node expressing *flo* corresponded to about leaf 20-24 in each case, based upon a rate of node initiation of 1-2 nodes/day. While *squa* was strongly expressed in the same axillary meristems as *flo*, it was only weakly expressed in the subtending leaf primordia. This made it

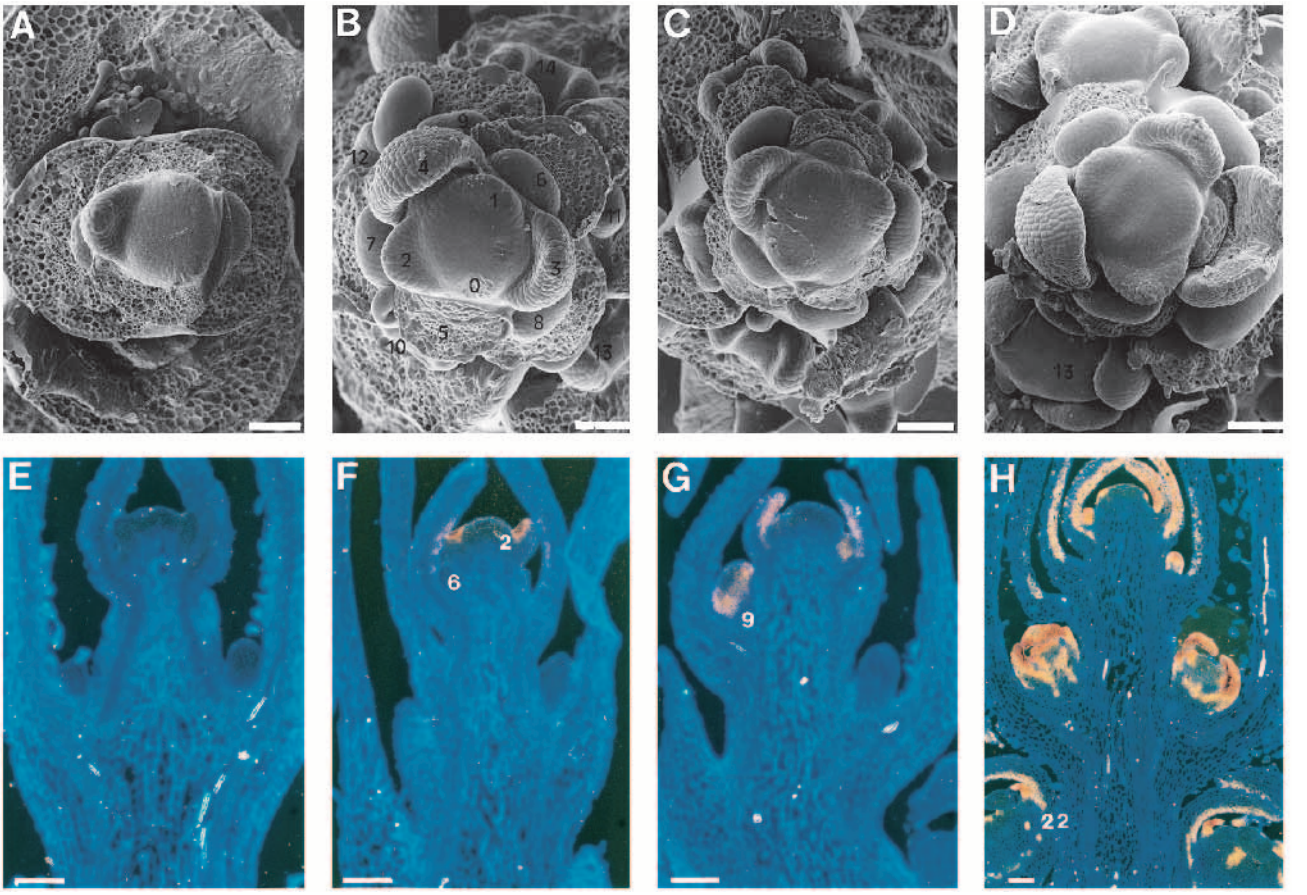

Fig. 2. *flo* expression precedes the morphological appearance of floral meristems. Plants were grown in continuous LD and harvested 28, 32, 36, 40 or 44 days after germination. Five plants with equal numbers of visible leaves were harvested at each time-point. A representative from 28, 32, 36 or 40 LD grown plants (A, B, C and D respectively) was moulded and looked at under the SEM. Nodes are numbered sequentially from the first visible leaf primordia at the apex (node 0). Note the change in phyllotaxis from opposite leaves to spiral (compare A with B, C or D) and that floral meristems are only recognisable morphologically after 40 LD (compare node 13 of C and D). (E-H) A further set of plants were harvested at each time-point and embedded, sectioned and probed with DIG-labelled antisense *flo* RNA, which viewed under dark-field optics gives the signal an orange colour on a blue background. Representatives from 28, 32, 36 and 44 LD grown plants are shown (E, F, G and H respectively). Nodes are numbered as above, though only a subset are present in any longitudinal section. Note that *flo* expression is first seen on day 32 (about nodes 0-6). All scale bars are equivalent to 100 μm.

difficult to detect the earliest node expressing *squa* by in situ hybridisation, since leaf primordia developed before the axillary meristems. PCR, however, revealed the time-course of *squa* induction to be very similar to that of *flo*, though a basal level of *squa* expression was always observed (data not shown). This basal level may have simply reflected the sensitivity of the RT-PCR for *squa* or a real difference in comparison to *flo*. One set of plants was left to flower in LD after transfer, and the position of the first flower (leaf number 21±1) correlated with the lowermost node found to express *flo* and *squa*.

