Compound leaves: equal to the sum of their parts?

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Summary

The leaves of seed plants can be classified as being either simple or compound according to their shape. Two hypotheses address the homology between simple and compound leaves, which equate either individual leaflets of compound leaves with simple leaves or the entire compound leaf with a simple leaf. Here we discuss the genes that function in simple and compound leaf development, such as *KNOX1* genes, including how they interact with growth hormones to link growth regulation and development to cause changes in leaf complexity. Studies of transcription factors that control leaf development, their downstream targets, and how these targets are regulated are areas of inquiry that should increase our understanding of how leaf complexity is regulated and how it evolved through time.

Introduction

The major light gathering organ in most plants is the leaf. Evolution has produced a variety of leaves with different shapes, sizes and arrangements that reflect the diverse conditions that plants grow in. Recently, significant progress has been made in understanding the molecular mechanisms that regulate leaf development in a few model plant species. This has been achieved by combining careful morphological observations and traditional genetic analyses with advances in molecular biology, such as genetic transformation, and with information from completed genome projects. The current challenge is to explore whether the regulatory mechanisms that control leaf development in model species have been conserved in non-model species and how these regulatory mechanisms have evolved to produce various leaf forms.

The leaves of seed plants can be classified as being either simple or compound according to their degree of complexity (see Box 1). Two hypotheses have been proposed to explain the homology of simple and compound leaves. The first hypothesis equates individual leaflets of compound leaves with simple leaves. In this model, compound leaves are seen as partially indeterminate structures that share properties with both shoots and leaves (Fig. 1A) (Sattler and Rutishauser, 1992). The second hypothesis suggests that the entire compound leaf is equivalent to a simple leaf and that leaflets arise by subdivisions of a simple blade (Fig. 1B) (Kaplan, 1975). Viewed in this way, leaf shape is seen as a continuum that ranges from simple leaves with entire margins, to serrated, lobed, or compound leaves. Both hypotheses can be used to guide investigators as to which genes might regulate compound leaf development. For example, if the genes that regulate shoot indeterminacy were shown to regulate compound leaf morphogenesis, this would support the hypothesis that compound leaves are partially indeterminate structures. Conversely, the alternative hypothesis would be supported by the finding that the genes that regulate blade development in simple leaves generate compound leaf pinnae.

Several recent studies have investigated the development of compound leaves in many non-model species. In this review, we discuss the mechanisms that determine leaf morphology, emphasizing those that govern differences between simple leaves and compound leaves. When possible, we will speculate upon the evolution of these mechanisms and propose avenues of future investigation.

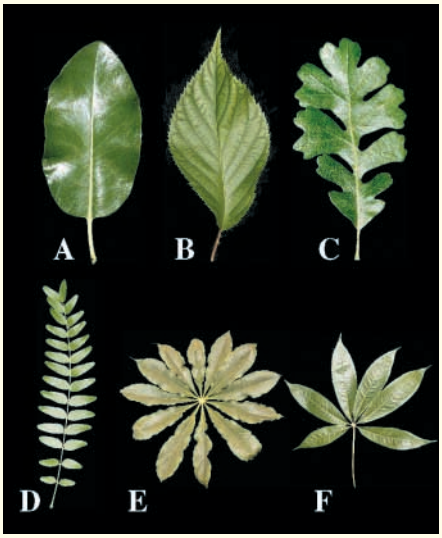
Genes controlling compound leaf development

Intensive research in model plant systems has identified numerous genes that control plant growth and development. The shoot apical meristem (SAM) of seed plants is an indeterminate structure that maintains itself and is the source of cells that give rise to determinate organs, such as leaves and flowers. Indeterminacy during vegetative and reproductive development is controlled by a suite of genes that function at different stages in the SAM. The process of leaf or floral organ initiation begins when cells in the incipient organ primordium alter their identity from being indeterminate to determinate. By comparing gene expression patterns between simple and compound leafed species during their development, it might be possible to assess the level of determinacy that each of these leaf types possesses.

The role of meristem genes

The indeterminate SAM is characterized by the expression of the Class 1 *KNOTTED1-LIKE HOMEOBOX* (*KNOX1*) genes. One of the earliest known indicators of a change in fate from indeterminate meristem cells to determinate leaf primordium cells is the downregulation of *KNOX1* genes. *KNOX1* genes have been implicated in the acquisition and/or maintenance of meristematic fate. Evidence for this is based on the phenotypes of loss-of-function mutants, misexpression mutants and overexpression transgenic plants. For example, loss-of-function mutations in the *KNOX1* genes *shoot meristemless* (*stm*) and *knotted1* (*kn1*) in *Arabidopsis* and maize, respectively, result in plants that are unable to maintain a SAM (Long et al., 1996; Vollbrecht et al., 2000). Maize plants that misexpress *KNOX1* genes outside of their normal domain have ectopic proliferation of tissue in leaves, described as knots, which often grow over veins (Vollbrecht et al., 1990;

Box 1. Simple and compound leaf forms



Simple leaves consist of a single blade borne on a supporting petiole that grows beneath, and in close association with, an axillary bud. Simple leaves can have margins that are entire (free from indentations), serrated or lobed. For example, *Arbutus menziesii* (A) has simple leaves with entire margins. The margins of *Prunus takesimensis* simple leaves (B) are serrated, and *Quercus lobata* (C) has simple leaves with deep lobes. Compound leaves have multiple blade units (called leaflets or pinnae), which are attached to a supporting structure called a rachis. Each compound leaf also subtends an axillary bud. Compound leaves vary depending on the arrangement of leaflets on the rachis and on the order of complexity. There are two main types of compound leaves: pinnate and palmate. Pinnate compound leaves have leaflets that occur in succession along a rachis, as seen in *Acacia spp* (D). Palmate compound leaves have leaflets borne at the tip of the rachis, and can be further categorized as being either peltate or non-peltate. Peltate leaves have leaflets that are present around the entire circumference of a radial, unifacial petiole, which is exhibited by *Arisaema taiwanensis* (E). Non-peltate leaves have leaflets present around a portion of a bifacial petiole, exemplified by *Chorisia speciosa* (F) (Kim et al., 2003b).

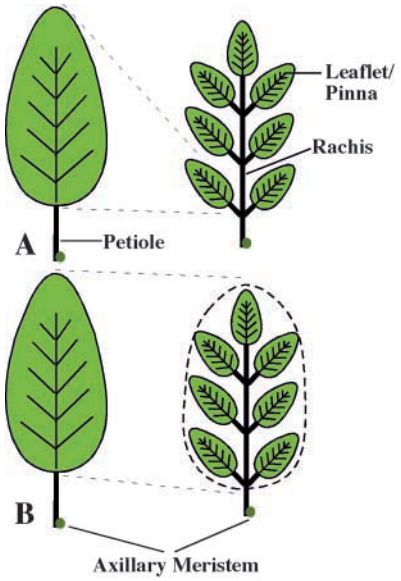


Fig. 1. Compound leaves can be viewed as (A) collections of simple leaves or (B) equivalent to simple leaves. [B redrawn, with permission, from Kaplan (Kaplan, 1975); see http://www.schweizerbart.de].

