Developmental processes shape plant morphologies, which constitute important adaptive traits selected for during evolution. Identifying the genes that act in developmental pathways and determining how they are modified during evolution is the focus of the field of evolutionary developmental biology, or evo-devo. Knowledge of genetic pathways in the plant model Arabidopsis serves as the starting point for investigating how the toolkit of developmental pathways has been used and reused to form different plant body plans. One productive approach is to identify genes in other species that are orthologous to genes known to control developmental pathways in Arabidopsis and then determine what changes have occurred in the protein coding sequence or in the gene’s expression to produce an altered morphology. A second approach relies on natural variation among wild populations or crop plants. Natural variation can be exploited to identify quantitative trait loci that underlie important developmental traits and, thus, define those genes that are responsible for adaptive changes. The possibility of applying comparative genomics approaches to Arabidopsis and related species promises profound new insights into the interplay of evolution and development.

EVO-DEVO

Evolutionary developmental biology (known as evo-devo) asks the question, “How do developmental genetic pathways diversify to give rise to different morphologies?” Put another way, evo-devo studies the subset of adaptive traits that relate to developmental processes. This field has grown dramatically in the past 20 years, in large part due to the discovery that a limited number of genetic pathways have been used and reused to build different body plans. This was first revealed for the Hox genes, which were shown to play similar roles in specifying anterior-posterior development in a wide variety of animal species (McGinnis et al., 1984; McGinnis and Krumlauf, 1992). Since then, a diverse array of developmentally important genes has been shown to play similar roles in specifying anterior-posterior development in a wide variety of animal species (McGinnis et al., 1984; McGinnis and Krumlauf, 1992). Since then, a diverse array of developmentally important genes has been shown to be conserved and to participate in analogous developmental processes in different organisms. This toolkit of developmentally important genes forms networks (developmental pathways) that are reutilized over and over again in different contexts.

HOW DO DEVELOPMENTAL PATHWAYS EVOLVE?

With the recognition that there are conserved toolkit genes, the focus is now shifting toward understanding how changes in these genes and their associated developmental pathways lead to diverse morphologies. Simply stated, evolution has two components: first, the generation of variation by mutation and, second, selection acting to fix such variation in populations. However, selection does not act on individual genetic changes but rather on the resulting phenotypes. This is where development comes in: genetic changes can cause reprogramming of developmental pathways, which in turn can result in alterations in phenotype. Therefore, understanding the ways in which developmental pathways can be remodeled has important implications for linking mutational changes to altered phenotypes.

Remodeling of developmental pathways can occur in a variety of ways. Gene duplication can provide the raw material for variation (Force et al., 1999). Alternatively, developmental pathways can be redeployed in other tissues (heterotopy) and at other developmental times (heterochrony). This occurs through modification of the expression patterns of component genes of the developmental pathway. The question then becomes whether there are biases to the kinds of developmental reprogramming that can occur. For instance, are the predominant types of changes in developmental pathway remodeling due to changes at the level of cis-acting elements or due to changes in the functions of developmentally important proteins? Are some kinds of changes more prevalent, while others are rarely seen (developmental constraint)? The first step in attempting to answer these questions is to accumulate evidence of developmental pathway remodeling and to pin down the specifics of differences between species. Below, we will discuss two main strategies that are being used to explore developmental differences between Arabidopsis and other plant species.
EXTENDING MODEL SYSTEMS USING A COMPARATIVE APPROACH

Model systems have provided an excellent basis for dissecting development using advanced genetic approaches. Among plant models, Arabidopsis has been utilized extensively to identify pathways involved in a variety of developmental processes, including flower formation, root organization, epidermal patterning, and meristem development. The host of genomics tools now available for Arabidopsis has greatly enhanced the ability to rapidly assess function using forward and reverse genetic approaches in this species. However, translating this information from Arabidopsis to other species still remains a considerable challenge.

