THE SPECIFICATION OF LEAF IDENTITY DURING SHOOT DEVELOPMENT

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ABSTRACT

A single plant produces several different types of leaves or leaf-like organs during its life span. This phenomenon, which is termed heteroblasty, is an invariant feature of shoot development but is also regulated by environmental factors that affect the physiology of the plant. Invariant patterns of heteroblastic development reflect global changes in the developmental status of the shoot, such as the progression from embryogenesis through juvenile and adult phases of vegetative development, culminating in the production of reproductive structures. Genes that regulate these phase-specific aspects of leaf identity have been identified by mutational analysis in both maize and Arabidopsis. These mutations have revealed that leaf production is regulated independently of leaf identity, implying that the identity of a leaf at a particular position on the shoot may depend on when the leaf was initiated in relation to a temporal program of shoot development.

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INTRODUCTION

The term leaf commonly evokes an image of the prominent photosynthetic foliage of most plants; however, a tremendous diversity of leaf form and function is found in nature (Gifford & Foster 1989). For example, leaves form elaborate insect traps in carnivorous plants; brightly colored floral bracts; the hollow trunks of banana trees; thorns and spines; inconspicuous protective scales on buds and rhizomes; vestigial fragments of tissue on tubers and cactus stems; nonphotosynthetic storage organs in bulbs; and many other unique shapes and structures. Although much of the variation in leaf shape observed in nature involves species-specific patterns of leaf development, the leaves formed by a single plant can also display remarkable diversity of form. In most, if not all, plant species, leaves formed early in shoot development (juvenile leaves) are morphologically and physiologically different from leaves formed late (adult leaves). These differences may be fairly subtle or quite dramatic. Eucalyptus albida provides a dramatic example of the remarkable dimorphism between juvenile and adult leaves. In this species, the juvenile foliage is opposite, elliptical in shape, and glaucous (covered in a white wax), which gives the leaf a dull whitish or blue-gray appearance in sharp contrast to the deep green, glossy, lanceolate, and sub-decussate adult leaves. In addition to developmentally determined variation in leaf identity, most plants have the capacity to produce different types of leaves in response to environmental conditions such as light quantity or intensity, nutrient availability, or, in the case of aquatic plants, terrestrial or aquatic environments. This phenomenon is termed heterophylly to distinguish it from developmental variation in leaf morphology, which is called heteroblasty.

Here we review research related to the various modifications of leaves that occur during the plant life cycle, with particular emphasis on the changes in vegetative leaf identity that take place during shoot ontogeny. In addition, we discuss spatial and temporal models for the specification of leaf identity. An excellent review of classical research on the regulation of heteroblasty is provided by Allsopp (1967), and recent reviews of leaf morphogenesis (Poethig 1997), cellular differentiation of leaf tissues (Hall & Langdale 1996), and the mechanisms that control the acquisition of regional identity within a leaf (Sylvester et al 1996) are also relevant to this discussion.

VARIATION IN LEAF IDENTITY

Developmental Variation in Leaf Identity

Higher plants develop in a modular and iterative fashion. Over the course of the life cycle of a plant, the shoot apex initiates coordinated and repeating sets
of structures (phytomers) that ultimately constitute the mature shoot. As a consequence of this growth strategy, structures formed late in shoot development occur in apical portions of the shoot whereas parts of the plant produced early in shoot development are retained basally. Thus, variation in the character of the leaves, buds, and internodes produced at different times in shoot development is recorded as spatial variation in these structures along the axis of the plant. Although many aspects of shoot anatomy and morphology change continuously throughout shoot development, other traits are expressed in a more discontinuous fashion (Borchert 1976, Hackett 1985, Poethig 1990, Haffner et al 1991, Greenwood 1995). The appearance of both continuous and discontinuous changes in traits suggests that at least two different developmental or physiological processes contribute to heteroblasty (Wareing 1959, Allsopp 1967). One of these is a gradual change in the physiological vigor of the plant, a process that Wareing (1959) termed aging, and the second is the progression from one discrete developmental phase to another, a process known as maturation or phase change.

Coordinated changes in particular sets of morphological, physiological, and biochemical traits make it possible to define four more-or-less distinct phases of shoot development (Greenwood 1987, Poethig 1990): (a) an embryonic phase where shoot and root meristems are first established, (b) a post-embryonic juvenile phase where the plant is incapable of sexual reproduction, (c) an adult (or mature) phase where reproductive potential is established, and (d) an adult reproductive phase. During the transition between phases, the traits that characterize one phase are gradually replaced by those that characterize the next, often resulting in the production of transition organs that combine traits from successive phases. This model of shoot development is likely to be an oversimplification, however, because intermediate stages within a particular phase of development might be distinguished by a unique trait or pattern of gene expression.

The developmental phases listed above are recorded most dramatically in the type of leaves produced by the shoot. During embryogenesis, the embryo produces one (in the case of monocotyledons) or two (in the case of dicotyledons) leaf-like structures known as cotyledons. Cotyledons form during embryo morphogenesis as the basic architecture of the seedling is established (for review, see West & Harada 1993, Goldberg et al 1994). Some debate has arisen as to whether cotyledons are products of an embryonic shoot apical meristem or form independently during embryogenesis. Kaplan & Cooke (1997) argue that cotyledons are indeed derived from an embryonic shoot apical meristem, that they are homologous to leaves, and that their formation reflects the initiation of the same iterative process of meristematic activity and leaf initiation that continues throughout plant development. Other investigators have argued
that cotyledons and foliage leaves arise from fundamentally different developmental processes (Wardlaw 1955, Barton & Poethig 1993). Whatever the case, cotyledons can be distinguished from so-called true leaves by their distinctive anatomy and morphology and by specific patterns of gene expression (Goldberg et al 1989, Thomas 1993). Although the structure and function of cotyledons vary greatly among species (for review, see Bewley & Black 1978), cotyledons are usually morphologically and anatomically simpler than true leaves. The cotyledons of *Arabidopsis*, for example, are round, glabrous, and have a simpler venation pattern than the elliptical, serrate, and pubescent leaves initiated after germination (Meinke 1992, Conway & Poethig 1997). In addition, cotyledons exhibit a variety of physiological and molecular traits that are not often found in foliage leaves. During later stages of embryogenesis, cotyledon development is often marked by the accumulation of nutrient reserves, the acquisition of desiccation tolerance, loss of photosynthetic pigments, and dormancy. Exceptions to this developmental strategy, however, are common among higher plants and include vivipary, found in mangroves and some other species, and the limited development characteristic of many orchid embryos. The structural genes responsible for some of the traits expressed late in embryogenesis have been identified, and their expression patterns have been characterized (Crouch 1988, Goldberg et al 1989, Thomas 1993).