Schneeberger et al., 1995; Muehlbauer et al., 1999). Transgenic overexpression of *KNOX1* genes often results in plants with curled, wrinkled and lobed leaves that form ectopic meristems (Sinha et al., 1993; Chuck et al., 1996; Tamaoki et al., 1997; Schneeberger et al., 1998). Ectopic expression of *STM* inhibits the differentiation of leaf cells, activates G1/S cell cycle markers (Gallois et al., 2002), and activates a CyclinB::GUS reporter gene (Lenhard et al., 2002). Thus, *KNOX1* expression within or outside of the meristem appears to be sufficient to promote stem cell proliferation and indeterminacy.

KNOX1 genes are downregulated in the incipient leaf primordia in both compound leafed and simple leafed species (Fig. 2). In most plants with simple leaves, such as *Arabidopsis*, tobacco, snapdragon and maize, this downregulation is permanent (Fig. 2A,B) (Smith et al., 1992; Lincoln et al., 1994; Nishimura et al., 1998; Waites et al., 1998; Nishimura et al., 1999). However, *KNOX1* gene expression is re-established later in the developing primordia of most plants with compound leaves (with the exception of pea, see below), such as in tomato and *Oxalis* (Fig. 2C,D) (Hareven et al., 1996; Chen et al., 1997; Janssen et al., 1998; Bharathan et al., 2002). Additionally, overexpression of *KNOX1* genes in transgenic plants or in naturally occurring tomato mutants results in leaves with increased numbers of leaflets (Hareven et al., 1996; Chen et al., 1997; Parnis et al., 1997). It has therefore been concluded that *KNOX1* genes are involved in regulating compound leaf development by establishing a more indeterminate environment within developing primordia. A survey of *KNOX1* gene expression in diverse seed plant taxa has indicated that *KNOX1* genes may have been recruited multiple times during evolution for the regulation of leaf complexity across the flowering seed plants (angiosperms) (Bharathan et al., 2002).

An important exception to the trend of *KNOX1* expression in compound leaf primordia is found in pea. In pea, *KNOX1* gene expression is permanently downregulated in the incipient primordium, and expression is not re-established in developing leaves (Gourlay et al., 2000; Hofer et al., 2001). Instead, *UNIFOLIATA* (*UNI*), an ortholog of *FLORICAULA* (*FLO*)/*LEAFY* (*LFY*), controls compound leaf development in pea (Hofer et al., 1997). *FLO/LFY* orthologs encode a group of plant-specific transcription factors. The *uni* mutant has a reduction in leaf complexity. Wild-type pea leaves usually consist of two or three proximal lateral leaflet pairs and three to four distal tendril pairs, followed by a terminal tendril. *uni* leaves range from being completely simple to being trifoliate (Marx, 1987; Hofer et al., 1997; DeMason and Schmidt, 2001). In all angiosperms studied to date, *FLO/LFY* orthologs have been found to play a crucial role in flower meristem identity by activating genes that specify whorls of organs within the flower (Coen et al., 1990; Weigel et al., 1992; Souer et al.,

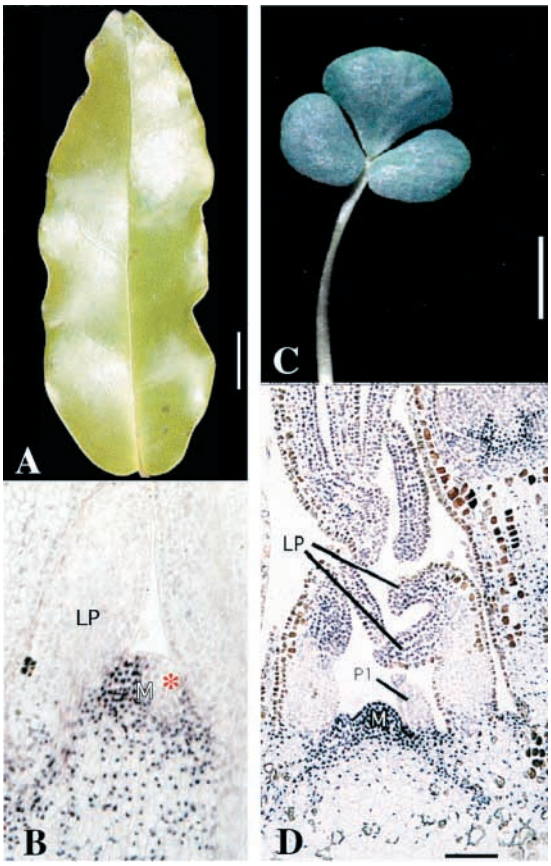


Fig. 2. Comparison of mature leaf form and KNOX1 expression patterns in simple and compound leaves. (A) *Amborella trichopoda* has simple leaves. (B) KNOX1 proteins accumulate in the shoot apical meristem (SAM) of *A. trichopoda*, except in the incipient leaf primordium (red asterisk). (C) *Oxalis* has compound leaves. (D) In *Oxalis*, KNOX1 proteins accumulate in the SAM and in developing leaves. LP, leaf primordia; P1, primordium 1; M, meristem. Images adapted, with permission, from Bharathan et al. (Bharathan et al., 2002).

1998; Molinero-Rosales et al., 1999). In addition to altered leaf development, the *uni* pea mutant has compromised floral development. Its transition to flowering is delayed, and when it does produce flowers, they are sterile and consist entirely of sepals and carpels (Marx, 1987; Hofer et al., 1997). Although expression of *FLO/LFY* is usually seen in vegetative SAMs and leaf primordia, in addition to in floral meristems, in simple leafed plants, such *Arabidopsis* and petunia, mutation of these genes does not cause altered leaf shape (Weigel et al., 1992; Souer et al., 1998). This suggests that the role of *FLO*/*LFY* orthologs in simple leafed plants is central to reproductive development but not to leaf development. Nonetheless, considering the expression patterns of *FLO*/*LFY* orthologs in simple leafed vegetative apices, their role, if any, in vegetative development remains unexplained.

