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Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control

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Summary Woody plants exhibit significant and predictable patterns of change in morphology and physiology as they become older and larger. Four models of potential pathways controlling these changes are presented: a stimulus–response model in which fully developed organs respond to changes in environment (defined here as everything external to the organ); an extrinsic model in which the attributes of developing organs are determined by environmental factors; an intrinsic model in which changes are a result of programmed changes in gene expression; and an extrinsic–intrinsic model in which changes in gene expression are induced by environmental factors. We review evidence that a genetic component is involved in controlling age- and size-related changes in foliar morphology and physiology and discuss the possibility of complex interactions among model pathways.

Keywords: life stages, maturation, phase change, photosynthesis.

Introduction

As a tree increases in size and complexity, its apical meristems respond to changes in both the external environment and its internal physiology. For example, shoots near the tops of large trees face more intense exposure to solar radiation and wind, and, as trees grow, water and mineral nutrients must be transported over ever-increasing distances between roots and shoot. Additionally, as trees age through seedling, sapling, mid-aged and old-growth life stages, challenges from the abiotic environment, pests and pathogens and competition for resources change in complex ways. Success at the seedling and sapling stages may be measured by the ability to capture belowground resources and maintain foliage above the shade of competitors, both achieved by maximizing allocation to growth. In contrast, success for a mature tree could be measured by long-term resistance to abiotic stresses, conservation of mineral resources and (at the species level) maintenance of high reproductive output.

Fully developed organs of trees are capable of responding to various environmental conditions or cues and adapting to changes in external stresses and resource availability. For example, stomatal conductance decreases during periods of high transpirational demand or low soil water availability. Developmental plasticity in organs produced by apical meristems provides a means of responding to changes in the external environment over the longer term. For example, expanding foliage adapts to the light environment in which it develops. Shade-adapted leaves generally have greater specific leaf area (SLA), lower respiration rates and decreased investment of resources in carboxylation enzymes than sun-leaves, all considered adaptations to maximize carbon gain in low light regimes (Givnish 1988). The morphology and physiology of trees and their component organs also exhibit predictable changes or trends that are correlated with increasing age or size. Like changes induced by environment, age- and size-related changes may enhance species fitness as trees face different challenges to survival at different life stages. Age- and size-related changes in foliar behavior have also been implicated in the phenomenon of declining productivity with tree age (see reviews by Ryan et al. 1997, Bond 2000).

Environmentally induced developmental changes, such as those associated with the adaptation of foliage to sun or shade, reveal the genetic potential for phenotypic plasticity in morphological and physiological characteristics. The meristems producing foliar primordia remain unaltered, but the new primordia are capable of developing into foliage with either sun- or shade-type attributes regardless of the form of foliage produced during previous flushes. The developmental trajectory of the leaves depends on the environment in which they expand. In contrast, age- and size-related changes appear to be more complex. Both indirect evidence from ecophysiological studies (e.g., Day et al. 2001) and direct evidence from genetic research (e.g., Woo et al. 1994) suggest that gene expression in meristems may be fundamentally altered as trees grow older and larger. However, developing organs in trees of different ages and sizes commonly experience substantially different external environments. The relative importance of plastic responses of developing organs to their environment and intrinsically regulated changes in gene expression remains unclear. If intrinsic age-related changes in gene expression occur in

meristems, they may be induced either by factors internal to the meristem or by factors external to the meristem (including conditions in other systems within the tree itself).

These observations suggest four models to describe the control pathway(s) of change in any particular age-related attribute over time (Table 1). In the following descriptions, external and internal factors are defined relative to the meristem or organs responding to the factors, i.e., external factors may include not only those in the plant's environment, but also conditions elsewhere in the plant. The first model is a stimulus–response model in which the behavior of a fully developed organ is modified in response to changes or stresses external to the organ (e.g., stomatal closure in response to increasing leaf-to-air vapor pressure deficit). In this case, response follows both the initiation of the organ by a meristem and its subsequent differentiation and expansion. The second model is an extrinsic model, in which a meristem remains inherently the same over time but produces leaf primordia with developmental plasticity to respond morphologically and physiologically to external factors as they expand and differentiate. The stimulus–response and extrinsic pathways correspond, respectively, to short-term dynamic responses and medium-term acclimation to environment that are commonly considered in plant ecophysiology. The third model, the intrinsic model, specifies that a meristem itself undergoes genetically programmed maturation over time and, with advancing age or tree size, produces leaf primordia that are intrinsically different, regardless of the external environment. Theoretically, such a genetic program would have evolved to optimize morphological and physiological attributes to meet the varied, but predictable, challenges faced by trees at different life stages. The fourth model combines the previous two, i.e., an intrinsic–extrinsic model in which meristematic gene expression is fundamentally altered by interactions of external and internal factors as trees age (Poethig 1990). Here, the underlying mechanism is not tree age, but changes in gene expression in response to factors external to the meristem.

