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UNIFOLIATA regulates leaf and flower morphogenesis in pea

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Background: The vegetative phenotype of the pea mutant *unifoliata* (*uni*) is a simplification of the wild-type compound leaf to a single leaflet. Mutant *uni* plants are also self-sterile and the flowers resemble known floral meristem and organ identity mutants. In *Antirrhinum* and *Arabidopsis*, mutations in the floral meristem identity gene *FLORICAULA / LEAFY* (*FLO*/*LFY*) affect flower development alone, whereas the tobacco *FLO/LFY* homologue, *NFL*, is expressed in vegetative tissues, suggesting that *NFL* specifies determinacy in the progenitor cells for both flowers and leaves. In this paper, we characterised the pea homologue of *FLO/LFY*.

Results: The pea cDNA homologue of *FLO*/*LFY*, *PEAFLO*, mapped to the *uni* locus in recombinant-inbred mapping populations and markers based on *PEAFLO* cosegregated with *uni* in segregating sibling populations. The characterisation of two spontaneous *uni* mutant alleles, one containing a deletion and the other a point mutation in the *PEAFLO* coding sequences, predicted that *PEAFLO* corresponds to *UNI* and that the mutant vegetative phenotype was conferred by the defective *PEAFLO* gene.

Conclusions: The *uni* mutant demonstrates that there are shared regulatory processes in the morphogenesis of leaves and flowers and that floral meristem identity genes have an extended role in plant development. Pleiotropic regulatory genes such as *UNI* support the hypothesis that leaves and flowers derive from a common ancestral sporophyll-like structure. The regulation of indeterminacy during leaf and flower morphogenesis by *UNI* may reflect a primitive function for the gene in the pre-angiosperm era.

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Background

Structural similarities between leaves and floral organs and between vegetative shoots and flowers have long been recognised [1,2]. A striking comparison can be made between the similar developmental units of compound leaves and flowers: both arise laterally from primordia derived from the shoot apical meristem; both produce lateral, leaf-like organs; and both are determinate (a leaf or flower primordium is said to be determinate if it has a limited growth potential, whereas a shoot primordium is considered to be indeterminate [3]). Pleiotropic mutants that are affected in leaf and flower development and combinations of floral homeotic mutations that result in the conversion of floral organs to leaf-like structures [4] suggest that there are common regulatory processes in the production of leaves and flowers. Further support for a common mechanism of regulation comes from transgenic studies in which leaf morphology is affected by ectopic expression of a floral homeotic gene [5,6], and the converse experiment in which floral organ formation is perturbed in plants that overexpress a gene demarcating leaf development [7]. In this paper, we describe the characterisation of *UNI*, a gene that regulates both leaf and flower morphogenesis in pea [8]. We show that *UNI* is the homologue of *FLO* [9] and *LFY* [10], which are both known to be floral meristem identity genes. We propose that *UNI* has a more general role in regulating indeterminacy in lateral primordia derived from apical meristems, and that this role may reflect an ancestral function for the gene.

The wild-type pea leaf is compound odd-pinnate; the petiole arises between a pair of stipules, and the leaf rachis supports pairs of leaflets, tendrils and a terminal tendril (Figure 1a,f). The wild-type flower is borne on an axillary peduncle (inflorescence), subtended by the compound leaf. The peduncle terminates as a collar of tissue with an adaxial spike and produces a pedicel (floral meristem) bearing a pentamerous arrangement of sepals and petals, ten stamens and a central carpel ([11]; Figure 1a,d). The recessive *uni* mutation alters the structure of leaves and flowers ([8]; Figure 1). Mutant leaves are simpler than wild-type leaves, having a shorter petiole bearing one to three pulvinate leaflets between a normal pair of stipules. Neither rachis nor tendrils are formed (Figure 1a,e). The peduncle and pedicel of the *uni* mutant are intact; however, the flower lacks petals and stamens and consists

of an incomplete sepal whorl, an open gynoecium and numerous iterations of axillary flowers of this kind. Bract-like laminae are often present in sepal whorls and the floral organs are commonly fused or mosaic (Figure 1a–c).

Similar flowers have been described on *Arabidopsis* plants that carry a mutation at the *LFY* locus [10] and to a lesser degree on *Antirrhinum flo* mutants [9]. Although no changes to vegetative leaf morphology were described for *flo* and *lfy* mutants, the resemblance of their floral phenotypes to those of *uni* mutants led us to hypothesise that the homologous gene from pea might correspond to *UNI*.