The tomato *FLO/LFY* ortholog is *FALSIFLORA* (*FA*). Like in other angiosperms, the *fa* tomato mutant has altered flowering time and inflorescence development. Floral meristem identity is lost in these mutants and flowers are replaced by secondary shoots. Interestingly, the *fa* mutant has a subtle leaf phenotype – the number of small intercalary leaflets is slightly reduced, which can be interpreted as a reduction in complexity (Molinero-Rosales et al., 1999). Known expression patterns of *FLO*/*LFY* orthologs in vegetative apices have been summarized recently (Busch and Gleissberg, 2003). In species with compound leaves, such as pea, tomato, grapevine and poppy, *FLO*/*LFY* expression is prolonged during leaf development and accompanies organogenesis at the marginal blastozone (Busch and Gleissberg, 2003). Therefore, it is possible that *FLO*/*LFY* also functions in compound leaf development in species other than pea. The regulation of both vegetative and floral meristem development by *FLO/LFY* may reflect the ancestral condition of seed plants, and, if this were the case, compound leaf development in most situations would be regulated by a combination of *KNOX1* and *FLO*/*LFY* genes. In pea, the role of *KNOX1* genes in regulating compound leaf development would have been completely taken over by the *FLO/LFY* ortholog, *UNI*. Thus, the role of *FLO*/*LFY* in regulating compound leaf development in all angiosperms is an area worthy of further investigation.

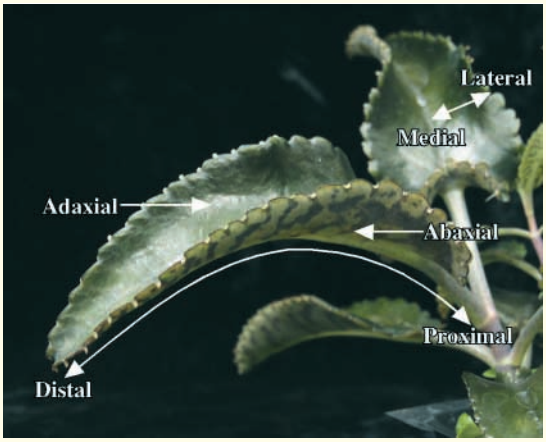
STAMINA PISTILLOIDA (*STP*) has been identified as another floral meristem gene that is involved in regulating compound leaf development in pea. Severe mutant *stp* alleles produce phenotypes similar to those observed in the *uni* mutant: flowers consisting of sepals and carpels, and a reduction in leaf complexity, in addition to other abnormalities. Weak mutant alleles of *stp* and *uni* act synergistically in pea, indicating that these two genes may act together to regulate common pathways (Taylor et al., 2001). *STP* is homologous to the *UNUSUAL FLORAL ORGANS* (*UFO*) gene of *Arabidopsis* and to the *FIMBRIATA* (*FIM*) gene of snapdragon (Simon et al., 1994; Ingram et al., 1995; Taylor et al., 2001). *UFO* is considered to co-regulate floral organ identity genes together with *LFY* (Lee et al., 1997). Overexpression of *UFO* in wild-type *Arabidopsis* leads to excessive leaf lobing, a phenotype that is also observed when *KNOX1* genes are overexpressed. However, overexpression of *UFO* in a *lfy* mutant background results in *Arabidopsis* plants with normal leaves, indicating that *LFY* is required to phenocopy the *KNOX1* overexpression results (Lee et al., 1997). *stm* mutants do not accumulate *UFO* transcripts, suggesting that expression of *UFO* depends on *STM*, and that these two pathways are linked (Long and Barton, 1998).

It is possible that *FLO/LFY* and *FIM*/*UFO* orthologs function together, and with *KNOX1* genes, to regulate compound leaf development in angiosperms (see also Tsiantis and Hay, 2003). Pea appears to be an excellent model species for revealing additional candidate genes that contribute to the regulation of compound leaf development. These additional regulators may be masked by *KNOX1* genes, which might act redundantly to control similar pathways in other angiosperms, such as tomato. The fact that meristem genes, like *KNOXI* and *LFY*, which regulate indeterminacy at the vegetative and reproductive SAM, also play a role in making compound leaves suggests that the acquisition of a level of indeterminacy is necessary for compound leaf development. This supports the hypothesis that individual leaflets of compound leaves are similar to simple leaves.

The role of leaf function genes

Leaf morphology is organized along three major axes: the

Box 2. Leaf polarity



The primordium and its resulting leaf have inherent polarities with respect to the meristem, as shown in these *Kalanchoë daigremontiana* leaves. The proximal region of the primordium or leaf is the region that is closest to the attachment point on the meristem or stem, and the distal region is the tip of the primordium or leaf, furthest away from the attachment point. The mediolateral axis spans across the leaf blade, from the middle region to the edge of the blade. The adaxial domain of a leaf, which corresponds to the top of the leaf, is the side of the primordium that is adjacent to the meristem. The abaxial domain is derived from the side of the primordium furthest away from the meristem, and forms the bottom of the leaf.

proximodistal axis, the mediolateral axis and the abaxial/adaxial axis (see Box 2) (Waites and Hudson, 1995; McConnell and Barton, 1998). It is thought that the juxtaposition of the adaxial and abaxial domains is required for blade outgrowth (Waites and Hudson, 1995; McConnell and Barton, 1998).

PHANTASTICA (PHAN) is a MYB-domain transcription factor that was first identified in snapdragon (Waites et al., 1998). Loss-of-function *phan* mutants have reduced adaxial domains. The most severe mutants have complete loss of the adaxial domain and radialized, needle-like leaves. Axillary buds, a marker of adaxial identity, are seen in *phan* mutants, suggesting that some adaxial identity is retained at the leaf base (Waites and Hudson, 1995; Waites et al., 1998). However, mutations in orthologous genes *ROUGH SHEATH2* (*RS2*) and *ASYMMETRIC LEAVES1* (*AS1*), in maize and *Arabidopsis*, respectively, usually do not cause major aberrations in the leaf adaxial domain in these plants (Schneeberger et al., 1998; Serrano-Cartagena et al., 1999). Nevertheless, the *as1-101* allele, in the L*er* background of *Arabidopsis*, occasionally produces plants that have lotus-like leaves, with the radial petiole attached to the abaxial surface of the leaf lamina, and the most severely affected *as1-101* L*er* plants have needle-like leaves (Sun et al., 2002; Xu et al., 2003). *PHAN* and its orthologs are expressed in the incipient leaf primordium, and in the developing leaves of simple leafed plants, in a pattern that is mutually exclusive to the expression pattern of *KNOX1* genes (Waites et al., 1998; Timmermans et al., 1999; Tsiantis et al., 1999; Byrne et al., 2000; Byrne et al., 2002).