It should be noted that while cotyledons possess a number of molecular and physiological traits that distinguish them from true leaves, some of these features are actually characteristic of tissues produced during seed development rather than specific to cotyledons. Seed storage protein mRNAs from many species are rigorously tissue specific during embryo maturation and normally do not appear during post-germinative growth or in vegetative tissues (Goldberg et al 1989, Perez-Grau & Goldberg 1989, Guerche et al 1990, Thomas 1993). Upon germination, cotyledons resume their metabolic activity and in some epigeal species expand and become photosynthetic. During this post-embryonic stage, seedlings also specifically express genes involved in the breakdown and mobilization of storage products located within the cotyledon or in adjacent endosperm tissue. In situ localization studies have shown, however, that while these embryo-specific and germination-specific programs of gene expression occur within cotyledons, the same genes may be expressed in other embryonic tissues, including the stem, root, and endosperm (Harada et al 1988, Dietrich et al 1989, Thomas 1993, Wobus et al 1995). As far as we know, no cotyledon-specific marker has been identified.

The first true leaves produced by the shoot often resemble cotyledons in some aspects of their anatomy or morphology, but they are generally larger and morphologically and anatomically more complex than cotyledons, and they do not possess storage products (Gifford & Foster 1989). As the shoot develops,
it usually produces increasingly larger and more complex leaves until a final climax leaf type is achieved (Figure 1). Many woody plants produce this climax leaf type throughout most of their lives. In plants with shorter life spans, leaf size and shape often vary continuously along the shoot axis. In most cases it is possible to identify three more-or-less discrete classes of leaves: juvenile leaves, transition leaves, and adult leaves. However, some strongly heteroblastic species produce a much wider range of leaf forms. For example, *Pseudopanax crassifolius*, a strongly heteroblastic tree from New Zealand, produces eight different types of leaves: five types of seedling leaves and juvenile, transition, and adult leaf forms (Gould 1993). In addition to these leaf types, plants may produce rudimentary scale leaves surrounding dormant buds and distinctive cauline leaves or bracts on inflorescence shoots.

Although leaf size and shape are the most obvious heteroblastic traits, leaves in different morphological categories usually exhibit a variety of other distinctive traits. These include differences in types of epicuticular wax (Franich et al 1977, Blaker & Greyson 1988), types of trichomes or patterns of trichome distribution (Brand & Lineberger 1992, Evans et al 1994, Moose & Sisco 1994, Telfer et al 1997), cuticle thickness/structure (Franich et al 1977,
Bongard-Pierce et al (1996), anthocyanin (Murray et al 1994), chlorophyll (Bauer & Bauer 1980, Hutchinson et al 1990, James & Mantell 1994) and terpene (Bryant et al 1991) production, photosynthetic rates (Bauer & Bauer 1980, Thiagarajah et al 1981, Hutchinson et al 1990), and a variety of histological features (Allsopp 1967, Bauer & Bauer 1980, Gould 1993, James & Mantell 1994, Lawson & Poethig 1995, Bongard-Pierce et al 1996). Although many chemical and metabolic differences between juvenile and adult shoots have been described (Haffner et al 1991), little is known about the molecular differences between organs produced during these phases or the factors that control the process of vegetative phase change (Poethig 1990, Greenwood 1995, Lawson & Poethig 1995). Differences in the protein composition of juvenile and adult leaves have been observed in several tree species by immunological approaches or by PAGE (Bon 1988, Snowball et al 1991, Huang et al 1992, Besford et al 1996), but none of these genes has been cloned. In Hedera helix, the absence of anthocyanin in adult leaves is correlated with the lack of transcription of dihydroflavonal reductase, an enzyme in the anthocyanin biosynthetic pathway (Murray et al 1994). Phase-specific differences in the induction or accumulation of mRNA for a chlorophyll a/b binding protein and a cell wall proline-rich protein have also been reported in this species (Woo et al 1994). In larch, immature juvenile and adult leaves have different amounts of mRNA for chlorophyll a/b binding proteins (Hutchinson et al 1990), but this difference does not persist in fully mature leaves (Hutchinson et al 1991). The only phase-specific gene cloned from a herbaceous plant is the Glossy15 (Gl15) gene of maize (Moose & Sisco 1996), which is specifically expressed in late juvenile leaves (leaves 3–6).

Goebel (1900) originally proposed that cotyledons and juvenile leaves are simply arrested forms of a foliage leaf. He assumed that there is a single morphogenetic pathway for leaf production and that heteroblastic variation in leaf morphology arises because different parts of a leaf primordium are arrested at different points along this pathway. In contradiction to this hypothesis, histological and morphological analyses of leaf development in a wide variety of heteroblastic species have shown that heteroblastic leaf forms become distinct very early in development (Foster 1935, Kaplan 1973, Bruck & Kaplan 1980, Kaplan 1980, Müller 1982, Richards 1983, Merrill 1986, Jones 1993, Clearwater & Gould 1994). This observation, and the qualitative differences in the patterns of cellular differentiation in heteroblastic leaf types described above, support the modern view that juvenile and adult leaves are specified by different developmental programs. As discussed below, this interpretation is also supported by the existence of mutations that affect the expression of some or all of the traits that distinguish juvenile and adult leaves without affecting the overall size of the leaf or its morphology.
Variation in Leaf Identity in Response to Environmental Conditions