To determine whether the activation of *flo* after 1 LD was reversible or whether it correlated with a stable commitment to flower, plants were given different lengths of LD pulses before return to SD (Fig. 1A). After growth for 43 SD, a pulse of 1 LD was enough to induce flowering in all plants. Upon flowering, all leaves above the lowermost flower also bore flowers in their axils. Most of these leaves were not visible as primordia at the time of the LD pulse, indicating that the pulse induced a persistent commitment to flower. RNA in situ hybridizations on plants harvested from the same experiment showed that *flo* was induced after 1 LD, although some weak expression was also detected on day 0. This weak expression was also observed in unpulsed plants at about leaf 32, yet these plants did not flower until about 70 leaves had been produced. In other experiments plants required a range of 1-3 days in LD to commit them to making floral meristems and induce *flo* expression. The reason for this range was not determined, but may have depended upon the age of the plants at the time of induction (Hedley and Harvey, 1975). These results show that strong induction of *flo* and *squa* expression is associated with the commitment of axillary meristems to become flowers.

Hairiness and leaf size but not phyllotaxy are dependent on daylength

The stem of wild-type *A. majus* can be divided into two principal regions. In the vegetative region, leaves are arranged in a decussate phyllotaxy, each pair at 90º to the adjacent. The leaves are separated by large internodes and bear vegetative shoots in their axils that may grow out or remain dormant.