Differences in the *PHAN* mutant phenotypes between species have raised uncertainties about the role of *PHAN* in regulating the adaxial domain of leaf primordia (Timmermans et al., 1999; Tsiantis et al., 1999; Byrne et al., 2000) and about the function of this domain in blade outgrowth (McHale and Koning, 2004). Downregulation of *PHAN* orthologs in mutant and transgenic plants is always accompanied by upregulation and ectopic expression of *KNOX1* in leaves. This has led to the proposal that, in plants with decreased levels of *PHAN*, there is a *KNOX1*-mediated displacement of stem identity into the leaf, causing it to become radial. In tobacco, *KNOX1*-expressing leaf blade cells maintain an immature identity, and juxtaposition of these cell types, with differentiated cells in the vein region of the leaf, leads to ectopic blade outgrowth along veins, and may also explain normal blade outgrowth (McHale and Koning, 2004). However, radial leaves and petioles do not show a stem-like vasculature because they are missing a central pith, which is normally present within the stem (Waites and Hudson, 1995; Sun et al., 2002; Kim et al., 2003c; Xu et al., 2003). While these data suggest a general role for *PHAN* in determining the adaxial domain, it is likely that *PHAN* also functions to regulate adaxial mesophyll development.

Recently, the role of *PHAN* orthologs in compound leaf development has been investigated. The tomato gene *LePHAN* is expressed in the SAM, developing vascular traces, and along the entire adaxial domain of developing leaves (Koltai and Bird, 2000; Kim et al., 2003b; Kim et al., 2003c). Transgenic tomato plants that express an antisense *LePHAN* construct have a diminished adaxial domain (Kim et al., 2003b). Various leaf phenotypes, such as needle-like or cup-shaped leaves, were generated depending on the amount and location of *LePHAN* production. Interestingly, some transgenic tomato plants produced peltate palmate leaves instead of pinnate leaves. In situ RNA expression analysis of plants with needle-like leaves showed that they had no *LePHAN* transcripts in developing leaves. Plants with cup-shaped leaves or with peltate palmate leaves had *LePHAN* expression restricted to the distal region of the leaf primordium. The most parsimonious explanation for these phenotypes is that the *PHAN* expression domain coincides with the adaxial domain, and that blades and leaflets only occur where an adaxial domain is present in these leaves (Kim et al., 2003b).

The results of altered *LePHAN* expression in tomato suggest that restriction of the adaxial domain in compound leafed species may be a natural mechanism to control compound leaf morphology. There is a high degree of sequence identity between *PHAN* orthologs from many species, and this indicates a conserved function for *PHAN* in defining the adaxial domain (Kim et al., 2003b). *PHAN* expression determines the placement and extent of this domain. Indeed, a broad survey of compound leafed species showed that pinnate leaves possess a distinct adaxial domain in the petiole and rachis, and *PHAN* is expressed along the entire adaxial region of the leaf primordium. Furthermore, peltate palmate leaf petioles are radial and do not have an adaxial domain. In these leaves, *PHAN* expression and the adaxial domain are restricted to the distal region of the primordium (Kim et al., 2003b). The common role of *PHAN* in simple leaf development and in compound leaf development is the regulation of adaxial domain identity, which, in the proper context, leads to blade expansion. An additional role for *PHAN* in compound leaves

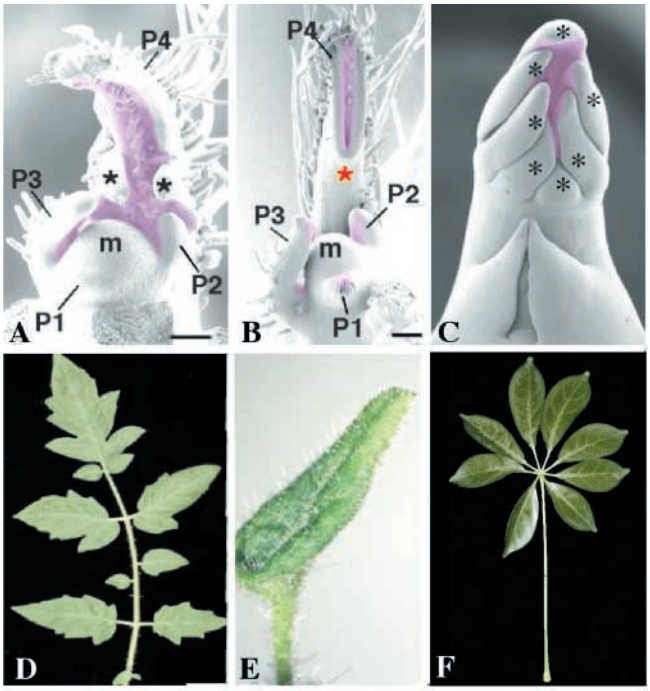


Fig. 3. The extent of the adaxial domain determines leaflet placement in compound leaves. (A-C) Scanning electron micrographs of vegetative apices. The adaxial domain has been colored pink. (D-F) Mature leaf form. (A) In the developing leaf blade of wild-type tomato, the adaxial domain extends from the base to the tip. (D) The mature tomato leaf has leaflets arranged along the edge of the adaxial domain. (B) The adaxial domain of transgenic antisense *PHAN* tomato plants is reduced to the tip of the leaf primordium, and these plants often produce cup shaped leaves (E). (C) The adaxial domain of *Schefflera actinophylla* is restricted to the tip of the developing leaf. (F) Consequently, leaflets are restricted to the tip of the petiole in this plant. m, meristem; P1, P2, P3 and P4, primordia 1, 2, 3 and 4, respectively. Asterisks indicate developing leaflets; red asterisk denotes region forming cup-shaped blade in antisense *PHAN* tomato plant. Figure adapted, with permission, from Kim et al. (Kim et al., 2003).

is the regulation of leaflet initiation and placement, as determined by the extent and placement of the adaxial domain (Fig. 3). The regulation, not only of blade outgrowth, but also of leaflet formation by *PHAN* suggests a common mechanism by which these two types of outgrowths occur, and that leaflets could arise by interruptions in blade outgrowth, supporting the hypothesis that the entire compound leaf is equivalent to a simple leaf.

Altered regulatory networks between meristem and leaf function genes

A negative regulatory network exists between *KNOX1* genes and *PHAN*/*RS2*/*AS1* in simple leafed species. In *Arabidopsis*, *STM* represses *AS1* and *AS2* in the SAM, confining their expression to developing primordia (Byrne et al., 2000; Byrne et al., 2002). *AS2* belongs to the *LATERAL ORGAN BOUNDARIES* (*LOB*) gene family (Iwakawa et al., 2002), and the AS2 protein can bind to AS1 (Xu et al., 2003). *AS1* and *AS2* together repress the expression of two other *KNOX1* genes, *BREVIPEDICELLUS* (*BP*; formerly called *KNAT1*) and

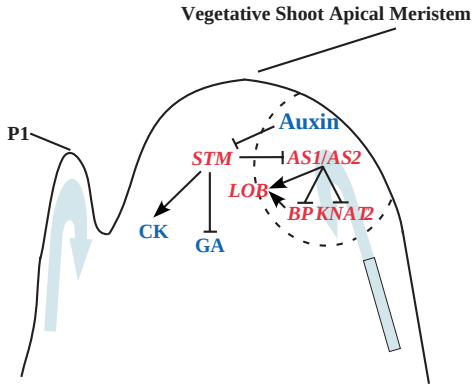


Fig. 4. Model of regulatory relationships between *KNOX1* genes, leaf genes and hormones in vegetative apices. Genes are shown in red and hormones are shown in blue. Arrows indicate positive regulation and lines with blunt ends indicate negative regulation. Light blue arrows show the path of polar auxin transport. The dotted line designates the incipient leaf primordium. *STM*, *SHOOT MERISTEMLESS*; *AS1*/*AS2*, *ASYMETRIC LEAVES1*/*2*; *BP*, *BREVIPEDICELLUS*; *KNAT2*, *KNOTTED-LIKE2* in *A. thaliana*; LOB, *LATERAL ORGAN BOUDARIES*; CK, cytokinin; GA, gibberellic acid.