Although the basic form of a leaf is largely dependent on the stage of shoot development in which the leaf is initiated, environmental conditions can have significant effects on leaf development. Temperature, photoperiod, light quality, light intensity, mineral and carbohydrate nutrition, and water availability have all been shown to affect the growth and morphology of leaves in a variety of species. Leaf development in aquatic plants is particularly susceptible to modification, making these species popular experimental systems. Many of the classic studies on the effects of environment on leaf shape have been reviewed in detail (Allsopp 1967, Vince-Prue & Tucker 1983) and therefore are not discussed here. In general, treatments that limit the supply of carbohydrate or mineral nutrients, e.g. defoliation (Ashby 1948a, Njoku 1956a), growth at low light intensity (Njoku 1956b, Cameron 1970), in vitro culture of shoots or leaf primordia in nutrient-poor media (Sussex & Clutter 1960, Feldman & Cutter 1970), or reduction in the activity of ribulose bisphosphate carboxylase by antisense RNA (Tsai et al 1997), cause the shoot to produce small, morphologically simple leaves. In species with lobed or pinnate leaves, these experimental conditions often reduce or eliminate leaf lobing or pinnae production (Allsopp 1967). Light quality also has significant effects on leaf anatomy and morphology. Leaves exposed to far-red enriched light are usually smaller, more elongated, less lobed, and thinner than leaves grown in white light (Vince-Prue & Tucker 1983). In the aquatic plant *Hippuris vulgaris*, submerged shoots can be induced to produce aerial leaves by exposure to a low ratio of red to far-red light either throughout their growth or for a brief period at the end of each photoperiod (Bodkin et al 1980). In these experiments, the effects of far-red light treatment have been shown to be reversible by exposure to red light, demonstrating that the effect is mediated by phytochrome.

The observation that leaf forms similar to those encountered in a normal heteroblastic series can be produced by varying nutritional conditions or light quality and intensity has led many investigators to conclude that developmental changes in leaf morphology are a consequence of quantitative or qualitative changes in the nutritional status of the shoot (Allsopp 1967). The validity of this hypothesis is difficult to evaluate, however, because most experimental analyses of leaf development have focused on leaf shape, and this trait is not necessarily a good marker of developmental identity. Recent studies have shown that similar types of leaves can arise by very different mechanisms (McLellan 1993; Jones 1993, 1995; Poethig 1997). A striking example of this is offered by *Cucurbita argyrosperma*. In this species, the overt similarity between shade leaves and juvenile leaves and between sun leaves and adult leaves is not reflected in the
developmental morphology of these leaf types. Sun and shade leaf primordia are initially indistinguishable and become morphologically divergent late in leaf development, whereas juvenile and adult leaves are morphologically distinct shortly after initiation (Jones 1995). Another difficulty with the concept that the morphology of juvenile and adult leaves is nutritionally regulated is the observation that adult phase shoots can be induced to produce juvenile leaf forms by a variety of conditions, but juvenile shoots cannot be readily induced to produce adult leaves (Cook 1969, Telfer et al 1997). To our knowledge, no one has identified experimental conditions (other than mutations) that completely block juvenile leaf production (Hackett 1985). Whether phase-specific features of leaf identity are regulated by the same mechanism that regulates environmentally induced changes in leaf identity remains an open question.

THE REGULATION OF LEAF IDENTITY

When Is Fate Specified?

Experimental studies of leaf development in both angiosperms and non-flowering plants have demonstrated that leaves are determined gradually, with different aspects of anatomy and morphology determined at different times in development. Major morphological features, such as the dorsiventral polarity of the leaf primordium and the subdivision of the lamina into lobes or pinnae, are usually specified very early in leaf development (reviewed in Sylvester et al 1996, Poethig 1997), whereas many histological aspects of leaf identity are determined only after a leaf primordium is already well established. Results from a variety of systems suggest that this temporal difference probably reflects the activity of several independently regulated genetic programs that are differentially responsive to developmental and environmental factors.

A dramatic demonstration of the developmental plasticity of leaf primordia is provided by heterophyllous aquatic plants that produce different types of leaves in aquatic and aerial environments. In *H. vulgaris*, leaves produced while the shoot is submerged have a relatively long, narrow lamina, a single layer of mesophyll cells, a single central vein, and elongated epidermal cells, and they produce a hydathode but not stomata. By contrast, leaves produced in an aerial environment are broad and have several layers of mesophyll cells, both central and lateral veins, relatively isodiametric epidermal cells, and they lack a hydathode but have stomata (McCully & Dale 1961, Goliber & Feldman 1990). In this species, submerged shoots can be induced to produce aerial leaves by growing them in a medium containing 5 \( \mu \)M abscisic acid (ABA). Using this technique, Goliber & Feldman (1990) found that submerged leaf primordia can be completely converted into aerial leaves if they are exposed to ABA before they reach a length of 300 \( \mu \)m and can be partially transformed into aerial leaves.
up until they are about one half their final length. Similar results were obtained for aerial shoots induced to form submerged leaf types. An interesting feature of the intermediate leaves produced in this study is that they possessed discrete sectors of submerged- and aerial-type tissue along the proximal-distal axis of the leaf in a pattern that corresponds to the basipetal pattern of leaf maturation. Leaves that were relatively large at the time the shoot was exposed to ABA had only a small basal region of aerial tissue. In successively younger leaves, the basal region of the leaf that was capable of responding to this treatment was progressively larger. This result implies that the particular traits examined in this study (stomatal density, lateral vein formation, and epidermal cell shape) are determined late in leaf development in a local region of the leaf, rather than at the level of the entire organ. In a similar study of the aquatic plant *Ranunculus flabellaris*, Bruni and coworkers (1996) found that different aspects of leaf anatomy and morphology were specified at different stages of leaf development. Lobe number was specified earliest, followed by lobe length and leaf area, and finally stomatal density, leaf thickness, palisade differentiation, and the volume of intercellular air space.

Gradual, local determination of different aspects of leaf identity has also been observed for phase-specific traits in the case of *Impatiens balsamina* (Battey & Lyndon 1988, Pouteau et al 1997). This species is somewhat unusual in that flower primordia can be readily induced to revert to leaf production or vice versa by photoperiodic treatments. As in the case of *H. vulgaris*, the susceptibility of organ primordia to this treatment depends on their size at the time of treatment and the maturity stage of the tissue within the primordium. Intermediate organs consist of a combination of petal and leaf-like tissue, with reverted tissue always being located at the base of the organ. Interestingly, cells and tissues in reverted regions exhibit a combination of traits not normally found in leaves or petals. For example, anthocyanin (a petal trait) is produced by cells that otherwise resemble leaf cells. This remarkable result demonstrates that different programs of cellular differentiation operate independently even in individual cells.