Results and discussion

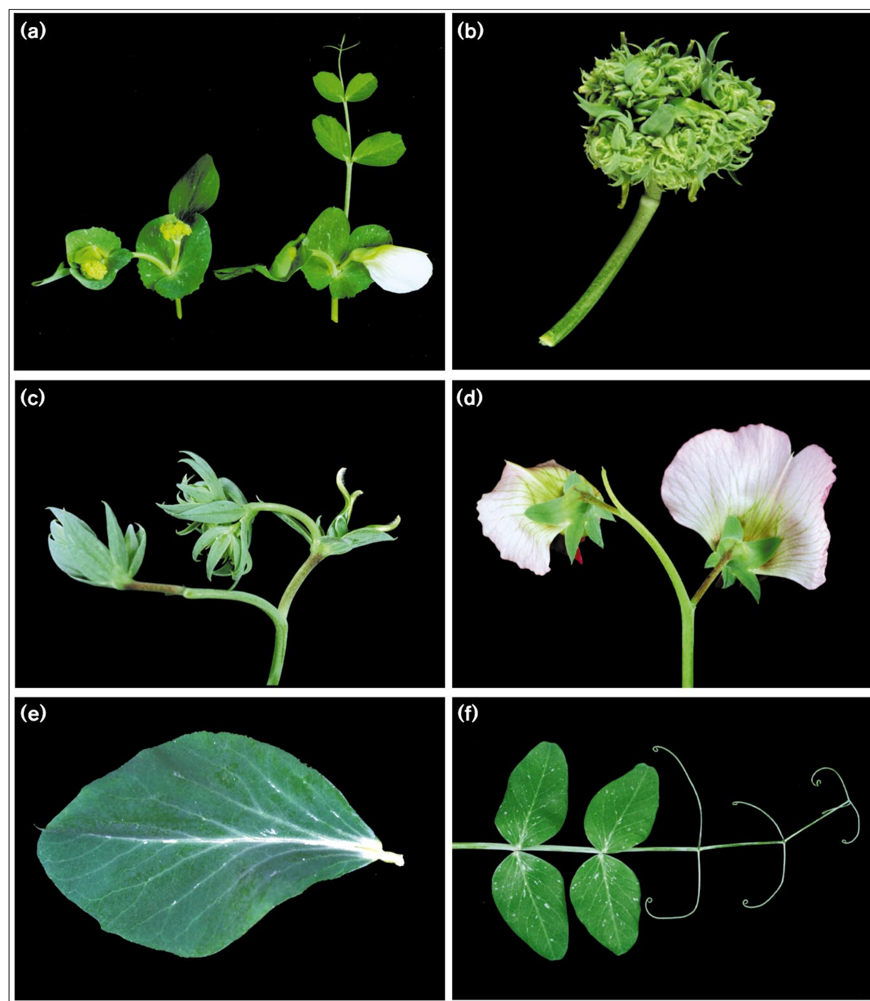
Map location of a pea *FLO/LFY* homologue

We isolated *PEAFLO*, a full-length cDNA homologue of *FLO*, from a bacteriophage library made from pea shoot-tip mRNA. The predicted protein sequence is 395 amino acids long and is similar to the cognate *Antirrhinum*, *Arabidopsis*, tobacco and cauliflower *FLO* sequences (76%, 71%, 78% and 68% identity, respectively; Figure 2). Restriction fragment length polymorphisms (RFLPs) detected by *PEAFLO* were used to map the single-copy gene in two independent recombinant inbred (RI) mapping populations (71 RI lines descended from the cross JI 281 × JI 399 and 44 lines from JI 813 × JI 1201; [12]). Alignment of the two RFLP maps with the classical pea linkage map [13] showed that the *PEAFLO* marker and the *uni* locus were in equivalent positions on these collinear maps. These results prompted us to screen segregating sibling populations for an RFLP that cosegregated with the *uni* phenotype.