KNAT2, in leaf primordia. *as1* and *as2* mutants have abnormal lobed leaves with ectopic expression of *BP* and *KNAT2* (Tsukaya and Uchimiya, 1997; Byrne et al., 2000; Ori et al., 2000; Semiarti et al., 2001; Lin et al., 2003). *BP* and *AS1/AS2* are positive regulators of *LOB* (the founding member of the *LOB* family), which is expressed between the SAM and organ primordia (Byrne et al., 2002) (Fig. 4).

The overlapping expression of *PHAN* and *KNOX1* orthologs in the SAM, and in developing leaves in tomato (Fig. 5A) and in other compound leafed species (Kim et al., 2003b), suggests that the regulatory relationship between these genes has been modified. Insights have come from examining *PHAN* expression in tomato mutants, such as *Mouse ears* (*Me*) and *Curl* (*Cu*), that overexpress the *STM* ortholog *LeT6*, and by crossing these mutants with antisense *PHAN* plants. *LePHAN* expression is reduced in heterozygous *Me* (*Me*/+) plants, indicating that *LeT6* represses *PHAN*. The relationship between *LeT6* and *LePHAN* is dose sensitive (Fig. 5B). In homozygous *Me* (*Me*/*Me*) mutants, the level of *LeT6* is increased further, and a corresponding reduction in *LePHAN* expression in leaf primordia often makes them radial. In the absence of *LePHAN*, leaflets are not initiated, masking the *LeT6* overexpression phenotype, suggesting that some *PHAN* activity, along with *KNOX1* expression, is required for leaflet initiation (Kim et al., 2003c). Additionally, some transgenic plants that overexpress *LeT6* at very high levels have radialized leaves (Janssen et al., 1998). Further support for the notion that *PHAN* activity is required for *LeT6* overexpression comes from crosses between *Cu* plants and transgenic antisense *LePHAN* plants that make cup-shaped leaves: the *Cu* phenotype is restricted to the distal region of the leaf, which coincides with the region of *PHAN* expression (Fig. 5C) (Kim et al., 2003c). Therefore, normal development of the tomato compound leaf requires a balance of these two antagonistic genes in overlapping domains (Kim et al., 2003c). Given that the relationship of *KNOX1* genes and *PHAN* orthologs is modified in compound leafed species, it would be interesting to see how

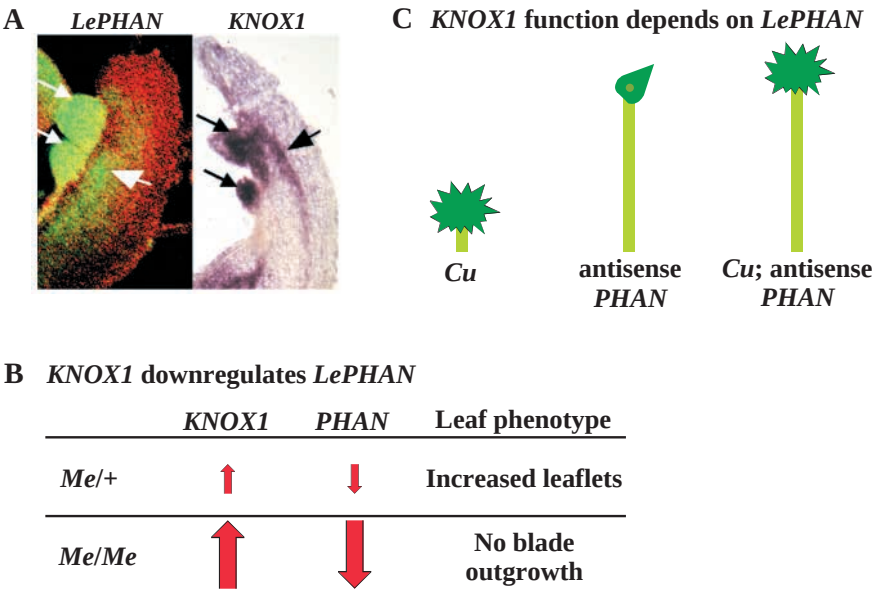


Fig. 5. *KNOX1* genes and *PHAN* genes are expressed in overlapping regions in developing tomato leaves. (A) The *KNOX1* gene *LeT6* and *LePHAN* are expressed in the same domain in wild-type tomato leaf primordia. In situ RT-PCR detection of *LePHAN* expression in a leaf primordium (left) and in situ hybridization with *LeT6* in a comparable leaf primordium (right). The small arrows show expression (green fluorescence in left, and purple in right, panel) in leaflet primordia, and the large arrows show expression in the developing vascular trace. (B) Heterozygous *Mouse ears* (*Me*/+) mutant tomato has increased levels of *LeT6*, decreased amounts of *LePHAN*, and an increased number of leaflets (*KNOX1* overexpression phenotype). Homozygous (*Me*/*Me*) mutants have even higher levels of *LeT6*, causing a greater decrease in *LePHAN* levels. Consequently, the leaves of these plants cannot produce blades (reduced *LePHAN* expression phenotype). A was adapted, with permission, from and Kim et al. and Janssen et al. (Kim et al., 2003; Janssen et al., 1998). (C) The *Curl* (*Cu*) phenotype (due to misexpression of *LeT6*), an antisense *LePHAN* phenotype (with cup-shaped leaf), and the *Cu* phenotype in an antisense *LePHAN* (with cup-shaped leaf) background. The misexpression phenotype of *Cu* in the antisense *LePHAN* background is restricted to the region of *LePHAN* expression.

this has affected the regulation of *LOB* orthologs. These analyses indicate a role for genes regulating SAM indeterminacy, as well as blade outgrowth, in compound leaf development. Studies such as these suggest that compound leaves share features with both branches and simple leaves.