When phase-specific vegetative traits become determined has not been carefully examined. It is significant, however, that the character of the transition leaves produced during the shift from juvenile-to-adult or from vegetative-to-reproductive development is often strikingly similar to the types of organs produced by the reversion experiments described above. Within this series of transition leaves, the change from one phase-specific form to the next may occur gradually for one particular group of traits and abruptly for another, resulting in combinations of phase-specific traits that do not normally occur in either juvenile or adult leaves (Hackett & Murray 1992). Furthermore, in many cases, the expression of phase-specific cellular markers of leaf identity in transition leaves occurs in discrete apical and basal domains of the leaf, as occurs in
transition leaves in *H. vulgaris* and *I. balsamina*. The anatomy and morphology of leaves produced during the juvenile-to-adult transition has been well documented in maize (Bongard-Pierce et al. 1996). In maize, cuticle thickness and cross-sectional cell shape change gradually from one transition leaf to the next, and there is no evidence for cell-to-cell variation in these traits within a transition leaf. In contrast, epicuticular wax production, the shape of lateral cell walls, and the staining pattern of epidermal cells change abruptly within single cells in predictable domains of the leaf (Moose & Sisco 1994, Lawson & Poethig 1995). In the basal-most transition leaves, adult tissue occupies a relatively small region at the base of the leaf, and this region expands acropetally in successively higher leaves. Frequently, individual cells express different phase-specific traits in the same region of the leaf. Thus transition leaves in maize may produce trichomes (an adult trait) in regions of the leaf that otherwise exhibit a juvenile pattern of cellular differentiation. The best evidence that different phase-specific aspects of leaf development are regulated independently in maize is provided by the phenotype of *gl15* mutants (Evans et al. 1994, Moose & Sisco 1994). Mutations in the *Gl15* gene replace juvenile epidermal traits with adult traits. Only certain phase-specific epidermal traits are affected with no effect on either the character of the mesophyll or the overall morphology of the leaf. Spontaneous wild-type sectors resulting from the excision of the *Spm* element in the *gl15-m1* allele demonstrate that *Gl15* gene product functions cell autonomously and is required late in leaf development (Moose & Sisco 1994). In summary, these observations suggest that developmentally regulated components of leaf identity are regulated in the same way as environmentally determined ones, namely, by parallel developmental programs that either operate early in development and affect the entire leaf or act later in local regions of the leaf. It must be reiterated, however, that the similarity in the temporal progression of leaf determination in these two instances does not necessarily mean that environmental cues operate by the same mechanism as developmental programs of leaf specification.

**Genetic Analysis of Leaf Identity**

**THE REGULATION OF COTYLEDON IDENTITY** In *Arabidopsis*, genes required for cotyledon identity have been defined by mutations that transform cotyledons into leaves. Recessive mutations in these so-called leafy cotyledon genes result in the partial or complete replacement of cotyledon-specific traits, such as storage product accumulation, desiccation tolerance, a simple vascular pattern, etc, with features normally restricted to vegetative leaves, i.e. trichomes, desiccation sensitivity, a complex vascular pattern (Meinke 1992, 1994; Bäumlein et al. 1994; Keith et al. 1994; West et al. 1994). Three such genes have been discovered and can be grouped into two classes based on the severity of their mutant
The LEAFY COTYLEDON2 (LEC2) locus appears to specifically regulate cotyledon/leaf identity because the single existing mutant allele of this gene has no other obvious effects on embryonic or post-embryonic development. lec2 mutant embryos are not viviparous, are desiccation tolerant, and produce storage products in approximately normal amounts and in the correct distribution in the embryo (except in the cotyledons). LEAFY COTYLEDON1 (LEC1) and FUSCA3 (FUS3) appear to have a more general role in embryogenesis (Meinke et al 1994, West et al 1994, Parcy et al 1997). Homozygous lec1 and fus3 mutant embryos are viviparous and completely desiccation intolerant. Lipid and protein storage bodies are reduced throughout the entire embryonic axis in these mutants, indicating their effect is not limited to cotyledons. This conclusion is also supported by the observation that leaf primordia are initiated precociously in these mutants. As is the case with LEC2, LEC1 and FUS3 are not essential for post-embryonic development, since viable and essentially normal plants can be grown to maturity from mutant embryos rescued in culture.

Two interpretations have been offered to explain the phenotypes of these mutations. Keith and coworkers (1994) have suggested that the leafy cotyledon phenotype is heterochronic and is a consequence of the precocious activation of a vegetative developmental program. Their hypothesis is that the premature exposure of cotyledons to vegetative signals causes them to differentiate as vegetative organs. An alternative interpretation is that LEC1 and FUS3 are required for cotyledon differentiation and thus the mutant phenotypes result from the loss of cotyledon identity functions rather than a change in developmental timing (Meinke et al 1994, Parcy et al 1997). West and coworkers (1994) emphasize that both interpretations may be correct because the leafy cotyledons in lec1 mutants express a combination of cotyledon and leaf traits. They note that if LEC genes function independently to promote the expression of cotyledon traits and to repress the expression of a post-embryonic pattern of development, then some aspects of their mutant phenotype may result from the failure of the cotyledon identity pathway, whereas others may result from the inappropriate expression of a post-germination pathway.

Whichever hypothesis is correct, the fact that the phenotype of lec2 is limited to cotyledons suggests that the programs for seed desiccation and dormancy are separable from the specification of cotyledon cell specialization and that cotyledon identity may be regulated by a hierarchical pathway in which genes that regulate the expression of general seed-specific traits activate downstream organ identity determinants such as LEC2. A major problem with this hypothesis is that only a single mutant allele of lec2 exists. If this mutation is leaky, then the relatively weak phenotype of lec2 may reflect the residual activity of the LEC2 gene product rather than the true function of this gene.
It is interesting that leaves initiated by the shoot meristem during embryogenesis may be morphologically and anatomically intermediate between cotyledons and true leaves formed after germination. In *Arabidopsis*, for example, the first two rosette leaves are morphologically similar to cotyledons and share with cotyledons a reduced potential for trichome production (Poethig 1997, Telfer et al 1997). Although maize does not produce a leaf-like cotyledon, the first two leaves produced during seed development have a pattern of epidermal differentiation similar to that of later-formed leaves but are molecularly (Moose & Sisco 1996), morphologically, and anatomically (Bongard-Pierce et al 1996) different from these leaves. This phenomenon raises the interesting question of whether factors that regulate the development of the embryo also have some influence on the developmental potential of leaves produced by the shoot apical meristem during embryogenesis.