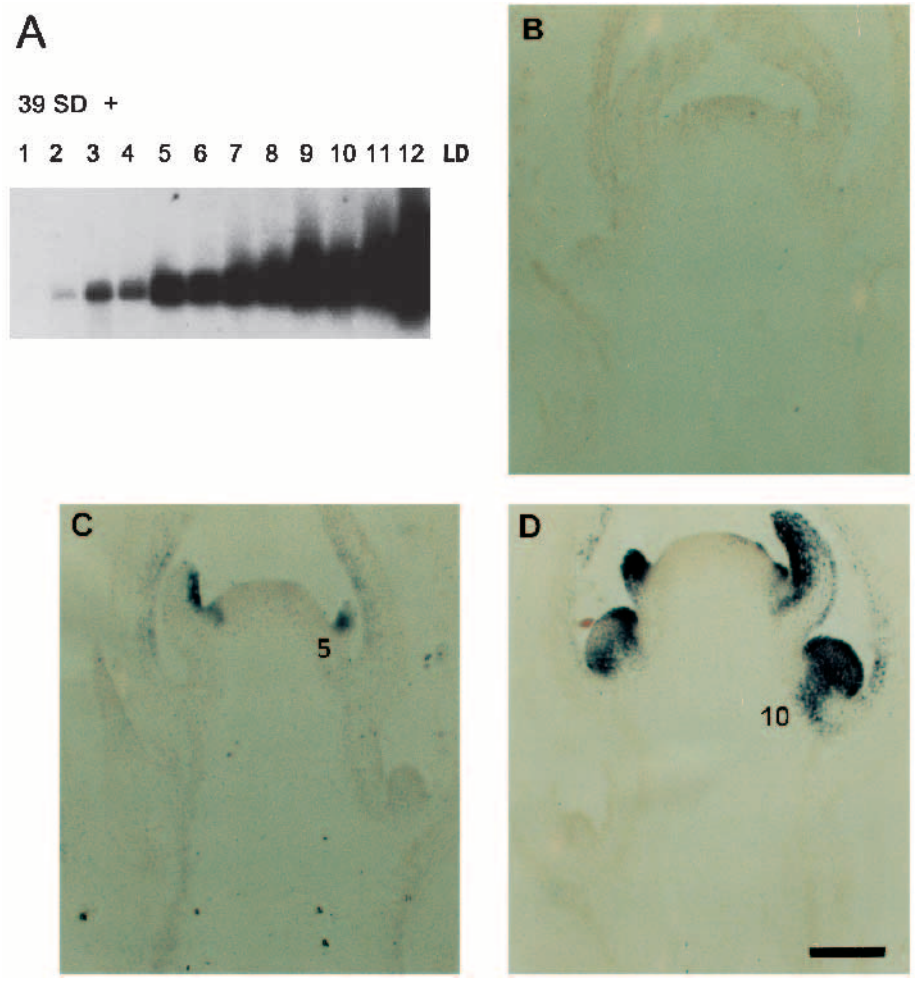

Fig. 3. The induction of *flo* expression in apices following transfer from SD to LD. Plants were grown for 39 SD and those with equal numbers of visible leaves were selected for transfer to LD. (A) Apices were harvested after 1-12 LD in pools of five and RNA was extracted for RACE-PCR, blotting and probing with *flo*. (B-D) Apices were embedded after 1, 4 or 6 LD (B, C and D respectively) and probed with DIG-labelled antisense *flo* RNA, which viewed under light-field optics gives a dark purple colour on a cream/light khaki green background. Nodes are numbered as in Fig. 2 and only a subset are present in any longitudinal section. Photographs B-D are at the same scale, with the bar in D equivalent to 100 μm.

Above the vegetative part lies the inflorescence region which has small leaves arranged in a spiral, separated by short internodes that have a high density of hairs. We wished to detail which features of these two regions are programmed independently and which are associated with daylength. Therefore, the traits of plants grown in environmentally controlled SD and LD were recorded upon flower opening. These traits included phyllotaxy, leaf length at each node, and hair density for each internode. Representative plants (median values) were chosen to show the general trends of growth under each condition (Fig. 4).

In continuous LD, the first 10-16 leaves of the vegetative region were arranged in pairs in a decussate phyllotaxy (Fig. 4A). Leaf length more than doubled over the vegetative region from a minimum at the first pair of leaves to a maximum that plateaued at about 80-90 mm. The stem was largely free of hairs in the vegetative region except over the internodes separating the first 1-2 pairs of leaves and the cotyledons. Above the decussate region, the next leaves were most often single and in a spiral, and from about leaf 20 upwards, flowers arose in the axils of leaves (Figs 1, 2). Between leaves 19-24, hair density increased and leaf length decreased, constituting a region of partial inflorescence traits. From about leaf 25, full inflorescence traits developed and these were defined as leaves of minimal size (10 mm) and maximal hair density. Internode lengths were measured for all six plants and proved quite variable for any particular region. However, by collating all measurements, the average internode length in the vegetative, decussate region was about 3 times that in the flowering region where hair density was maximal.

Under continuous SD, many more leaves were made before the appearance of flowers (Fig. 4B). As with LD, the vegetative stem had an initial region (2-3 leaves above the cotyledons) marked by hairs and an increase in leaf length. The decussate phyllotaxy extended over about 10-14 leaves and leaf length reached a maximum. Leaves were then single, in a spiral, and of roughly constant length, over about 50-60 nodes, until the first flower was produced at around leaf 75. As in LD, there was a region of a few nodes, having partial inflorescence traits, from the appearance of the first flower to full inflorescence traits. The only difference was that in SD, flowering seemed to occur slightly before hairs appeared on the stem and the region of partial inflorescence traits was extended by about 2-3 nodes. The switch from decussate to spiral phyllotaxy therefore occurred at the same position in SD as in LD, but the reduction in leaf length and increase in hairiness was delayed in a similar way to flowering time. Therefore, the defining characteristics of the inflorescence meristem under both SD and LD are the production of small leaves separated by short and hairy internodes. The switch to spiral phyllotaxy is programmed independently of floral induction by daylength.