For example, decreases in net primary productivity in forest stands have been associated with a decline in net photosynthesis (Ryan et al. 1997, Bond 2000). This phenomenon could result from extrinsic (to the foliage) physical factors such as changes in hydraulic conductivity (Ryan and Yoder 1997, Hubbard et al. 1999) acting through a stimulus–response pathway. Alternatively, the low photosynthetic rates may indicate that shoots have intrinsically less capacity to grow as they mature (Takemoto and Greenwood 1993, Day et al. 2001). To distinguish between these possibilities, it is necessary to determine if reduced growth is a direct result of reduced photosynthetic capacity, or if an inherent reduction in growth potential leads to a reduction in the tree's investment in photosynthetic capacity.

In this review, we provide evidence for the involvement of a genetic component in age-related change, and examine some potential pathways by which genetic and external factors might act and interact to effect the observed trends in morphology and physiology. Because explanations based on stimulus–response and extrinsic pathways have been explored by Ryan et al. (1997), Ryan and Yoder (1997) and Bond (2000) and other papers in this issue (Hubbard et al. 2002, Köstner et al. 2002, Mencuccini 2002, Niinemets 2002, Rust and Roloff 2002, Tegischer et al. 2002, Wieser et al. 2002, Wirth et al. 2002), they are discussed only briefly here.

Evidence for genetic control of foliar traits during development

Woody species commonly exhibit changes in foliar morphological characteristics with increased size or age (see reviews by Gatsuk et al. 1980, Hackett 1985, Greenwood and Hutchison 1993). In conifers, these trends generally include decreasing SLA, increasing needle width-to-length ratios, and may include other aspects of needle morphology and foliage display (Steele et al. 1989, Rebbeck et al. 1992, Day et al. 2001). It is also well established that photosynthetic rates and related physiological attributes differ between juvenile and reproductively mature individuals in nearly every woody species examined (Bond 2000). In tree species, the general trend is toward lower instantaneous or integrated photosynthetic rates in reproductively mature individuals, but the inverse has also been reported in several species (Bond 2000).

Evidence from several conifer species suggests that the morphological and physiological trends that characterize the transition from juvenility to reproductive maturity continue as trees progress to the old-growth life stage. Post-maturational changes in both foliar morphological and physiological attributes have been described in *Picea rubens* Sarg. (Day et al. 2001) and *P. englemannii* × *sitchensis* × *glauca* hybrids (Richardson et al. 2000), for foliar morphology in *P. sitchensis* (Bong.) Carr. (Steele et al. 1989) and for photosynthetic physiology in *Sequoiadendron giganteum* (Lindl.) Bucholz (Grulke

Table 1. Summary of key characteristics of suggested pathways for control of age-related changes in morphology and physiology of shoot meristems and their derivatives.

Model	Change in state of meristem	Controlling factor(s)	
		Location relative to meristem	Time of action relative to organogenesis
Stimulus–response	No	External	Post-development
Extrinsic	No	External	During development
Intrinsic	Yes	Internal	Pre-development
Extrinsic–intrinsic	Yes	External + Internal	Pre-development

and Miller 1994). Generally, rates of change between mid-aged (reproductively mature) and old-growth life stages are substantially slower than those between the juvenile stage and early reproductive maturity (e.g., Steele et al. 1989, Day et al. 2001).