Evidence that leaf identity may be regulated by factors that also operate to regulate cotyledon identity is provided by the production of “extra cotyledons” in precociously germinating *Brassica napus* embryos and in several mutants of *Arabidopsis*. When immature *B. napus* embryos are transferred to minimal medium (without added hormones), they germinate precociously, and the shoot meristem begins to initiate lateral organs (Finkelstein & Crouch 1984). In older immature embryos, these organs develop into vegetative leaves, but in embryos cultured at an early stage of development, they develop as either cotyledons or chimeric organs with sectors of cotyledon and leaf character (Finkelstein & Crouch 1984, Bisgrove et al 1995, Fernandez 1997). These extra cotyledons are recognizable as cotyledons because they have the anatomy and morphology of cotyledons and accumulate storage protein mRNAs in the same spatial and temporal pattern as true cotyledons. However, like leaves, they arise sequentially from the shoot meristem in a spiral phyllotaxy. Thus these unusual embryos simultaneously express an embryonic program of cotyledon identity and a post-embryonic program of shoot growth.

In situ hybridization analysis indicates that embryo-specific genes and germination-specific genes are expressed simultaneously in mosaic organs but in spatially separate domains (Fernandez 1997). This feature implies that these identities are regulated by one or more local cell-autonomous factors that act on the entire suite of genes involved in cotyledon differentiation. One of the striking features of this phenomenon is the spatial relationship between sectors in different organs. When sectors occur on more than one organ, they are always located in adjacent regions of these organs rather than in discontinuous domains. This feature was originally interpreted to mean that embryonic tissue is capable of influencing the fate of nearby cells. An alternative possibility is suggested by the fact that these sectors more closely reflect the cell lineage of a leaf than its temporal pattern of determination. These sectors may be derived
from one or more adjacent cells that simultaneously underwent a stable change in fate very early in leaf development. The observation that this transformation takes place in leaves that are already present at the time the embryo is placed in culture implies that the cells in these primordia are not determined at this stage and that there is stochastic, local variation in the conditions that predispose cells to undergo this fate change.

Three mutations in Arabidopsis—extra cotyledon 1 (xtc1), extra cotyledon 2 (xtc2), and altered meristem program 1 (amp1)—display a phenotype similar to that of precociously germinated B. napus embryos, namely the formation of leaves that have been partially transformed into cotyledons (Conway & Poethig 1997). The extra cotyledons produced in these mutants can be distinguished from true cotyledons by their position on the shoot (formed at 90° from the cotyledons, the normal position of the first leaf), by their time of emergence (after cotyledons emerge, but before the first leaves of a wild-type plant), and by their small size and often irregular shape. They resemble true cotyledons in that they have few or no trichomes, a simple venation pattern, and they possess storage products normally present in cotyledons but not in leaves. This phenotype is different from mutations or natural variants of Arabidopsis that produce more than two cotyledons. In the case of fass mutants, which produce extra cotyledons and partially uncouple cell division and morphogenesis (Torres-Ruiz & Jürgens 1994), the production of additional cotyledons is presumed to result from an increase in the size of the shoot meristem, with the cotyledons all arising at the same nodal position and at the same time.

As in the case of B. napus, the extra cotyledons produced by xtc1, xtc2, and amp1 mutants are a consequence of a change in the relative timing of embryo and shoot development (Conway & Poethig 1997). In both xtc1 and xtc2, the morphogenesis of the embryo is delayed between the globular and heart stages of embryogenesis, and the shoot apical meristem enlarges precociously, producing one or two prominent leaf primordia. amp1 has a different effect on embryogenesis in that it causes the upper half of the embryo to proliferate abnormally. It is similar to xtc1 and xtc2 because it also causes the precocious production of leaf primordia in the developing seed. The transformation of leaf primordia into cotyledons in these mutants is believed to be a consequence of this change in the timing of leaf initiation because only leaf primordia that are initiated precociously undergo this transformation. Furthermore, in the case of amp1, this developmental transformation is prevented by another mutation, paused, that delays leaf production.

Although the transformation of leaves into cotyledons can result from a variety of different abnormal conditions in Arabidopsis and Brassica embryos, a common feature of these conditions is the precocious activation of the shoot meristem that leads to the premature initiation of leaf primordia. Whether these
leaf primordia then take on cotyledon identity owing to proximity to differentiating cotyledons or from some other source of phase information is unknown. It should be emphasized that the specification of an organ primordium as a cotyledon undoubtedly involves more than simply being initiated during embryogenesis, especially when one considers that mature seeds of many species contain numerous leaf primordia in addition to the cotyledons. In maize, for example, five to seven leaf primordia can be found in a mature dry seed. These vegetative leaves form in close proximity to the cotyledon and undergo the same processes of seed maturation and desiccation, yet display morphological and physiological features distinct from the cotyledon.

THE REGULATION OF FOLIAGE LEAF IDENTITY Mutations that affect the production of juvenile or adult leaves have been identified in both maize and Arabidopsis. These mutations can be divided into two broad categories. One group of mutations affects a large number of phase-specific traits (including flowering) and may therefore represent genes involved in regulating the transition between juvenile and adult phases of shoot development. The other category includes mutations that affect a more limited set of phase-specific traits. This group is predicted to represent genes that play roles in establishing juvenile or adult leaf identity in response to phase change signals.