Hormone networks in compound leaf development

Plant growth regulators (PGRs) are small molecules that regulate many aspects of plant growth and development. PGRs such as gibberellic acid (GA), cytokinin and auxin have been implicated in controlling leaf morphology. Meristem genes like *KNOX1* and *FLO*/*LFY* orthologs may be regulated by plant hormones and may coordinate hormone networks (Fig. 4). For example, *KNOX1* misexpression phenotypes are similar to cytokinin overexpression phenotypes (Estruch et al., 1991; Sinha et al., 1993). In addition, there are several examples of overexpression of *KNOX1* genes stimulating cytokinin synthesis (Kusaba et al., 1998b; Frugis et al., 1999; Ori et al., 1999; Hewelt et al., 2000). A clear relationship between GA and *KNOX1* genes has also been established. Their interaction was first noted in studies that showed that ectopic expression of *KNOX1* in various species resulted in decreased levels of GA (Tamaoki et al., 1997; Kusaba et al., 1998a; Kusaba et al., 1998b; Tanaka-Ueguchi et al., 1998). Subsequently, it was demonstrated that the tobacco *KNOX1* gene *NTH15* directly binds to, and represses the transcription of, a *GA20-OXIDASE* gene, which is involved in GA biosynthesis (Sakamoto et al., 2001). *KNOX1* genes from *Arabidopsis* and tomato also repress *GA20-OXIDASE* (Hay et al., 2002). Thus, one role of *KNOX1* genes is to inhibit GA biosynthesis in the meristem.

Me and *Cu* tomato mutants both exhibit ectopic expression of *LeT6*, the tomato *STM* ortholog, and a concomitant reduction in *GA20-OXIDASE*, leading to reduced GA levels. The exogenous application of GA, or constitutive GA signaling (as exhibited in the tomato *procera* mutant), results in a reduction in leaf compounding in wild-type and *Me* backgrounds, indicating that leaf complexity in tomato is regulated by GA (Hay et al., 2002; Hay et al., 2004). Recruitment of *KNOX1* genes into developing primordia, and the preservation of the interaction between *KNOX1* genes and GA biosynthesis, may have been a mechanism that has been used several times in evolution to promote the partially indeterminate state that is required for compound leaf development.

Polar auxin transport and auxin gradients regulate the site of primordia formation on a SAM, and control the arrangement of leaves on the stem (phyllotaxy) (Reinhardt et al., 2000; Kuhlemeier and Reinhardt, 2001; Stieger et al., 2002; Reinhardt et al., 2003). In maize, a polar auxin transport inhibitor, called N-1-naphthylphthalamic acid (NPA), prevents leaf initiation and inhibits the downregulation of KNOX1 proteins in the incipient primordium of cultured shoots (Scanlon, 2003). It is possible that *KNOX1* genes in simple and compound leaves are downregulated in response to an auxin gradient (Scanlon, 2003; Hay et al., 2004). Recently, the role of auxin in pea leaf development has been examined. Wild-type and *uni-tac* (a mild allele of *uni*) plantlets were cultured on auxin transport inhibitors and an auxin antagonist. Both wild-type and mutant plantlets displayed reduced leaf complexity, and had reduced *UNI* transcript levels within the shoot apex (DeMason and Chawla, 2004). In young pea leaves, auxin concentrations are highest at the tip. Pinna type (either leaflet or tendril) is primarily determined by the position of the pinna along the rachis, and thus may respond to the auxin gradient. DeMason and Chawla speculate that *UNI* is regulated by auxin concentration gradients and/or auxin transport. In wild-type pea, *UNI* expression correlates with the predicted site of auxin action (DeMason and Chawla, 2004). Interestingly, in *Arabidopsis*, *LFY* is regulated by GA via MYB-domain proteins (Gocal et al., 2001). DeMason and Chawla propose that auxin may regulate *LFY*/*UNI* expression through GA in pea (DeMason and Chawla, 2004), as it has been established that auxins regulate GA biosynthesis.

Future research in compound leaf development

Evolution of meristem and leaf genes

The evolution of expression domains

Changes in the expression domains of key morphogenetic regulators, such as *KNOX1* genes and *PHAN* orthologs, correlate with, and may have contributed to, the evolution of compound leaves. Changes that might have this effect include those to promoters and regulatory regions of these genes (cis-alterations), and/or changes in the proteins that interact with the regulatory regions of these genes (trans-alterations). Phylogenetic analyses and comparisons of non-coding regions from genes such as *KNOX1*, *FLO*/*LFY* and *PHAN* orthologs might help address this issue. At this time, there are no known proteins that directly interact with the promoters of *KNOX1* genes. In *Arabidopsis*, a MYB domain protein (AtMYB33), which mediates response to GA, binds to a specific sequence in the *LFY* promoter (Gocal et al., 2001). The identification of trans-factors that interact with the promoters of *KNOX1* and *FLO*/*LFY* orthologs will be crucial to our understanding of how the expression domains of these genes are controlled.

Two other mechanisms that may control where important regulators are expressed are RNA and protein movement. *KNOX1* RNA and protein movement has been well documented. *KN1* mRNA expression in maize was not detected in the tunica layer of the meristem, although expression of the KN1 protein was observed in these cells (Jackson et al., 1994). Lucas et al. used microinjection studies in both maize and tobacco to demonstrate that labelled KN1 protein is transported between cells via plasmodesmata (Lucas et al., 1995). The movement of GFP-labelled KN1, BP and STM is differentially regulated within leaf tissue and the meristem (Kim et al., 2002; Kim et al., 2003a). Additionally, the long-distance movement of a *LeT6*-fusion transcript from a tomato *Me* mutant stock to a wild-type scion across a graft union has been reported and is developmentally significant (Kim et al., 2001). Likewise, *LFY* is also capable of moving between cells. In wild-type *Arabidopsis*, *LFY* mRNA is expressed in all cell layers of young flower primordia. Using a promoter that restricts transcription of *LFY* to the outer cell layer of the meristem rescues *lfy* mutants, indicating that LFY protein can move between cell layers (Sessions et al., 2000). Movement of a LFY-GFP fusion protein across several layers is considered to be non-targeted and driven by diffusion (Wu et al., 2003). Wu et al. suggest that diffusion of macromolecules within the apex of *Arabidopsis* may be the default state and the retention of certain macromolecules may be significant (Wu et al., 2003). Movement (or retention) of RNA and protein between cells and over long distances could have multiple points of regulation, which, if altered, could influence the localization of transcription factors and the regulation of downstream targets.

The role of meristem signals and polarity genes

Changes in the timing, concentration and location of signals that establish patterns and gradients may also contribute to the expression domains of factors that regulate leaf morphology. A prime candidate for investigation is auxin, which may control the expression of *KNOX1* and *LFY* orthologs. Additionally, signals that emanate from the meristem act to promote development of the adaxial domain of leaves. Incisions that isolate the incipient leaf primordium from the meristem result in radialized leaves that lack an adaxial domain (Sussex, 1954; Sussex, 1955; Snow and Snow, 1959). This suggests that in the absence of the signal(s) from the meristem, the default state is development of the abaxial domain. To date, the identity of the adaxial-promoting signal(s) has remained elusive.