We reviewed the literature to evaluate whether these observations can be explained solely by stimulus–response or extrinsic models, with factors associated with environment or tree size acting on developed or developing organs, or whether there is a genetically regulated component to age-related change (i.e., intrinsic or extrinsic–intrinsic models). The most convincing indirect evidence for a genetically regulated developmental component comes from studies that controlled for both the effects of tree size and environment by grafting scions from donors of various ages onto rootstock of common age, and maintaining the grafted individuals in a common environment. This common-rootstock approach, although subject to several limitations, has the potential of minimizing the influence of both external factors (e.g., light, wind) and internal factors that are extrinsic to the meristem (e.g., differences in efficiency of water conduction pathways). Therefore, if the development of attributes is purely under extrinsic control, organs produced by the meristems of scions after grafting should no longer show differences related to the age or size of scion-donors. Common-rootstock experiments using scions from juvenile and reproductively mature donors indicate that age-related patterns of morphological and physiological attributes continue in foliage produced by scions after grafting in *Pinus taeda* L. (Greenwood 1984), *Larix laricina* (Du Roi) C. Koch (Hutchison et al. 1990), *Picea rubens* (Rebbeck et al. 1992), and in clones of two European oak species (McGowran et al. 1998). Furthermore, these age-related patterns persist for at least several years, suggesting differential patterns of gene expression between juvenile and reproductively mature life stages. Day et al. (2001) used the common-rootstock approach to evaluate differences between mid-aged and old-growth *P. rubens* trees and found that the age-related trends in foliar morphology and photosynthetic attributes described for a multicohort field population persisted for three growing seasons in foliage produced by scions grafted onto common rootstock.

Although common-rootstock experiments provide a means of controlling for the influence of size or environment, they are based on the assumption that graft unions do not differ by scion-donor age class in their ability to knit with rootstock vascular systems and provide developing scions with water and mineral nutrients. A similar assumption underlies the attempt to overcome differences in size and environment by vegetative propagation of mature and juvenile explants of similar size. Whenever these assumptions have been evaluated during common-rootstock trials, no significant age-related differences in graft union hydraulics have been reported. Hutchison et al. (1990) found significant differences in growth and photosynthetic rates of juvenile and mature larch scions, and subsequent tests of water flux through graft unions showed no differences related to scion age (M.S. Greenwood, unpublished data). Similarly, Day et al. (2001) reported significant differences in photosynthesis, stomatal conductance and growth among scions from juvenile, mid-aged and old-growth *P. rubens*, but graft unions exhibited no significant differences in specific hydraulic conductivity and no clear age-related trends (mid-aged > old-growth > juvenile).

Evidence for intrinsic changes and pathways that could affect meristem development

Table 2 lists examples of stable physiological phenotypic changes in woody plants that could, in turn, regulate age- and size-related developmental changes. These changes are grouped into three categories: hormone sensitivity, changes in DNA and patterns of gene expression. In all the examples shown, the effect of increased size and complexity was controlled by the production of equal-sized plants from juvenile or mature donors by grafting, use of rooted cuttings or tissue culture. The data in Table 2 provide strong circumstantial evidence that gene expression patterns differ between the juvenile and mature state. The pronounced decline in regenerative potential of mature cuttings or tissue culture explants and the changed reproductive competence of plants propagated from mature tissue are paralleled by differences in rooting response to auxin (Diaz-Sala et al. 1996) and gibberellin-induced induction of female flowering (Eysteinsson and Greenwood 1993). The observations that DNA methylation increases with maturation (Fraga et al. 2002), and that mitochondrial genotypes may also change (Huang et al. 1995) suggest intrinsic mechanisms for altering gene expression.

All developmental processes require precise patterns of differential gene expression over time. Differential gene expression requires mechanisms for silencing some genes while promoting the expression of others. Developing an understanding of the mechanism for silencing the dihydroflavonol reductase (DFR) gene in the mature phase of *Hedera helix* L. may be a paradigm for determining the effects of maturational processes on other genes. Dihydroflavonol reductase catalyzes the reduction of dihydroquercitin to leucocyanidin, a key step in the biosynthesis of anthocyanin, a readily visible maturation marker (Murray et al. 1994). Although attempts to characterize the regulatory sequences of this gene and its methylation state in juvenile and mature tissues have been unsuccessful, there is evidence that the inhibitory effects of inorganic phosphate (P_i) on DFR (Dedaldechamp et al. 1995) are relieved in juvenile plants compared with mature plants (W.P. Hackett, University of California at Davis, personal communication). Juvenile tissue may more readily sequester P_i by phosphorylation of monosaccharides, thereby permitting DFR to function, or mature tissue could be more sensitive to the effects of P_i. At present, we have little understanding of what regulates differential sequestration of, or increased sensitivity to, P_i.