Segregation analysis

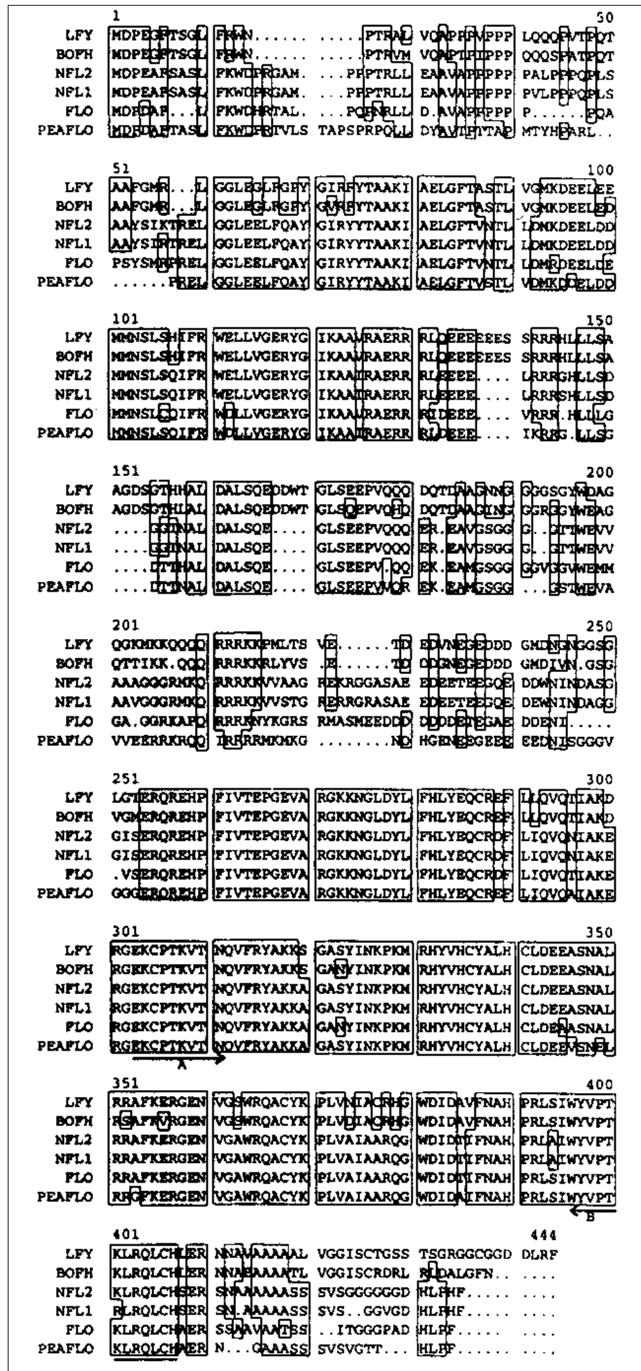
The *uni* line JI 2171 is descended from the original spontaneous mutant described by Eriksson [8] and is maintained in the John Innes *Pisum* Germplasm Collection as a heterozygote. Another spontaneous mutant with a similar *uni* phenotype was identified in the cultivar 'Honey' at the John Innes Institute in 1968, crossed into a different

Figure 1



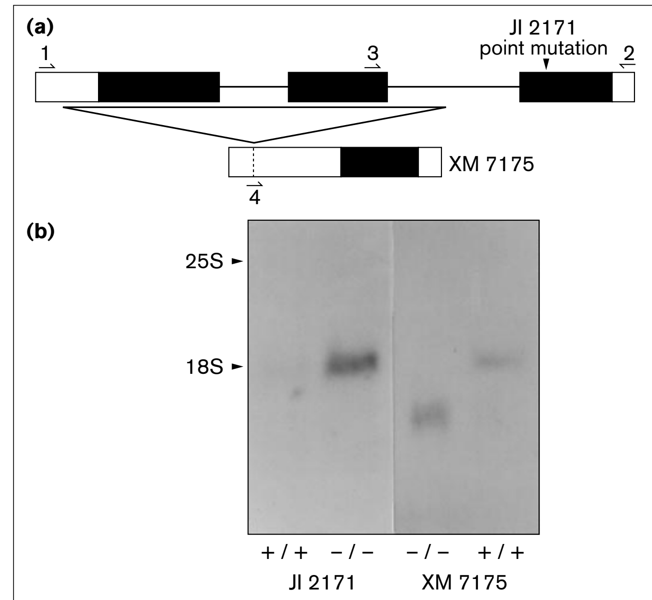
Phenotypes of wild-type and *uni* mutant peas. **(a)** Wild-type flowering node (right) and *uni/uni* flowering node (left), line JI 2171. In both specimens, the subtending compound leaf is behind the flower and the growing tip of the main shoot axis and subsequent nodes are bending left. **(b)** A *uni/uni* flower (XM 7175) showing a profusion of sepalloid and carpelloid floral organs. **(c)** A *uni/uni* flower from a cross (XM 7175 × JI 1396) showing anthocyanin pigmentation of pedicels. The pedicel in the centre is faintly pigmented. The central flower shown here arises axillary to an adaxial sepal and gives rise to further axillary flowers. **(d)** Wild-type flower (JI 813) showing an unpigmented peduncle terminating in an adaxial spike and anthocyanin-pigmented pedicels bearing flowers. Five sepals and an adaxial petal, called the standard, are visible. **(e)** *uni/uni* unifoliate leaf (XM7175) consisting of a short petiole and pulvinis bearing a single leaflet. Stipules are not shown. **(f)** Wild-type leaf (JI 813) with two pairs of leaflets, three pairs of tendrils and a terminal tendril (bending away from view). Stipules are not shown.

Figure 2



Predicted amino-acid sequence of PEAFLO aligned with the coding sequences of *FLORICAULA* (FLO; [9]) from *Antirrhinum*, and its homologues from tobacco (NFL1 and NFL2; [15]), cauliflower (BOFH;[33]) and *Arabidopsis* (LFY; [10]). Identical amino acids are boxed. Arrows indicate the positions of degenerate primers used to obtain a partial-length *PEAFLO* probe. Sequences were aligned using the programme PILEUP and percentage identities with *PEAFLO* were calculated using BESTFIT [34].