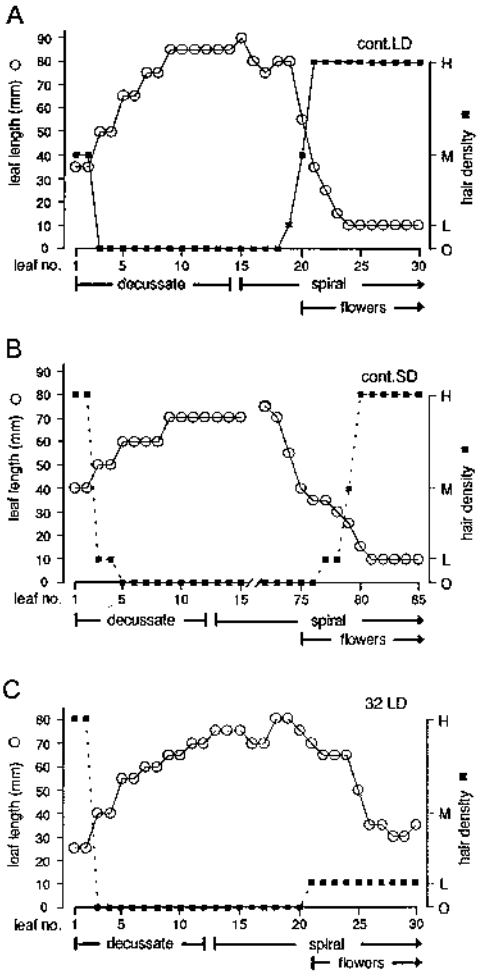

Fig. 4. Traits and their distribution in wild-type *Antirrhinum* grown under continuous LD or SD, or given 32 LD before transfer to SD. Plants were germinated and grown in continuous (cont.) LD (A) or cont. SD (B). A set of 6 plants in cont. LD, with equal numbers of visible leaves, were transferred after 32 LD to cont. SD (C). When all plants flowered, each was scored for leaf size, position of flowers, internode length (see text) and the density of hairs on the stem above each node. One representative (out of 6 plants), having the median value for the position of the first flower, was chosen and graphed. Hair density was measured as O (no hairs), L (less than 10 hairs on the stem of the internode region), M (10-50 hairs) and H (>50 hairs).

Floral meristems can arise in the absence of full inflorescence traits

Under both continuous SD and LD, inflorescence and floral meristems always appeared together. To determine whether both meristems were committed together and if one was a prerequisite for the other, traits on adult plants grown for 28, 32, 36 or 40 LD before transfer to continuous SD were recorded. As indicated earlier, commitment to flower occurred between 28-32 LD growth, with 28 LD grown plants flowering at a position similar to continuous SD grown plants (Fig. 1A). Plants transferred after 28 or 40 LD developed inflorescence traits and floral meristems together. However, plants transferred after 32 or 36 LD produced flowers but only had partial inflorescence traits of low hair density and a small reduction in leaf size (Fig. 4C). The vegetative region of 32-36 LD grown

Fig. 5. Flowering region of plants grown in continuous LD or for 34 LD before transfer to SD. The flower-bearing regions of a representative plant grown under continuous LD (right) or 34 LD before transfer to SD (left) had most of their flowers removed to reveal the stem and subtending structures in the flowering region.

plants was very similar to the continuous LD grown plants, but upon the appearance of flowers, the subtending leaves remained long and the internodes were long and almost without hairs for more than about 15 nodes (Fig. 5). To look more closely at the hairs, an SEM of the stem in the flowering region of the 32 LD transferred plants was compared to that of continuous LD grown plants (Fig. 6). The major differences were in the reduced density of hair cells and greater internode lengths in the 32 LD plants. The 36 LD plants showed a similar, but less marked phenotype to the 32 LD transferred plants. Internode lengths were significantly greater over the first six flowers in 32 LD transferred plants (21±9 mm) than in 40 LD transferred or continuous LD grown plants (8±6 mm). This indicates that plants were committed to produce flowers after 32 LD, but full inflorescence traits only after 40 LD.