In *Arabidopsis*, *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*) and *REVOLUTA* (*REV*), a group of closely related Class III HD-ZIP proteins with sterol/lipid-binding domains, promote the adaxial domain. One hypothesis is that these genes, which positively regulate adaxial identity, act as receptors for the meristem signal. Upon receiving the signal, they promote the adaxial domain and SAM maintenance, and repress abaxial identity (Talbert et al., 1995; McConnell and Barton, 1998; Eshed et al., 2001; McConnell et al., 2001; Otsuga et al., 2001; Bowman et al., 2002). Two groups of genes that promote abaxial cell fate appear to be the likely targets of such repression: the YABBY family of putative transcription factors (Siegfried et al., 1999) and the three GARP transcription factors called KANADI1, 2 and 3 (Eshed et al., 2001; Kerstetter et al., 2001; Eshed et al., 2004). All *YABBY* genes are expressed in abaxial domains, and all asymmetric lateral organs express at least one *YABBY* gene (Bowman et al., 2002). Gain-of-function *kan* alleles result in radialized organs with abaxial tissue in place of adaxial tissue (Eshed et al., 2001; Kerstetter et al., 2001). Interestingly, expression of *PHB*, *PHV* and *REV* are regulated by microRNAs, and this regulation occurs in all land plants (Reinhart et al., 2002; Rhoades et al., 2002; Emery et al., 2003; Floyd and Bowman, 2004). To our knowledge, the roles of these other polarity genes remain uninvestigated in compound leafed species. It will be fascinating to see whether changes in *PHB*, *PHV* and *REV* expression correlate with compound leaf morphology and, if so, whether the altered expression patterns are mediated through microRNAs.

Competence to respond

The evolution of downstream targets of key regulators, such as *KNOX1* genes, *FLO/LFY* orthologs and polarity genes, may also drive modifications to leaf shape. The acquisition or loss of targets of these genes through changes in their regulatory regions would be significant for leaf evolution. Even changes in the affinity of a regulator for its target sequence could alter the amount of product produced. If the product is required at a certain threshold level to be effective, this alteration could be important as well. In addition to directly regulating *GA20-OXIDASE*, *KNOX1* genes also appear to regulate the biosynthesis of lignin, a component of the cell wall (Mele et al., 2003). Apart from these genes, little is known about the targets of KNOX1 transcription factors. More is known about the genes regulated by *LFY* in *Arabidopsis*. For example, *AGAMOUS*, *APETALA3* and *APETALA1* are direct targets of *LFY* (Busch et al., 1999; Wagner et al., 1999; Lamb et al., 2002). Microarray analysis has identified 15 additional candidates that respond to *LFY* (William et al., 2004). However, targets of *FLO*/*LFY* orthologs have not been investigated in compound leafed species. Comparisons of *KNOX1* and *LFY* targets between simple and compound leafed species should be a useful future research avenue.

The regulation of target genes by factors that control leaf complexity is also subject to epigenetic control that is exerted by chromatin remodeling factors. *as1* and *as2* single mutants

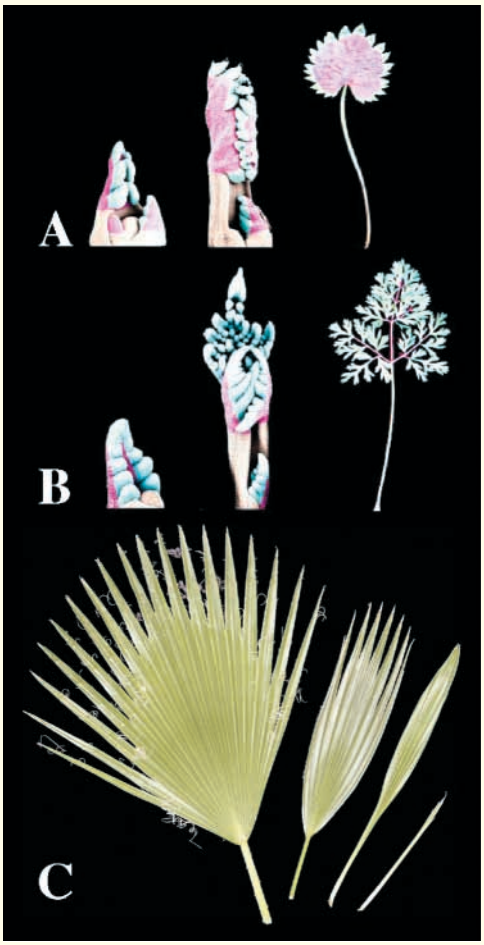
in *Arabidopsis* misexpress *BP* and *KNAT2*, and have mild *KNOX1* overexpression phenotypes (Ori et al., 2000). Ori et al. found that crossing each single mutant with either *serrate* (*se*) or *pickle* (*pkl*) dramatically enhanced the overexpression phenotypes of the progeny (Ori et al., 2000). *PKL* encodes a CHD chromatin-remodeling factor (Eshed et al., 1999; Ogas et al., 1999). *SE* encodes a putative single 2Cys-2His zinc finger transcription factor, which also might modify chromatin structure (Prigge and Wagner, 2001). *BP* and *KNAT2* are not misexpressed in *se* single mutants, nor were *BP* and *KNAT2* expression levels increased in *se*/*as1* or *se*/*as2* double mutants (Ori et al., 2000). It is possible that *SE* and *PKL* negatively regulate *KNOX1* target genes. In support of this, *GA20-OXIDASE* transcript levels are reduced in the *pkl* mutant (Hay et al., 2004). This suggests that other *KNOX1* targets may also be subject to epigenetic control.

In addition, the presence or absence of interacting partners could temper the response to transcription factors that regulate leaf complexity. KNOX1 proteins belong to the TALE (three-amino acid loop extension) family of homeodomain transcription factors. In simple leafed species, KNOX1 proteins can form heterodimers with another group of TALE proteins belonging to the BELL (BEL) family (Bellaoui et al., 2001; Muller et al., 2001; Smith et al., 2002; Chen et al., 2003; Smith and Hake, 2003). This interaction occurs in compound leafed species as well. The potato KNOX1 protein POTH1 interacts with several BEL-like proteins (Chen et al., 2003). StBEL5-POTH1 heterodimers bind to the *GA20-OXIDASE* promoter with greater affinity than the individual proteins (Chen et al., 2004). It is possible that KNOX1/BEL heterodimers in simple and compound leafed species may have a different subset of targets. The availability of interacting partners could limit the activity of KNOX1 transcription factors. Additionally, different interacting partners may allow the complex to behave as an activator or a repressor.