There have been few studies on how differential gene activity is affected by extrinsic and intrinsic factors. Colot and Rossignol (1999) showed that genes with methylated promoter sequences are not expressed, and that methylation can

Table 2. Examples of differences between juvenile and mature phenotypes, including changes in hormone sensitivity, DNA sequence, DNA methylation and gene expression patterns, that occur after the juvenile to mature transition in woody plants. In all cases, effects of increased size and complexity were minimized by grafting scions of juvenile and mature plants onto common rootstock or by rooting cuttings.

Type of difference	Species	Reference	Difference between juvenile and mature phenotypes
Hormone sensitivity	*Pinus taeda*	Diaz-Sala et al. 1996	Seedling hypocotyl cuttings quickly organize adventitious root meristems in response to auxin; epicotyl cuttings do not
Hormone sensitivity	*Castanea sativa* Mill.	Ballester et al. 1999	Juvenile cuttings have less IAA in the cutting base than mature cuttings, but form adventitious roots more readily
Hormone sensitivity	*Hedera helix*	Geneve and Kester 1991	Petiole cuttings from stock plants rooted from juvenile and mature phases show only juvenile cuttings form roots in response to auxin
Hormone sensitivity	*Larix laricina*	Eysteinsson and Greenwood 1993	Grafted scions from older trees are less sensitive to the promotive effect of GA4/7 on female flowering
DNA changes	*Sequoia sempivirens* (D. Don) Endl.	Huang et al. 1995	Mitochondrial DNA differs between juvenile and mature in terms of restriction fragment analysis (grafted scions)
DNA changes	*Larix laricina*	Greenwood et al. 1989	No difference in % methylcytosine in long shoot foliage between juvenile and mature grafted scions
DNA changes	*Pinus radiata* D. Don	Fraga et al. 2002	Lower % methylcytosine in basal portions of juvenile needles; no difference between apical portions (grafted scions)
Gene expression	*Larix laricina*	Greenwood et al. 1989	Greater expression of chlorophyll a/b binding protein gene in developing juvenile foliage than mature foliage (grafted scions)
Gene expression	*Hedera helix*	Woo et al. 1994	Greater expression of chlorophyll a/b binding protein gene in petioles of juvenile foliage than of mature foliage (rooted cuttings)
Gene expression	*Hedera helix*	Woo et al. 1994	Auxin decreased expression of a proline rich protein gene in juvenile petioles but not in mature petioles (rooted cuttings)
Gene expression	*Hedera helix*	Murray et al. 1994	Gene for dihydroflavanol reductase is expressed in petioles of juvenile foliage but not in mature foliage (rooted cuttings)
Gene expression	*Sequoia giganteum*	Bon and Monteuuis 1991	Using SDS-PAGE, showed 16 kDa protein produced only in juvenile or rejuvenated meristems cultured in vitro
Gene expression	*Prunus avium* L.	Hand et al. 1996	Using polyclonal antibodies, found 28 kDa protein expressed in greater amount in juvenile shoot tips than in mature shoot tips (rooted cuttings and seedlings)

inhibit the binding of transcription factors. Shortening telomeres, which are repeat sequences of DNA that cap chromosome ends, alters cell proliferative capacity. In animals, cell cycles arrest and senescence and programmed cell death (apoptosis) increase as telomeres shorten. Parallel processes may occur in plants (Riha et al. 2001). Thus, changes in telomere length may occur as cell division progresses in the apical meristems of long-lived woody plants. In addition, age-related changes in mitochondrial DNA could alter mitochondrial function, and thereby affect development. Furthermore, changes in hormone sensitivity, e.g., the loss of ability to organize root meristems in response to auxin (Geneve and Kester 1991, Diaz-Sala et al. 1996), may affect organ development as meristems age. The genetic regulation of age-related changes in meristem behavior may be a complex function of many interacting factors.