In maize, genes in the latter category include Teopod1 (Tp1), Teopod2 (Tp2), Teopod3 (Tp3)/Corngrass (Cg), and Gl15. The first three genes (Tp1, Tp2, Tp3/Cg) are defined by dominant gain-of-function mutations that result in prolonged expression of a large number of juvenile traits. These mutations affect all known juvenile vegetative traits (including leaf shape, epicuticular wax production, and hair or trichome initiation) and have profound effects on the overall vegetative morphology of the plant (Lindstrom 1925, Galinat 1954a,b, Poethig 1988a, Bongard-Pierce et al 1996). They have relatively little effect, however, on the timing of the expression of adult traits or on the reproductive competence of the shoot. The leaves of Tp2 mutants, for example, are morphologically and anatomically intermediate structures, combining features that are normally specific to either juvenile or adult leaves (Bongard-Pierce et al 1996). In addition, although Tp2 increases the number of leaves produced by the shoot, it does not affect the time at which the shoot becomes reproductively competent or the time at which the primary shoot meristem finally ceases growth (Bassiri et al 1992). This aspect of their phenotype implies that the Tp genes regulate a juvenile program of leaf identity that operates in parallel to, and to some extent independently of, the programs that specify adult and reproductive traits. Mosaic analysis demonstrates that Tp1 and Tp2 function non–cell-autonomously and may therefore regulate a diffusible factor (Poethig 1988b, Dudley & Poethig 1993).
By contrast, recessive mutations in the \textit{Gl15} locus result in a premature switch in the expression of a subset of vegetative phase-specific traits (Evans et al. 1994, Moose & Sisco 1994). Mutant plants cease displaying juvenile epidermal traits (e.g. epicuticular wax) and begin to display adult epidermal traits (e.g. macrohairs and bulliform cells) much earlier than their normal siblings, with no other effects on overall vegetative morphology. Double mutants between \textit{gl15} and \textit{Tp1} and between \textit{Tp2} and \textit{Cg} indicate that \textit{Gl15} is required for the effects of these dominant mutations on epidermal traits but not for other aspects of the \textit{Tp} phenotype (Evans et al. 1994, Moose & Sisco 1994). \textit{gl15} mutations, therefore, uncouple epidermal differentiation aspects of leaf identity from the overall process of phase change in the vegetative shoot. This result implies that \textit{Gl15} acts downstream of the \textit{Tp} genes and is required specifically to promote the expression of a subset of juvenile epidermal traits and to repress the expression of an alternative set of adult traits. \textit{Gl15} has been cloned and is predicted to encode a product that shows significant similarity to the putative DNA binding domain of the \textit{APETALA2} gene of \textit{Arabidopsis} (Moose & Sisco 1996).

Genes that may be involved in regulating the transition from juvenile-to-adult growth in maize include \textit{viviparous8} (\textit{vp8}) and genes involved in gibberellin (GA) biosynthesis, such as \textit{dwarf1} (\textit{d1}), \textit{dwarf3} (\textit{d3}), \textit{dwarf5} (\textit{d5}), and \textit{anther ear1} (\textit{an1}). Mutations that block GA production prolong the production of juvenile leaf traits, delay the expression of adult leaf traits including flowering, and dramatically enhance the phenotype of the \textit{Tp} mutations (Olson 1954, Evans & Poethig 1995). Exogenous applications of GA\textsubscript{3} suppress the effect of \textit{d3} on \textit{Tp1} and \textit{Tp2}, demonstrating that the effect of \textit{d3} on these dominant mutations is mediated by a reduction in GA (Evans & Poethig 1995). These results suggest that GA acts to promote the adult phase of vegetative development and reproductive development in maize. However, the observation that the effect of GA-deficient mutations on phase-specific traits is relatively minor, even in double mutants of \textit{d1} and \textit{d3}, indicates that GA is not the only factor involved in this process (Evans & Poethig 1995). At present, the \textit{Vp8} gene is a candidate for encoding such an additional factor (Evans & Poethig 1997). Like GA-deficient mutations, the \textit{vp8} mutation prolongs the expression of juvenile traits and delays the expression of adult traits, although its effect on these traits is more pronounced than that of the \textit{dwarf} mutations. \textit{vp8} interacts synergistically with both the \textit{Tp} mutations and the \textit{dwarf} mutations, implying that it is not in the same pathway as these genes. Furthermore, \textit{vp8} differs from the \textit{dwarf} mutations in that it does not have a significant effect on flowering time. Thus \textit{Vp8} appears to act in a pathway that promotes vegetative phase change but that does not directly regulate reproductive maturation. Another gene that is believed to be involved in regulating phase transitions in maize is defined by the dominant mutation \textit{Hairy sheath frayed1-O} (Bertrand-Garcia & Freeling...
1991). This mutation has a complex phenotype that includes the prolonged expression of many juvenile traits and the accelerated expression of some adult traits. It is expressed non–cell-autonomously in genetic mosaics, although to a more limited extent than *Tp1* and *Tp2* (Saberman & Bertrand-Garcia 1997).

The morphological changes that accompany vegetative phase change in *Arabidopsis* have only recently been investigated (Telfer et al 1997). As a result, only a few traits that differ between early and late stages of shoot development have been identified. In *Arabidopsis*, the first leaves produced are small and round, whereas later rosette leaves are larger, more elliptical, and more serrated (Röbbelen 1957, Poethig 1997) (Figure 1). Although these changes in leaf shape are predictable and easily observed, they occur in a continuous fashion, making them of limited utility as an unambiguous marker of leaf identity. Currently, the most readily quantifiable difference detected between leaves produced at different times in shoot development is the distribution of trichomes on the leaf blade (Chien & Sussex 1996, Telfer et al 1997). Trichomes are normally formed on the adaxial surface of all rosette leaves. Abaxial trichomes, however, are absent from the early rosette leaves and only appear later in vegetative development. While the first appearance of abaxial trichomes differs from one ecotype to the next, the presence or absence of abaxial trichomes serves as a useful trait to distinguish juvenile and adult phases of vegetative growth in *Arabidopsis*.

One of the reasons that abaxial trichome production in *Arabidopsis* is considered a good marker for the developmental phase of the shoot is that this trait responds to factors known to affect phase change in other species. In *Arabidopsis*, as in maize (Evans & Poethig 1995), GA acts to promote the adult form of this trait. Leaves with abaxial trichomes appear precociously in plants treated with regular applications of GA, as well as in *spindly-4* mutant plants that display a constitutive GA response. Conversely, GA-insensitive and GA-deficient mutants delay the production of leaves possessing abaxial trichomes (Chien & Sussex 1996, Telfer et al 1997). Abaxial trichome production is also affected by environmental conditions (e.g. day length) and by some mutations that affect flowering time (Chien & Sussex 1996, Telfer et al 1997). However, changes in the size of the shoot or the rate of leaf initiation do not affect the time at which the first abaxial trichomes appear (Telfer et al 1997).