To determine if the inflorescence meristem simply required longer exposure to the inductive conditions or is programmed by some other change occurring after 36-40 LD, plants were given inductive LD pulses. Plants were grown in continuous SD and given pulses of 1 to 12 LD before returning them to SD. Representative plants from 1, 4 and 8 LD pulses were photographed upon flowering and compared to a control plant left in SD and given no pulse (Fig. 7). As described above, all plants given a pulse of 1 LD or more, produced flowers earlier than the control (unpulsed) plants and at about the same position for each treatment (Fig. 1A). The appearance of inflorescence traits, however, depended upon the length of the inductive pulse (Fig. 7). A pulse of only 1 LD gave flowers in the presence of partial inflorescence traits, long leaves on a stem with a low density of hairs, similar to the effect of transferring plants to SD after 32-36 LD. Increased exposures to LD gave progressively more hairs, shortening of internode length and a decrease in leaf length (Fig. 8). By pulsing for 8 or more LD, a sharp transition zone from vegetative to full inflorescence traits was obtained,

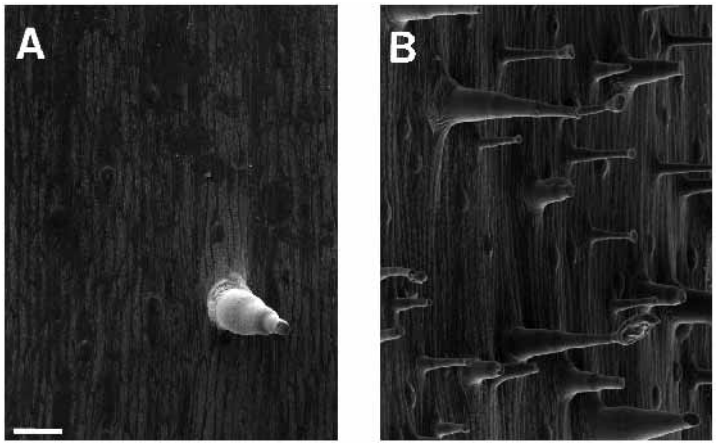

Fig. 6. Stems of plants grown in continuous LD or for 32 LD before transfer to SD. Moulds were taken of stems between flowering nodes of plants grown either for 32 LD before transfer to SD (A) or in continuous LD (B). Both photographs are at the same scale, with the bar in A equivalent to 100 μm.

similar to that seen under continuous LD. Thus the inflorescence meristem was inducible by LD, but required more LD than floral meristems for commitment.

Full inflorescence traits are promoted by *flo* under SD

The previous analysis of wild-type plants under SD and LD clarified which traits were independent of flowering or daylength conditions. The *flo* gene is expressed in floral meristems, in the transition zone, before the appearance of full inflorescence traits. Any role for *flo* in the appearance of these traits was investigated by detailing the growth characteristics of *flo* mutant plants compared to their wild-type segregants under SD or LD. Under continuous LD, *flo* segregants showed no significant difference to wild type in the position of full inflorescence traits; the minimum leaf size and maximal hair density occurring at about the same position (Fig. 1C). The overall growth characteristics were also similar, though *flo* plants appeared to have a longer transition zone (2-4 nodes more than wild-type plants) from maximal to minimal leaf size and zero to maximal hair density (Fig. 9A,B). Under continuous SD, the vegetative regions were similar but wild-type plants reached maximal hair density and minimal leaf size significantly earlier than the *flo* mutant (Fig. 1C). However, partial inflorescence traits developed at about the same node in wild-type and *flo* plants (about leaf 75-80). Therefore, the transition to full inflorescence traits was much longer and more gradual in the *flo* mutant under SD. Full inflorescence traits are therefore *flo*-dependent under SD conditions.