Extrinsic and extrinsic–intrinsic pathways

Although there is indirect evidence for the action of intrinsic genetically based pathways, there has been no direct attempt to differentiate between the importance of intrinsic (strictly maturational) or extrinsic–intrinsic (environmentally induced) pathways. Likewise, if extrinsic–intrinsic pathways are implicated, it is unclear what factors associated with increased age or size lead to changes in meristematic gene expression, and to what extent these factors vary by species. Internal water relations are a candidate. Photosynthetic attributes of fully developed foliage can be altered by changes in properties of the soil–plant–air hydraulic continuum, and there is substantial evidence that leaf-specific hydraulic conductivity decreases with age in some tree species (Hubbard et al. 1999). Ryan and Yoder (1997) have suggested that water conduction becomes a principal physiological challenge as trees age, and may limit species-specific maximum heights. Decreasing hydraulic conductivity with tree age could have a direct effect on stomatal conductance (a stimulus–response model) or could induce genetically based changes in morphology and physiology as an adaptive response to predictable age-related changes in internal water relations (an extrinsic–intrinsic model).

Day et al. (2001) have proposed that lower photosynthetic rates in older trees are a consequence of inherently lower growth rates at old-growth life stages. Inherently lower growth potential could limit photosynthetic carbon gain through non-stomatal mechanisms, such as sink strength or end product accumulation effects on carboxylation efficiency. The results of both field studies and common rootstock experiments on *P. rubens* provide indirect evidence for a model based, at least in part, on non-stomatal limitations to photosynthesis. Day et al. (2001) described consistently low growth, stomatal conductance and photosynthetic rates in old shoots, but did not find a corresponding difference in internal CO_2 concentrations under ambient conditions between age classes, suggesting non-stomatal limitations to photosynthesis (Kubiske and Abrams 1993). Takemoto and Greenwood (1993) concluded that age-related decline in diameter growth of larch shoots grafted to common juvenile rootstock was not the result of decreased rate of cell division. They attributed the phenomenon to reduced sink strength for resources that limited radial expansion of tracheids in scions from old donors compared with scions from younger donors. Although high growth rates are generally advantageous to young trees that are competing for upper-canopy status, low growth rates in older trees may optimize resource-use efficiency or other attributes associated with long-term survival and reproductive success. Age-related decreases in growth potential could decrease photosynthesis by either direct (mechanistic) or indirect (regulation of gene expression) pathways. Direct inhibition of photosynthesis by end-product accumulation has been described in many herbaceous species (see reviews by Farrar and Williams 1991, Stitt 1991), and may occur in coniferous trees (Leverenz 1981, Myers et al. 1999). Accumulation of photosynthetic end products may result in down regulation of genes associated with components of the photosynthetic system (Sheen 1989, Krapp et al. 1993, Krapp and Stitt 1995, Jang et al. 1997).

Challenges to understanding age-related trends and directions for research

The observed trends in age-related changes in morphology and physiology are likely the result of complex interactions among extrinsic, intrinsic and intrinsic–extrinsic pathways. For any specific trait, these interactions may be further complicated by the influence of stimulus–response pathways controlled by environmental variables. For example, Hutchison et al. (1990) found that age-related trends varied between scions maintained in a greenhouse after grafting and scions maintained outdoors. Additionally, age-related changes in various aspects of morphology and physiology may be regulated by multiple induction pathways, resulting in age-related trends in different components occurring along different trajectories relative to maturational states. Steele et al. (1989) described such variation in the timing of changes of various morphological attributes in *P. sitchensis*.

The relative importance of pathways controlling age-related trends may also vary by species or species-groups, or across life stages within species. The challenges to survival differ with life stage and among species with different growth habits (e.g., rapidly growing, short-lived species compared with slowly growing, long-lived species), crown forms (e.g., tight, conical crowns compared to expansive crowns) and foliar habits (e.g., low foliar density compared to high foliar density). As with other evolved responses to environmental changes, different species in differing habitats are likely to have adapted to the changes associated with increasing age and size through diverse strategies, suggesting that a universal model may not exist.

The relevance of the four model pathways for regulating age-related trends in a particular trait should be evaluated in species representing a range of life strategies, life spans and habitat types. The most promising approach will likely be a combination of in situ morphological and physiological observations and experimental approaches that use grafted scions or rooted cuttings to control for effects of size and environment. Aside from differentiating among the models, such an approach may help determine (1) the most important extrinsic or intrinsic pathways, or both, affecting shoot and foliar behavior; (2) whether extrinsic or intrinsic controls, or both, differ by species or adaptation to habitat-type; (3) whether a universal model can account for age- and size-related changes in morphology and physiology; and (4) whether age- and size-related changes are reversible, and if so, what is the time course of reversibility.

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