Figure 3



Map of *uni* alleles and northern-blot analysis of *UNI* (*PEAFLO*) mRNA in flowers. **(a)** Wild-type *uni* allele shown with exons (boxes, shaded for open reading frame) and introns (horizontal lines). The single nucleotide substitution in the JI 2171 *uni* mutant allele is represented by a vertical arrowhead. The deletion in the XM 7175 *uni* mutant allele is represented by a large triangle and the resulting truncated genomic product is shown below the full-length map with a potential open reading frame starting at methionine 297 (corresponding to position 330 in Figure 2). PCR primers used for segregating progeny and sequence analysis are represented by horizontal arrows. **(b)** Northern blot of RNA from wild-type (+/+) and *uni/uni* (–/–) flowers. *UNI* mRNA was more abundant in JI 2171 *uni/uni* flowers compared with wild-type flowers. A smaller transcript was detected in XM 7175 *uni/uni* flowers. 25S and 18S ribosomal RNA size markers are shown on the left.

genetic background and maintained in the collection as line XM 7175. The Honey mutant was shown to be allelic to *uni* by examining F1 progeny from a cross between heterozygous JI 2171 and XM 7175 plants. Offspring confirmed as double heterozygotes exhibited a *uni* phenotype, indicating that the mutations were non-complementary and therefore allelic. The *PEAFLO* cDNA probe detected an RFLP that cosegregated with five *uni* mutants among 17 sibling progeny derived from a selfed XM 7175 heterozygous plant. Furthermore, a PCR-generated marker (Figure 3) cosegregated with the mutation in all five *uni* progeny in a larger population of 40 sibling plants, derived from a different self-pollinated heterozygous XM 7175 parent (data not shown).

Molecular characterisation of *UNI* alleles

Genomic DNA spanning the *PEAFLO* coding region was amplified from mutant and wild-type XM 7175 siblings (Figure 3). Sequence analysis showed that *uni* individuals had a 1503 bp deletion when compared with wild-type plants. The deletion begins 145 bp upstream of the initiation methionine and ends in the second intron, removing one of a duplicated pair of 5 bp sequences. As predicted by

sequence analysis, a smaller *PEAFLO* transcript is generated in XM 7175 *uni* mutants (Figure 3). There is some evidence that the deletion transcript may be more labile, seen as a smeared band in northern analysis (Figure 3b) and as a weaker hybridising signal in *in situ* hybridisation analysis (Figure 4c). PCR-amplified genomic DNA from JI 2171 *uni* plants contained a single nucleotide change in the highly conserved 3′ end of the *PEAFLO* coding sequence compared with wild-type sibling sequences (Figure 3), resulting in the substitution of a tryptophan residue for an arginine at position 318 in the mutant protein (corresponding to position 351 in Figure 2). Transcripts of equal size were detected in JI 2171 wild-type and *uni* mutant flowers, although the mutant mRNA was in greater abundance (Figure 3b). A likely explanation for this is that a larger number of initiating primordia expressing *PEAFLO* are present in *uni* flowers (Figures 1,4). *PEAFLO* expression levels are also correlated with organ age (Figures 3–5). Wild-type JI 2171 flower buds harvested for northern analysis in Figure 3b were older than wild-type XM 7175 flower buds, consistent with the lower level of *PEAFLO* expression in JI 2171 flowers.

FLO/LFY function

The functions of *FLO* and *LFY* have been determined by phenotype analysis and gene expression studies of mutant and transgenic plants. The inflorescence-like flowers of *flo* and *lfy* mutants that have been described [9,10] are in accordance with a loss of determinacy in flowers. The primary and lateral shoots of *Arabidopsis* plants over-expressing *LFY* are converted into flower meristems, demonstrating that *LFY* is sufficient for the formation of determinate floral meristems from indeterminate shoot meristems in a wild-type genetic background [14]. *FLO* homologues are expressed in both floral and non-floral tissues, however, suggesting that the gene has a wider role in plant development than simply to confer floral meristem identity. For example, *FLO* and *LFY* are expressed in bract and cauline leaf primordia, respectively [6,9,10], and the tobacco and *Impatiens* homologues are expressed in floral and vegetative meristems [15,16]. A more extensive developmental role has been postulated for the tobacco homologue of *FLO*, *NFL*, in the establishment of determinacy in all recent derivatives of apical initial cells that are destined for both floral and vegetative lateral structures [15].