The induction of inflorescence traits by LD pulses requires *flo*

We have defined the inflorescence meristem by the production of short leaves and short, hairy internodes. The *flo* gene has a role in the activation of the inflorescence meristem under SD. Partial inflorescence traits of reduced leaf size and low hair density were initiated independently of *flo* under SD. Similar partial traits were induced in wild-type plants given LD pulses (Figs 7, 8). We tested whether their induction by LD was due to a *flo*-independent or *flo*-dependent pathway by growing *flo* mutant and wild-type plants under SD and giving them pulses of LD. Progeny from plants heterozygous for *flo* were sown

Fig. 7. Response of SD grown plants to 0, 1, 4 or 8 LD pulses. After 43 days growth in SD, plants were selected with equal numbers of visible leaves and then groups of 8 were left in SD as controls or pulsed for 1, 4 or 8 LD. Plants were arranged from left to right, 0, 1, 4 or 8 LD pulses and photographed. Note that the control SD plants eventually flowered but only after 3 to 5 times the number of leaves in pulsed plants had been produced.

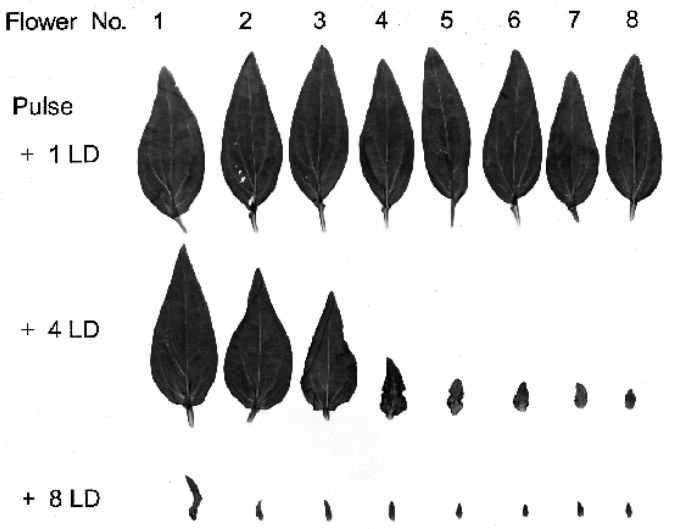

Fig. 8. Leaves subtending flowers from 1, 4 or 8 LD pulsed plants. The leaf at each flowering node from the first up to the eighth flowering node were removed from representatives of plants that had been pulsed for 1, 4 or 8 LD, and aligned.

and grown under continuous SD and given pulses of 3 or 8 LD before return to SD. The response of wild-type segregants was similar to earlier experiments: all pulsed plants flowered earlier (about leaf 45) than control unpulsed plants (leaf 85; Fig. 1C). As before, the region of partial inflorescence traits was more extended for the 3 LD pulsed plants (about 11 leaves) than for the 8 LD pulsed plants (about 5 leaves). The *flo* mutant segregants did not show a significant response to any of the pulses and minimal leaf size was attained much later (about 50 more leaves produced) than in wild type. Therefore, induction of inflorescence traits by LD pulses appeared to be dependent upon *flo*.

DISCUSSION

We tested two models for the induction of inflorescence and floral meristems, linear or parallel. The linear model, largely based on genetic data, predicts that the induction of an inflorescence meristem precedes that of floral meristems. In contrast, the parallel model allows for independent induction of inflorescence and floral meristems. Under continuous SD or LD growth conditions, both of these models were applicable because the appearance of inflorescence and floral meristems was synchronised in wild-type plants; only 3-5 flower-bearing nodes occurred between the transition from partial inflorescence traits (characterised by a reduction in leaf size and the appearance of hairs on the stem) to the development of full traits (defined by a minimal leaf size of 10 mm and a maximal density of stem hairs). However, by manipulating the growth conditions of wild-type plants, this transition region can be extended by 20-30 nodes. This suggests that the switch to full inflorescence traits is a separate programme from the development of partial inflorescence traits and floral meristems.

The development of partial inflorescence traits is dependent upon *flo*. Analysis of the *flo* mutant showed that, in contrast to