A number of mutations that alter the appearance of abaxial trichomes have been described. Seedlings homozygous for the recessive mutation *paused* (*psd*) produce a normal embryo but fail to produce true leaves for several days after germination (Telfer et al 1997). The first leaves eventually formed are morphologically distinct from the first leaves made by wild-type seedlings. Instead, they resemble normal leaves initiated at the same time by wild-type siblings, although in a different position on the shoot. The *psd* mutation therefore generates a specific deletion of the first two to three juvenile leaves of the *Arabidopsis*
shoot (Telfer et al 1997). The \textit{amp1} mutation has an opposite phenotype; it increases the rate of leaf production, thereby increasing the number of juvenile leaves produced by the shoot. As in the case of \textit{psd}, \textit{amp1} has relatively little effect on the time at which the shoot begins to produce adult leaves. Thus both mutations alter leaf production without affecting the timing of the juvenile-to-adult transition.

In contrast to \textit{psd} and \textit{amp1} mutations, \textit{hasty} (\textit{hst}) mutations accelerate the first appearance of abaxial trichomes without significantly affecting the rate of leaf production (Telfer & Poethig 1998). \textit{hst} mutations also accelerate the loss of adaxial trichomes (a trait typical of bracts), reduce the total number of leaves produced by the shoot, and have a number of other effects on shoot morphology. The interactions between \textit{hst} and genes that regulate floral induction or floral morphogenesis (such as \textit{LEAFY}, \textit{APETALA1}, the GA-deficient mutation \textit{ga1-3}, and the GA-insensitive mutation \textit{gai}) indicate that \textit{hasty} increases the reproductive competence of the shoot and does not require GA or a GA response for its effect on vegetative or reproductive maturation.

Another class of genes that may function to regulate vegetative phase change in \textit{Arabidopsis} has been identified based on effects on flowering time. Several of the late flowering mutations affect vegetative morphology as well as the timing of flower initiation (Martínez-Zapater et al 1995, Telfer et al 1997). Although most of these mutations have a relatively small effect on abaxial trichomes, most of the extra leaves in the \textit{fpa1} mutant lack abaxial trichomes, indicating that this mutation delays the transition to the adult vegetative phase (Telfer et al 1997). Most of these genes are defined by only a single allele, so it is possible that more severe alleles of the other late flowering loci will also reveal a role for these genes in vegetative phase change.

**A Temporal Model for Vegetative Leaf Identity**

In recent years, rapid progress has been made in understanding the factors that regulate some of the changes in meristem and organ identity that accompany the transition to reproductive growth. The ABC model for the regulation of floral organ identity postulates that the floral meristem is divided into four concentric domains by interactions between genes with three types of functions (A, B, and C), and that the fate of floral organs is specified by the domain in which they originate (Coen & Meyerowitz 1991, Weigel & Meyerowitz 1994). The model assumes that the demarcation of the meristem into these four domains occurs prior to the initiation of floral organs and that this process is independent of the process of organ initiation because mutations that change organ identity generally have no effect on the number, growth rate, size, and cell lineage of the organs located in the affected whorl of the floral primordium. The major exception to this rule is \textit{AGAMOUS}, which specifies stamen and carpel identity.
and is also responsible for suppressing organ initiation in the central-most region of floral primordium. Thus floral morphogenesis is generally viewed as involving two processes: one that establishes a precise pattern of expression of organ identity genes within the floral primordium (meristem patterning) and a subsequent process that regulates the production of organs within these domains (Coen & Meyerowitz 1991, Weigel & Meyerowitz 1994).

Our current understanding of the regulation of vegetative organ identity is consistent with this view insofar as it predicts that organ identity and organ initiation are separable processes that can be altered independently of one another. However, it is difficult to apply all aspects of this model to vegetative development because there is no evidence that the vegetative meristem is divided into domains that are fated to produce juvenile, adult, and reproductive organs (Figure 2A). This is particularly obvious in the case of reproductive organs. Clonal analyses of the seedling meristems of maize (Johri & Coe 1983, McDaniel & Poethig 1988), sunflower (Jegla & Sussex 1989), and Arabidopsis (Johri & Coe 1983, Furner & Pumfrey 1992, Irish & Sussex 1992) have shown that at this stage in development there is no lineage restriction between adult vegetative organs and organs in the inflorescence. Unfortunately, the cell lineage relationship between juvenile and adult parts of the shoot has never been explicitly examined. Nevertheless, a reevaluation of data from a clonal analysis of embryogenesis in maize (Poethig et al 1986) suggests that there is no cell lineage restriction between these regions prior to the initiation of leaf one. The observation that adult phase maize shoots can be readily induced to revert to the juvenile phase in culture (Irish & Karlen 1998) further supports the conclusion that the shoot meristem does not possess developmentally determined juvenile and adult domains.

Perhaps the strongest argument against a meristem patterning model for the specification of vegetative leaf identity comes from a consideration of the type of intermediate organs produced during the transition from one developmental phase to the next. In those cases in which juvenile and adult leaves are distinguished by obvious cell-autonomous traits, transition organs are often divided into a distal region that expresses juvenile traits and a basal region that expresses adult traits (Goebel 1900, Bongard-Pierce et al 1996, Telfer et al 1997). In successively higher leaves, the juvenile domain is confined to a progressively smaller region at the distal end of the leaf blade. This proximal-distal pattern of cell identity is not predicted by a meristem patterning model; instead, transition organs are predicted to straddle the boundary between different domains in the meristem. Clonal analysis demonstrates that the adaxial and abaxial surfaces of a leaf are derived from different cells in the radial dimension of the shoot (Poethig & Sussex 1985, Poethig & Szymkowiak 1995). Thus a meristem patterning model would predict that in a transition leaf, cells with different
Figure 2  (A) Meristem patterning model for the regulation of organ identity. This model assumes that organ identity is specified by the spatial pattern of expression of organ identity genes in the apical meristem and that organ initiation is independent of this pattern. Organs that arise at the boundary between two domains are predicted to display different patterns of cellular differentiation on their adaxial and abaxial surfaces. (B) An alternative model for the regulation of organ identity. This model assumes that cells in the meristem only express a single fate at any given time in development. When the shoot apex switches to a new developmental phase, all of the cells in the shoot meristem and all of the undetermined cells in pre-existing leaf primordia adopt this new identity. This model predicts that the distribution of cell types in transition organs reflects the determination state of the organ at the time of the transition. Thus organs that mature in a basipetal fashion will possess proximal and distal domains with different developmental identities.

devvelopmental identities will be located on the adaxial and abaxial sides of the leaf, not in distal and proximal regions as is generally observed.