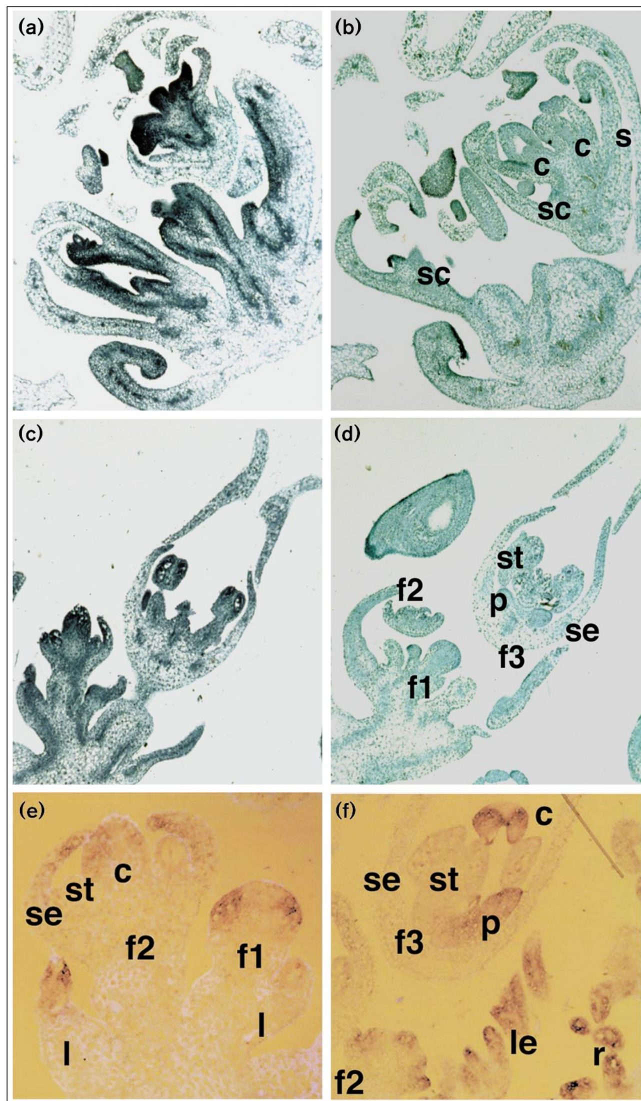
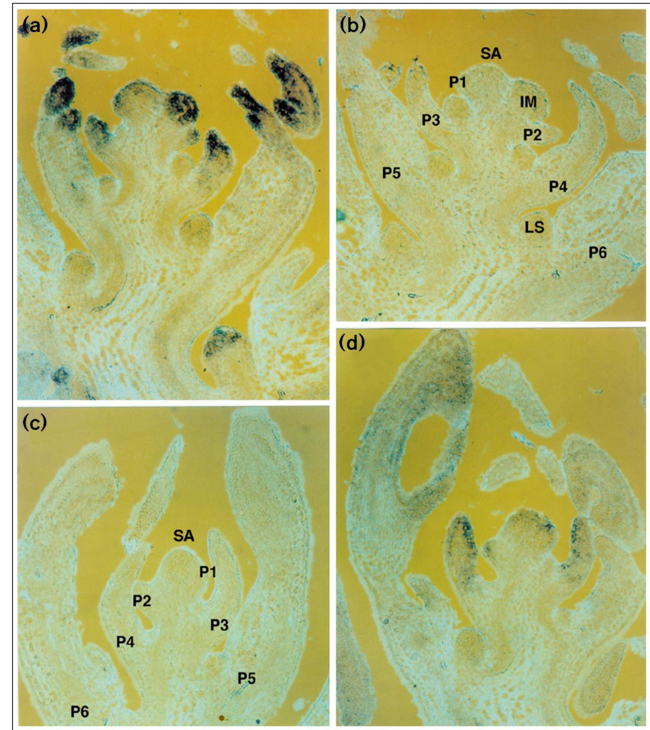