Secondary morphogenesis

Final leaf morphology provides only an incomplete picture of the true nature of the leaf. Studies that analyze all stages of leaf development are crucial for obtaining an accurate view of leaf morphogenesis (see Box 3). Cell division and cell expansion both contribute to growth. One way to control the spatial and temporal distribution of growth is to regulate cell-cycle arrest. If cell-cycle arrest is precocious, morphogenesis would rely solely on cell expansion. The delay to or absence of cell-cycle arrest could result in abnormally shaped leaves, or leaves that grow indeterminately. Mutations have been isolated in *Arabidopsis* and snapdragon in which entry into cell-cycle arrest has been perturbed. The *CINCINNATA* (*CIN*) gene from snapdragon encodes a TCP transcription factor (that belongs to a group of plant-specific basic helix-loop-helix DNA binding proteins) that promotes cell-cycle arrest. It is expressed in a dynamic pattern in actively dividing cells, in front of, or overlapping with, the arrest front. The perimeter of *cin* mutant leaves grows faster than can be accommodated in flat leaves, resulting in crinkled, uneven leaves (Nath et al., 2003). Studies in *Arabidopsis* reveal that a microRNA encoded by the *JAW* locus can cleave several *TCP* mRNAs that control leaf development. *jaw* mutant plants are reminiscent of the *cin* mutant in that they have uneven leaf shape and abnormal curvature (Palatnik et al., 2003). The *JAGGED*

Box 3. Secondary morphogenesis



The ultimate morphology of a leaf is a culmination of both the primary elaboration of primordia and secondary morphogenesis. For example, leaves that have a single blade at maturity may develop from simple primordia, or from compound primordia that are simplified by secondary morphogenesis (Bharathan et al., 2002). Interestingly, plants that have secondary simplification of compound primordia resulting in simple leaves also have *KNOX1* expression in primordia (Bharathan et al., 2002). Both anise (A) and carrot (B) have compound primordia, as shown in these scanning electron micrographs. Carrot has compound leaves at maturity. However, through a process of secondary morphogenesis, anise leaves become simple. Leaflet primordia have been colored green, and the remaining primordium magenta, for comparison across the developmental stages. Therefore, *KNOX1* expression patterns correlate with the morphology of developing primordia and not final leaf shape.

The palms present an interesting case of a simple primordium giving rise to a compound leaf by secondary morphogenesis that includes folding and abscission of part of the primordium (Kaplan et al., 1982a; Kaplan et al., 1982b). Palm leaves (C; viewed from oldest to youngest) develop from a simple primordium. As the leaf grows, the primordium folds, and, at later stages, the abscission of cells along one surface produce a compound leaf. Similarly, non-peltate palmate compound leaves can be considered a variation of pinnate compound leaves, as the two differ from one another as a result of differential rachis expansion during secondary morphogenesis (Kim et al., 2003b). Therefore, final leaf morphology does not necessarily correlate with initial primordium morphology, but is also a consequence of the spatial and temporal distribution of post-primordial growth. A and B were adapted, with permission, from Barathan et al. (Barathan et al., 2002).

(*JAG*) gene in *Arabidopsis* also functions to control entry into cell-cycle arrest. *JAG* encodes a putative C_2H_2 zinc-finger transcription factor that suppresses cell-cycle arrest. Lateral organs do not develop completely in loss-of-function *jag* mutants. As a consequence, leaves have serrations, especially in distal regions. Dinneny et al. speculate that the serrations could be due to a reduction in growth in regions of blade between the hydathodes (pores that exude water) (Dinneny et al., 2004).

The regulation of cell-cycle arrest and cell expansion could contribute to compound leaf evolution. It is possible that the inhibition or promotion of cell-cycle arrest could result in the formation of leaflets, or the growth of entire margins, respectively. It would be interesting to evaluate and compare the roles of genes that control the cell cycle in simple leafed species with simple primordia, in simple leafed species with compound primordia that undergo secondary simplification, and in compound leafed species.

Discovering other loci that regulate leaf complexity

Researchers have used the knowledge gained from model organisms like *Arabidopsis*, maize and rice to identify genes that might play a role in compound leaf development. However, there must exist genes that have, as yet, unknown functions in these model species that could be important for compound leaf morphogenesis. The present challenge is to identify these unknown genes. One possible fruitful approach involves using the genetic variation in naturally occurring species to identify quantitative trait loci (QTL) that might regulate leaf complexity. The analysis of segmental introgression lines between two tomato species, *Lycopersicon esculentum* and *Lycopersicon pennellii*, led to the identification of 30 QTL that contribute to leaf size and complexity (Holtan and Hake, 2003). These, and other, QTL studies could eventually lead to the discovery of relevant genes and add to our knowledge of compound leaf development. Tomato and pea have served as useful model species for studying compound leaf development. Numerous mutations that alter the compound leaf exist in both species (Marx, 1987; Kessler et al., 2001). For instance, the semi-dominant mutation *Lanceolate* regulates leaf morphogenesis and shoot meristem activity. Heterozygotes have simple leaves, whereas homozygous mutants have no SAM (Mathan and Jenkins, 1962). Continued genetic and molecular studies of this and other mutations should eventually identify new pertinent genes and provide additional tools with which to study compound-leaf evolution.

Conclusions

The ancestral angiosperm is thought to have had simple leaves, and compound leaves are believed to have arisen numerous times in this group, with several reversions back to the simple state. This suggests that the conversion from simple to compound leaves and back can be attained with relative ease. Yet saturation mutagenesis in *Arabidopsis* has not yielded any single mutation that can convert the simple leaf into a compound one. Certain mutant combinations produce deeply lobed leaves that often have accompanying *KNOX1* gene expression in the leaves, thereby mimicking the situation of *KNOX1* gene expression seen in most compound leaves. A mutation in the *UNI* gene can lead to an almost simple leaf. Collectively, these data support the partial shoot homology of compound leaves by indicating that genes regulating indeterminacy are required to make compound leaves. However, *PHAN/RS2/AS1* (a gene that regulates blade development in simple leaves) also regulates adaxial identity in tomato and determines leaflet placement in various compound leaves. In addition, although *PHAN/RS2/AS1* expression is excluded from the SAM in simple leafed species, all compound leafed species examined thus far show *PHAN/RS2/AS1* expression in the SAM (Kim et al., 2003b). These data suggest that there may be a blurring of the boundary between the determinate leaf and the indeterminate SAM, as suggested by Arber (Arber, 1950). Because *KNOX1* and *PHAN* are mutually antagonistic but may also be co-dependent in manifesting phenotypes (Fig. 5), studies of these genes do not allow us to clearly distinguish between the two proposed hypotheses for compound leaf development. Perhaps other genes that play specific roles in either blade outgrowth or SAM function need to be analyzed in order to understand the true nature of compound leaves.

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