An alternative model for the regulation of vegetative organ identity that is consistent with the character of the transition leaves is illustrated schematically in Figure 2B. This model is similar to the one proposed by Hempel & Feldman (1995, Hempel 1996) to explain the morphology of the inflorescence in Arabidopsis and is based on the observation that the character of a leaf can be modified even after the leaf has been initiated. As noted previously, there is considerable evidence that this is true both in aquatic plants (Goliber & Feldman 1990, Bruni et al 1996) and in Impatiens balsamina (Battey & Lyndon 1988), and preliminary results indicate that it is also true for juvenile and adult traits.
in maize (H Passas & RS Poethig, unpublished observations). The most important assumption of this model is that all the undetermined organs on the shoot meristem are in a single identity state (juvenile, adult, reproductive) at any given time. A second assumption of the model is that the factors responsible for the transition to a different developmental state (e.g. the juvenile-to-adult transition or the vegetative-to-reproductive transition) act both on the meristem and on all undetermined organs (leaves, buds, internodes) at the shoot apex. All of the cells in the meristem switch to the next developmental state immediately upon induction, as do all of the undetermined cells in preexisting organs. Because leaves mature basipetally, regions at the tip of preexisting leaves are likely to have already become determined for the previous developmental state, whereas basal regions of the leaf will still have the capacity to switch to the new state. Thus, variation in the amount of juvenile and adult tissue in successive transition leaves is assumed to result from the relative age of these leaves at the time the phase transition occurs.

A prediction of this model is that the progression of heteroblastic leaf forms during shoot development depends on the way in which the timing of leaf initiation is coordinated with temporal expression patterns of genes that regulate organ identity (Figure 3). As described above, the effect of xtc1, xtc2, and amp1 on leaf identity appears to be a result of the inappropriate production of leaves during embryogenesis (Conway & Poethig 1997). Similarly, the psd mutation of Arabidopsis reduces the number of juvenile leaves by blocking leaf production, not by affecting the timing of the transition from juvenile-to-adult development (Telfer et al 1997). Mutations that affect the expression of phase-specific traits without affecting the onset or rate of leaf production include the dwarf (Evans & Poethig 1995), gl15 (Evans et al 1994), and Tp2 (Bassiri et al 1992) mutations in maize, and the hst mutation in Arabidopsis (Telfer & Poethig 1998). These mutations define genes that function independently of leaf initiation and are involved either in regulating the switch between different developmental phases or in regulating the expression of a set of phase-specific leaf traits.

Given that floral organs evolved from leaves (Gifford & Foster 1989) and, at least in Arabidopsis, can be transformed into leaves by loss-of-function mutations in floral organ identity genes (Bowman et al 1991), it is not unreasonable to expect that the mechanism of vegetative organ specification and the mechanism of floral organ specification may be similar in some respects. Whereas highly evolved flowers are characterized by the rapid production of four different types of organs in discrete whorls, more primitive flowers initiate floral organs in a spiral and produce a graded series of organ types. A striking example of this is provided by Astrobaileyea scandens, in which each floral organ is unique (Endress 1980). This pattern is similar to that observed during vegetative phase
Figure 3  A model for heteroblastic leaf development. This model assumes that leaf initiation is regulated independently of organ identity. Organ identity is regulated by a mechanism that specifies different morphogenetic patterns at different times in shoot development. In this figure, the timing of leaf initiation is represented by the distance between leaves on the schematic illustration of an Arabidopsis plant, and the timing of organ identity programs is represented by the line to the right of each plant. The identity of a leaf at any particular position on the shoot depends on the way in which these processes are coordinated. (A) Wild-type plant. (B) Acceleration of leaf initiation relative to the organ identity program leads to the transformation of leaves into cotyledons and the production of extra juvenile leaves; this is the phenotype of the amp1 mutant (Telfer et al 1997). (C) A delay in leaf initiation causes the shoot to produce adult leaves at basal nodes, as in the psd mutant (Telfer et al 1997). (D) Truncation of the juvenile phase of shoot development leads to the precocious production of adult leaves, as in the hasty mutant (Telfer & Poethig 1998).

transitions and suggests that floral morphogenesis in primitive flowers may involve the same sort of temporal progression of identity states that we propose for vegetative development. Whether this model is correct, it is worth noting that the ABC model does not readily explain the morphogenesis of flowers in which organ identity changes gradually. The mechanism of vegetative organ specification is therefore of interest both in its own right and because of what it may tell us about the evolutionary origin of the mechanism of floral morphogenesis.

CONCLUSIONS

Heteroblasty is an ancient and universal feature of plant development. Even primitive plants, such as green algae and bryophytes, exhibit heteroblastic patterns of organ development similar to those observed in more highly evolved species (Goebel 1900, Mishler 1986, Nishimura & Mandoli 1992). In 1790,
von Goethe proposed that all of the organs on the shoot arise from a single foliar structure, leaving it to succeeding generations of biologists to define the nature of this structure and the mechanism of its metamorphosis into cotyledons, juvenile leaves, adult leaves, bracts, and foliar organs. Although considerable progress has been made in elucidating the molecular-genetic mechanism of floral morphogenesis, the way in which vegetative organ identity is regulated is still largely unknown. Two of the major problems in trying to define the factors that regulate leaf identity have been the paucity of cellular or molecular markers for vegetative organ identity in some species and the difficulty of distinguishing phase-specific traits from those that are regulated by reversible changes in the physiological status of the shoot. Techniques that allow investigators to visualize patterns of gene expression make it possible to observe the extent of the differences between organs and tissues and should resolve some of these problems. In combination with genetic analysis of model plant systems, which provides a means for identifying the factors that regulate organ identity, these tools offer new opportunities for studying this fundamental aspect of plant development.

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