Figure 4

Expression pattern of *UNI* (*PEAFLO*) mRNA in flowers. RNA *in situ* hybridisations were performed on longitudinal sections of flowers. **(a)** *uni/uni* flower (JI 2171). An antisense *UNI* probe detected mRNA in sepalloid carpels, carpel-like and sepal-like organs, labelled on a consecutive section in (b). **(b)** *uni/uni* flower (JI 2171) hybridised to the *UNI* sense probe. Abbreviations: sc, sepalloid carpels; c, carpel-like organ; s, sepal-like organ. **(c)** Wild-type flower (JI 2171). At stage f2, an antisense *UNI* probe detected mRNA in sepal and carpel primordia and outer stamen primordia derived from common primordia. At stage f3, the *UNI* signal was strong in carpels, petals, see also (f), and weaker in sepals and stamens, labelled on a consecutive section in (d). **(d)** Wild-type flower (JI 2171) hybridised to the *UNI* sense probe. f1, floral meristem approximately 200 μm across at initiation of sepal primordia; f2, floral meristem with expanding sepals, central carpel primordium and common primordia dividing into petal and outer stamen primordia; f3, floral bud with expanding organs; se, sepal; p, petal; st, stamen. **(e)** Wild-type flower (XM 7175). An antisense *UNI* probe detected mRNA in cells of an f1 floral meristem that would give rise to sepal and common primordia and not in the intervening central region. In an adjacent f2 floral meristem, *UNI* mRNA was detected in sepal and carpel primordia and was weakly detected in outer stamen primordia. *UNI* mRNA was also detected in leaves subtending floral meristems. se, sepal; st, stamen; c, carpel; l, leaf; f1 and f2 as in (d). **(f)** Wild-type flower (XM 7175). In this f2 meristem, which is less mature than those shown in (c,e), *UNI* mRNA was confined to common primordia and is absent from inner stamen progenitor cells. Strongly hybridising signals were also detected in petals and carpels of an f3 floral bud and in leaflets (le) and rachillae (r) at the bottom right. Images (a–d) are at ×22 magnification, and the mRNA signal appears dark on a green tissue background. Images (e,f) are at ×60 magnification, and the mRNA signal appears dark on a white tissue background.

Like *flo* and *lfy*, *uni* flowers exhibit a loss of determinacy, in that supernumary flowers arise in the axils of first whorl organs; however, reiterative flowers of this kind are also characteristic of *squamosa* (*squa*)/*apetala1* (*ap1*) and *unusual floral organs* (*ufo*)/*fimbriata* (*fim*) mutants [4,17]. It has been proposed that *FLO/LFY* interacts with and activates *SQUA/AP1* and *UFO/FIM* [14,17–19], so the indeterminate *uni* floral phenotype may be a secondary consequence of a failure to activate downstream genes or interact with cofactors. Multiple genes with overlapping roles, such as *FLO/LFY*, *SQUA/AP1*, *FIM/UFO* and *CAULIFLOWER* [20], make functional analysis difficult in flowers. We decided that it would be easier to define *UNI* function in compound leaves because these have a simpler structure than flowers.

Figure 5



Expression pattern of *UNI* (*PEAFLO*) mRNA in XM 7175 shoots. RNA *in situ* hybridisations were performed on median longitudinal sections of pea shoots that were fixed before they flowered. **(a)** An antisense *UNI* probe detected mRNA in leaf primordia P1–P8 (P1–P6 are shown and labelled on the consecutive section in (b). As leaf size increased, *UNI* expression became localised to distal, adaxial regions where rachillae and leaflets were developing. *UNI* mRNA was also detected in lateral shoot primordia (LS) and in a newly initiated inflorescence meristem (IM). The shoot apex is indicated by SA. **(b)** Consecutive section to (a), hybridised to the *UNI* sense probe. **(c)** A *uni* mutant vegetative shoot hybridised to the *UNI* sense probe. **(d)** Consecutive section to (c), hybridised to the antisense probe. A smaller *UNI* transcript (Figure 3) was detected in leaf primordia (P1–P6 shown and labelled in Figure 5c). The plants shown are sibling progeny of genotypes *Uni/Uni, afila/afila* (*af*), *tendril-less/tendril-less* (*tl*) (a,b) and *uni/uni, af/af, tl/tl* (c,d). The same pattern of expression, but at lower levels, is observed in wild-type (*Uni/Uni, Afila/Afila, Tendril-less/Tendril-less*) vegetative shoots. All images are at ×55 magnification, and the mRNA signal appears dark on a white tissue background.

Pea leaves are 'partially determinate'

Surgical experiments on isolated pea leaf primordia have shown that the characteristic shape of a leaf is resolved gradually and acropetally, over four plastochrons, from the time a primordium is initiated on the shoot apical meristem [21,22]. Likewise, the four distinctive whorls of a pea flower are developed within four plastochrons [11]. A primordium is defined as indeterminate if it has an unlimited growth potential and emerges as a shoot or whole plant when cultured in isolation from the shoot apical meristem. In contrast, a determinate primordium is developmentally constrained and has only the potential to develop into one particular organ, for example, a leaf [3]. The degree of branching, or bifurcation, on a mature vegetative structure thus reflects the initial growth potential of the primordium from which it was elaborated. A vegetative primordium of pea has three developmental possibilities: it can become a unifoliate leaf, a multifoliate leaf or a shoot. If each of these increasingly branched structures reflects the status of the primordium from which it was derived, the shoot primordium being indeterminate and the unifoliate leaf primordium being determinate, then the multifoliate compound leaf primordium has some intermediate, 'partially determinate' condition, or, it has a changing developmental potential that is transiently indeterminate, then determinate. A transient phase of indeterminacy may be characteristic of all lateral primordia derived from apical meristems. This transient phase would be prolonged in compound leaf and flower primordia, curtailed in simple leaf and floral organ progenitors, and would not occur in groups of cells that are fully determinate and fail to develop into primordia.