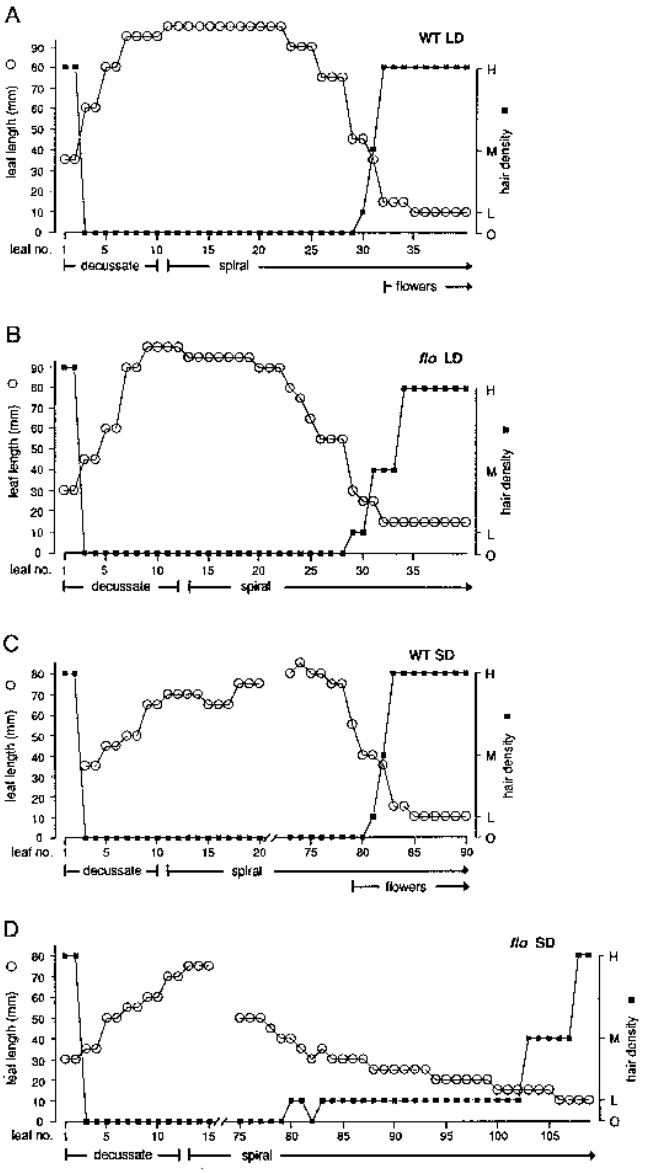

Fig. 9. The traits of wild-type *Antirrhinum* and *flo* mutants grown under continuous LD or SD. A family segregating for wild type and *flo* mutants were grown in continuous LD (A,B) or continuous SD (C,D) respectively. Adult plants were scored as in Fig. 4 for leaf size, position of flowers (on wild-type plants) and the density of hairs on the stem above each node. One representative (out of 12 to 15 plants), having the median value for the position of the minimal leaf size, was chosen and graphed.

wild-type plants, partial inflorescence traits were not inducible by a few LD pulses. Thus, both the development of partial traits and floral meristems by LD pulses was dependent upon *flo*. Therefore, two pathways appear to act in parallel in *Antirrhinum*, one *flo*-dependent and one *flo*-independent. The *flo*-dependent pathway requires a lower threshold of inductive signal to be activated, allowing the two pathways to be separated by exposing plants to short pulses of LD.

The activation of the *flo*-dependent pathway was further investigated by analysing the distribution of *flo* transcripts in meristems. Expression of *flo* can be induced in SD grown

plants by a single LD pulse (8 hours extra of daylength) and this expression is limited to nodes that will bear flowers. The induction of *flo* is discrete, with only the youngest nodes at the apex (0-4) responding, suggesting that there is a small window of competent leaf primordia and axillary meristems. Older nodes do not respond to the inductive stimulus, reflecting either their insensitivity to some signal or the spatial restriction of the signal in the apex. The induction of *flo* expression is not only rapid but persists, since plants pulsed for a short time before return to non-inductive SD, express *flo* at all nodes subsequently arising at the apex. The level of *flo* expression may be critical for commitment. Weak expression of *flo* at nodes that did not give rise to floral meristems, such as nodes 0-4 in 43 SD grown plants (corresponding to about leaf number 32), suggests that a threshold level of expression may be required. This may reflect autoregulation of *flo* such that if the initial level is not over the appropriate threshold then it may not persist (Carpenter et al., 1995). Partial flowers (most organs identifiable but not expanded) or floral-like structures (a whorled arrangement of floral-like organs) can be generated in *Antirrhinum* by giving plants short periods of inductive pulse, suggesting that there is a second level of control in the development of complete floral organs.