One general strategy has been to identify important developmental genes in Arabidopsis and compare them to their orthologs in other species. There are several hurdles to surmount with this deceptively simple approach. First of all, gene duplications can make it difficult to decide which genes to compare. About two-thirds of all Arabidopsis genes are members of gene families (Arabidopsis Genome Initiative, 2000), reflecting the fact that over evolutionary time, gene duplications have contributed extensively to the number of tools in the toolkit. For instance, there are 107 MADS box genes present in the Arabidopsis genome (Parenicova et al., 2003). Furthermore, it is clear that MADS box genes have independently duplicated in different plant lineages (Theissen et al., 1996; Stellari et al., 2004), contributing to the difficulties in making accurate comparisons between genes from different species.

Even if orthologous genes can be identified, comparing their roles can be a challenge. Ideally, one would want to compare the functions of such genes using forward and reverse genetic approaches in their respective species. While such analyses can be carried out with ease in Arabidopsis, such functional analyses can be difficult to carry out in nonmodel systems. This has led to various stand-ins being used, for example analyzing the expression patterns of orthologs, heterologous overexpression, or heterologous rescue experiments. All of these alternatives have potential pitfalls and may not reflect the function of a particular gene in its native context.

Despite these experimental limitations, progress has certainly been made. Plants clearly possess key master regulatory genes that have been reprogrammed across species to evoke different morphologies. Many comparative expression analyses have been carried out and have been used to suggest hypotheses for how a variety of different morphologies might have evolved. Several examples serve to illustrate this type of analysis. The Arabidopsis homeobox-containing KNOX genes have partially redundant roles in specifying meristem identity, and their down-regulation is required for leaf development (Long et al., 1996; Byrne et al., 2002). However, the observation that a number of species with compound leaves display KNOX gene expression in leaf primordia has led to the suggestion that such expression changes may underlie the evolution of different leaf forms (Bharathan et al., 2002). Similarly, shifts in the expression patterns of the B-class MADS box genes have been suggested to underlie the independent origins of petals in several flowering plant lineages (Kramer and Irish, 1999). In a third example, the developmental pathways controlling flowering have been investigated in rice (Oryza sativa), which flowers in response to short day conditions, and compared to Arabidopsis, a long day responsive species. In Arabidopsis, the CONSTANS transcription factor activates FT, which encodes a key integrator of flowering signals (Samach et al., 2000). By contrast, the rice homolog of CONSTANS functions to repress the FT homolog, suggesting that a switch in the mode of transcriptional regulation of FT is responsible for long day versus short day flowering (Hayama et al., 2003). Such examples provide support for the idea that cis-regulatory changes affecting the expression of master regulatory genes may have been instrumental in the origins of new morphologies and changes in developmental timing (Doebley and Lukens, 1998).

However, changes in the expression patterns of developmentally important genes are unlikely to be the only mechanism by which changes in developmental pathways occur. There is mounting evidence that changes in the protein coding sequences of developmentally important genes also play a role. For instance, while the amino-terminal DNA binding domain of the plant MADS box genes appears to be highly conserved, frameshift mutations in duplicated members of several MADS box gene lineages have led to the acquisition of different carboxy-terminal motifs (Litt and Irish, 2003; Vandenbussche et al., 2003). In at least one case, these changes have been shown to be responsible for alterations in gene function (Lamb and Irish, 2003).

An important corollary to comparing developmentally important genes from various species is assessing such comparisons within a phylogenetic framework. In other words, the relationships of the species in question should be well understood so that the direction of any evolutionary change can be inferred. This can be done by mapping changes in the relevant character (for instance, a particular expression pattern) onto a species phylogeny. As a result, one can hypothesize what is likely to have been the ancestral state versus the derived state. This means, though, that comparisons should be made among species that span the evolutionary tree. So, for instance, when assessing the role of a gene thought to be involved in flowering, comparisons should be drawn from species across the angiosperm phylogeny. However, while it is relatively easy to clone genes and perform sequence and expression analyses from virtually any species, carrying out functional analyses are far from trivial for most nonmodel plant species. Developing stable and transient transformation methods for a wide range of
phylogenetically informative plant species is necessary if truly comparative functional analyses are to be carried out.