UNI function

The hypothesis that *FLO* homologues act to specify determinacy in the progenitor cells for flowers and leaves [15] predicts that the leaves of a loss-of-function mutant would be more branched, or indeterminate, than wild-type. The more determinate, unifoliate leaf of the *uni* mutant counters this prediction, however, and instead *UNI* might have an opposite function in the specification of indeterminacy in compound leaves. In accordance with this, the addition of increasing amounts of *UNI* function to XM 7175 mutant pea leaves resulted in increased branching [23]. We propose that the role of *UNI* and other *FLO* homologues is to maintain a transient phase of indeterminacy that precedes determination of lateral derivatives of an apical meristem. *In situ* hybridisation experiments showed that *PEAFLO* was expressed strongly in leaf, leaflet, inflorescence and lateral shoot primordia on the main shoot axis (Figure 5) and in floral organ primordia (Figure 4), consistent with this proposed role for *UNI* in pea development.

FLO expression in wild-type *Antirrhinum* [9], *Arabidopsis* [10] and tobacco [15] inflorescence and floral meristems has been described and its possible function in the cascade of events leading to activation of genes required for determinacy has been suggested [9]. Floral ontogeny in the pea is very different from these three species in that, in the pea, five petal and five outer stamen primordia are derived from four common primordia, and the carpel primordium is initiated relatively early [24]. Like *LFY* in *Arabidopsis* [10], *PEAFLO* expression in floral meristems occurred in developing primordia as they arose and declined as organs expanded. Unlike *NFL* in tobacco [15], *PEAFLO* was strongly expressed in carpel primordia.

Three stages of floral development are shown in Figure 4. At stage f1, when the floral meristem is approximately 200 μm wide, *PEAFLO* expression was observed in cells on the meristem flanks that will give rise to sepal and common primordia [24] and was absent from the intervening central region (Figure 4e). At stage f2, characterised by the expansion of sepals, the division of common primordia into petal and outer stamen primordia and the initiation of the central carpel, *PEAFLO* mRNA was detected in all of these primordia but was absent from the region of the meristem from which inner stamen primordia would arise (Figure 4c,e,f). At stage f3, when the organs of the floral bud are expanding, expression of *PEAFLO* was strong in petals and the distal portion of the carpel, weaker in stamens, and weak in sepals (Figure 4c,f).

The novel function of *UNI* in regulating leaf morphogenesis is supported by the presence of *PEAFLO* mRNA during the first four plastochrons after leaf primordium initiation (Figure 5), at the time when pea leaf pattern is established [21,22]. Strong *PEAFLO* expression was detected in petiole–rachis progenitor cells of the P1 primordium, at a stage prior to the differentiation of leaf lateral organs [25,26]. In older primordia, P4–P6, *PEAFLO* transcript levels were reduced in the rachis; strong expression was confined to newly developed lateral appendages, corresponding to rachilla and leaflet primordia in this genotype (Figure 5a).

This previously uncharacterised function of *FLO*/*LFY* homologues in leaf development is revealed in pea presumably because it is a plant species that has compound leaves. Leaf shape in tomato, another species with compound leaves, can be manipulated by overexpression of a different gene, *KNOTTED* [6]. It is possible that different regulatory mechanisms operate in pea and tomato, which are distinguished by acropetal and basipetal leaf development, respectively [7,26]; analysis of a range of species will be required to ascertain whether *FLO*/*LFY* homologues play a role in the morphogenesis of all types of compound leaves, or only in leaves with acropetally-initiating lateral organs.

Origins of leaves and flowers

The fossil record first chronicles a variety of compound leaf forms during the adaptive radiation of angiosperms in the Cretaceous period [27], concomitant with the major phase of diversification of whorled angiosperm flowers [28]. Arguments in favour of the atavistic derivation of modern angiosperm leaves from the compound fronds of Carboniferous seed ferns have also been made, however [29]. Parsimony analyses support the contention that anthophyte [29] flowers were derived from the sporophyllous fronds of seed ferns; one interpretation is that carpels can be homologised with the sporophyll rachis and ovules with primitive cupules [27]. Compound leaves and flowers can thus be considered to be derivatives of the same ancestral structure. The proposed common function of *UNI* in regulating indeterminacy during leaf and floral development may reflect a primitive function for this gene in the fronds of seed ferns in the pre-angiosperm era.

Materials and methods

Plant material

The recombinant inbred mapping lines JI 281 × JI 399, JI 813 × JI 1201 and the *uni* mutant type line JI 2171 were obtained from the John Innes Germplasm Collection. The spontaneous *uni* mutant identified in JI 385, cultivar 'Honey', was crossed into a genetic background containing two further leaf morphology mutations, *afila* [30] and *tendril-less* [31] and was maintained as line XM 7175. Plants were grown in glasshouses, individually potted in John Innes No. 1 potting mix plus 30% extra grit.