The *squa* gene is induced by the same inductive conditions as *flo* and in a similar manner, suggesting that it is also an early marker for floral meristems. Although neither *flo* nor *squa* appear to influence each other's early expression, similar to their homologues in *Arabidopsis*, both are necessary for the correct development of floral meristems and may reinforce each other once activated (Huijser et al., 1992; Huala and Sussex, 1992; Weigel et al., 1992; Carpenter et al., 1995).

Several observations indicate that activation of *flo* and *squa* may not only be markers for floral meristems but may also mark commitment of an axillary meristem to become a flower. First, only when plants are competent to flower in LD or SD, is *flo* activated. Secondly, by manipulating growth conditions (continuous LD or SD, or giving LD pulses), the position on the stem where the axillary meristems become flowers can be manipulated and these correspond to where *flo* is expressed. Thirdly, *flo* is activated very early and before any morphological change in the axillary meristem distinguishes a shoot from a floral meristem (Carpenter et al., 1995). Finally, the *flo* phenotype defines a key role for *flo* in the switching of axillary meristems to becoming floral meristems. In mutants such as *centroradialis*, the shoot apical meristem is converted to a flower and this correlates with altered expression of *flo* and *squa* (Huijser et al., 1992).

In *Arabidopsis*, *lfy* and *ap1* expression appears to be limited to floral meristems similar to *flo*, supporting the idea that these genes are markers for floral meristems (Weigel et al., 1992; Mandel et al, 1992). However, a tobacco gene, *NFL*, of very similar sequence to *flo* and *lfy*, is expressed in vegetative as well as floral meristems (Kelly et al., 1995). This may reflect a difference in *NFL* function, or a shift in control to the protein level, or that *NFL* is not the functional homologue of *flo*. It is also possible that plants with determinate, cymose inflorescences, such as tobacco, may have modified the action of their *flo* homologue reflecting a change in inflorescence structure, or that some other factor, such as the *squa* homologue, is not active in the vegetative meristems (Kelly et al., 1995).

The level of *flo* or *squa* activity may be crucial to the stable commitment of meristems to form flowers. Reducing the level of *flo* or *squa* may account for the effect of reducing the dosage of *lfy* under certain environmental conditions and obtaining flowers that return to forming a shoot (Okamuro et al., 1993). Similarly, perhaps the level of *flo* or *squa* activity is important in species such as *Impatiens* or *Silene* which fail to generate all whorls of the flower and revert to producing a shoot under certain environmental conditions (Battey and Lyndon, 1990; Donnison and Francis, 1994).

In addition to committing axillary meristems to floral development, the *flo*-dependent pathway gives rise to partial inflorescence traits. The action of *flo* is to make the transition region from partial to full inflorescence traits occur over fewer nodes and thus more discrete. In *flo* mutants grown under SD, the duration of partial inflorescence traits was greatly extended compared to wild type. The *flo* gene is not evidently expressed in the inflorescence meristem, yet it has an influence on the inflorescence traits. This could be a direct effect of *flo* in the subtending leaves, where *flo* is expressed. However, this does not explain the effect of *flo* on stem hairs or internode length. Another possibility is that *flo* influences the apex even though it is not expressed there, or that it is weakly expressed in the apex, at a level below our detection methods. The *flo* and *lfy* mutants have already revealed some role for these genes in the apex, including the inhibition of carpel development or the expression of genes normally limited to axillary meristems (Huala and Sussex, 1992; Weigel et al., 1992; Carpenter et al., 1995; Simon et al., 1994).

Both the *flo*-dependent and *flo*-independent programmes are separate from that controlling phyllotaxy. The change from decussate to spiral phyllotaxy, that occurs under SD or LD, is independent of daylength or *flo*. This third programme may act as a marker for plants competent to flower, although manipulation of conditions in some species can induce flowers without such changes in phyllotaxy (Bernier et al., 1981). Nevertheless, it is striking that the change in phyllotaxy corresponds approximately to the appearance of the inflorescence and floral meristems under optimal LD conditions. Although all three programmes, inflorescence, floral and phyllotaxy, are independent, they may have evolved to appear synchronously in *Antirrhinum* for optimal presentation of flowers on a modified stem. By varying the relative timing of these three programmes in *Antirrhinum* or other species, a wide variety in flowering times, plant heights and traits could be generated.

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