EXTENDING MODEL SYSTEMS THROUGH NATURAL VARIATION

A complementary strategy to dissecting how developmental traits have evolved relies on identifying relevant developmental genetic differences between closely related species or cultivars. This approach attempts to identify adaptive traits that evolved in natural populations or were bred into crops, to uncover the genetic basis for developmental processes. A prerequisite is a thorough knowledge of the range of phenotypic variation present in natural populations. This natural variation can be thought of as nature’s mutant collection. The traditional approach to identifying the genetic basis for specific traits in natural populations is by quantitative trait locus (QTL) analysis. This involves crosses between different subspecies or cultivars and determination of the rates of co-occurrence of traits in the progeny. While very powerful in uncovering pathways, particularly those in which multiple genes are involved, the difficulty is in getting from QTL to gene. The problem is analyzing a sufficient number of recombination events to narrow the chromosomal interval sufficiently to identify candidate genes. A complementary approach is the production of recombinant inbred lines through multiple backcrosses and, thus, isolation of small chromosomal regions from one subspecies in the genetic background of another. For instance, this type of approach has been utilized to identify allelic variants at the CRY2 blue light photoreceptor locus as being responsible for differences in flowering time between two Arabidopsis ecotypes (El-Din El-Assal et al., 2001). While QTL analyses have not yet been carried out extensively to explore natural variation in developmental traits in Arabidopsis species or cultivars, such approaches have been extremely useful to define genes of large effect involved in various developmental processes in crop species. These include the identification of a locus controlling fruit size in tomato (Lycopersicon esculentum) cultivars (Frary et al., 2000) and the teosinte branched 1 gene as a major player in conditioning branching differences between maize (Zea mays) and its wild relatives (Doebley and Stec, 1991; Doebley et al., 1997). These types of analyses are powerful in that they explicitly define loci that are responsible for morphological variation; in turn, the identification of such loci allows one to explore what kinds of genetic alterations are responsible for such changes. For instance, nucleotide polymorphisms at the teosinte branched 1 locus are predominantly found in the regulatory regions of the gene, again pointing to the primacy of regulatory change in driving morphological evolution (Wang et al., 1999).

A third approach, which ultimately may prove to be the most powerful, is to use the tools of comparative genomics. The basic idea is to compare the adaptive traits of closely related species and determine the genetic origins of the traits by comparing genomes and how they are expressed. The advantage is that crosses do not have to be performed. The problem is that determining what has changed between two species requires knowledge of the full sequence of both genomes as well as how they differ in gene (and possibly protein) expression. These types of genome-level comparisons between Drosophila species have been extremely powerful in defining the suites of developmental genes correlated with differences in metamorphosis (Rifkin et al., 2003). However, such approaches have not yet been exhaustively employed for Arabidopsis or its close relatives. However, as sequencing costs continue to drop, the cost of full genome coverage for nonmodel organisms, particularly for close relatives of Arabidopsis, becomes affordable. Many closely related species have diploid genomes that are only slightly larger than that of Arabidopsis. Moreover, the Brassica crop plants as well as papaya (Carica papaya) and cotton (Gossypium hirsutum) are all relatively closely related to Arabidopsis. Comparing genome sequences with expression data across closely related species will likely lead to profound new insights in how developmental processes have evolved in plants.

PROSPECTS

Advances in evo-devo rely on identifying key developmental differences between species. Identifying such differences will depend first on correlating differences in the sequence and expression of relevant genes with developmental differences, using the various strategies we have outlined. However, the next step is to demonstrate that such differences are actually responsible for phenotypic variation. Ultimately this will require functional analyses, using genetic or transgenic approaches, in a wide range of species. Ideally, a set of amenable and informative species should be identified that would serve as a common ground for comparative investigations. In our judgment, there are several criteria that would serve to identify these exemplars. First, such species should occupy key phylogenetic positions, in that they would be representatives of different major clades, or alternatively, closely related to model species such as Arabidopsis. Second, they should experimentally tractable, easy to grow, and have a manageable generation time. Third, they should be of some agronomic or horticultural interest. Finally, such species should ideally have a variety of molecular and/or genetic tools already available. Of the more than 250,000 extant angiosperm species, only a handful is currently being utilized for developmental, genetic, or molecular analyses. In addition to Arabidopsis, the major experimental focus is on members of the Poaceae, the Solanaceae, and the Fabaceae. A concerted effort to develop genetic and transgenic technologies for other
taxa that meet the criteria listed above would provide a rich resource for future investigations of the evolution of developmental pathways.

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LITERATURE CITED