Gel blot analyses

RNA and DNA were extracted from pea tissues [32]. For Southern blots, genomic DNA was digested overnight with *Hin*dIII or *Eco*RI and separated on a vertical 0.8% agarose gel in 25 mM Tris-acetate buffer. DNA was transferred to nitrocellulose and hybridised with a ^{32}P-labelled probe. *PEAFLO* probes were excised from plasmid DNA, isolated by gel electrophoresis and labelled by extension of random hexamers. Filters were washed twice in 0.1× SSC, 0.1% SDS at 65°C and exposed to X-ray film. For northern blots, samples of total RNA were fractionated on a formaldehyde agarose gel, transferred to a nitrocellulose membrane and hybridised to a ^{32}P-radiolabelled *PEAFLO* probe. An rDNA control probe was used to verify that the amount of RNA (20 μg) in XM 7175 wild-type and mutant lanes was approximately equal. Ethidium bromide staining confirmed that more RNA (50 μg) was loaded in the JI 2171 wild-type lane compared to the JI 2171 mutant lane (20 μg), to enable detection of the less abundant JI 2171 wild-type *PEAFLO* transcript.

cDNA library preparation

Shoot apices, including apical meristems, inflorescence meristems and leaf and flower primordia, were harvested from JI 813 pea plants. PolyA$^+$ RNA was isolated on an oligo(dT)–cellulose affinity column (Pharmacia). A cDNA library of approximately 2.5 × 10^5 clones was constructed from 5 μg polyA$^+$ RNA using a lambda Unizap cDNA cloning kit (Stratagene). Size-selected cDNA (100 ng; Pharmacia SizeSelect-400 spin column) was ligated with 1 μg *Eco*RI/*Xho*I-digested lambda vector and packaged with GigapackII packaging extract (Stratagene).

Isolation of a FLO/LFY *cDNA homologue*

Degenerate PCR primers were used to obtain a 315 bp probe, which was cloned into pBluescript, sequenced and then used to isolate the full-length *PEAFLO* cDNA from a cDNA library (see above). The 315 bp probe was amplified from first-strand cDNA, made from 1 μg of polyA$^+$ shoot tip mRNA, using primers A and B in 40 cycles of PCR at 94°C, 60 sec; 50°C, 60 sec; 72°C, 120 sec. Primer A: 5′GA(A/G)AA

(A/G)TG(T/C)CCACIAA(A/G)GT(T/C/G/A)AC(T/C/G/A)AA3′. Primer B: 5′TGGCA(A/G)AGCTGACG(C/A)AGCTT(A/G/C/T)GT(A/G/C/T) GG(A/C/G/T)AC(A/G)TACCA3′. The *PEAFLO* cDNA sequence is available as Genbank accession number AFO10190.

Linkage analysis

Segregation analysis was carried out on F_8 generations of the recombinant inbred populations derived from the crosses JI 281 × JI 399 and JI 813 × JI 1201. These populations and the methods for estimating linkage distances [12] used the Haldane correction for multiple meioses in the generation of recombinant inbred lines and the Haldane mapping function for the calculation of map distances.

Analysis of mutant UNI *alleles*

For PCR analysis of XM 7175 populations segregating for *uni*, three oligonucleotides were used together. Primer 3 (5′CATCGCTAAAGAGCGCGGTG3′), primer 4, spanning the deleted region (5′GTTCAAAACCATGCAACACGTG3′) and primer 2, in the 3′ non-coding region (5′CTCCCGTCCATTGGTGGAA3′), generated 788 bp and 837 bp products from homozygous *uni/uni* and wild-type plants, respectively. For sequence analysis of *UNI* alleles, three independent PCR amplifications from wild-type JI 2171 genomic DNA, using primer 1 (5′CAACCTCAACTAGTCTCG3′) in the 5′ non-coding region and primer 2 generated 2314 bp products. These products were cloned into pBluescript SK (Stratagene) and their sequences were compared with three independently amplified PCR products from JI 2171 *uni/uni* genomic DNA. The same two primers were used to generate PCR products from XM 7175 genomic DNA for sequencing.

In situ *hybridisation*

Digoxygenin-labelled sense and antisense probes were generated from a full-length *PEAFLO* cDNA clone, cleaved with *Xho*I or *Eco*RI and transcribed with T3 or T7 RNA polymerase (Stratagene), respectively. Samples were embedded in wax and 8 μM sections were hybridised to probes as described previously [9]. XM 7175 sections were counterstained with 0.1% (w/v) calcofluor white M2R (Sigma) and viewed by light microscopy and ultraviolet illumination, JI 2171 sections were counterstained with 1% (w/v) fast green FCF (Sigma) in 95% ethanol and viewed by light microscopy.

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