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Citation
Kramer, Elena M. 2007. Understanding the genetic basis of floral diversity. BioScience 57(6): 479-487.

Published Version
http://dx.doi.org/10.1641/B570605

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Understanding the Genetic Basis of Floral Diversity

ELENA M. KRAMER

The major radiations that punctuate angiosperm evolution are often associated with innovations in floral architecture. The field of plant developmental evolution seeks to understand the generation of this diversity using comparative genetic approaches that build on work done in model species such as Arabidopsis. *This strategy has been particularly successful when applied to the genetic program controlling floral organ identity, but is also making advances in the area of floral symmetry. As useful as the "candidate gene" approach has been, alternatives are required to investigate morphological phenomena that are not represented in model species. Other options include methods based on quantitative trait loci and DNA sequencing, which have the additional benefit of facilitating the development of new model species. The combination of all of these approaches across a wide range of species has the potential to elucidate the genetic underpinnings of both recent and ancient radiations of flowering plants.*

Keywords: floral evolution, gene duplication, ABC model, MADS-box genes, CYCLOIDEA

The 240,000-plus species of angiosperms that now dominate most of Earth's terrestrial ecosystems were derived from a series of major radiations, starting with their enigmatic appearance approximately 130 million years ago (Dilcher 2000). In many cases, these waves of speciation were driven by physiological factors, such as adaptation to new soil types or innovation in light responses (Mathews et al. 2003, Feild et al. 2004), but variability in floral morphology is particularly notable for its direct connection to factors that affect plant fitness (e.g., pollinator interactions). As perhaps the most charismatic aspect of angiosperm diversity, variation in floral structure has become a prominent topic within the field of plant evolutionary developmental biology, or "evo-devo." However, this is due as much to scientists' detailed understanding of the genetic pathways controlling floral development as it is to the wealth of floral diversity. Expanding the genetic work done in *Arabidopsis* and *Antirrhinum* to nonmodel species has led to a complex picture of strict conservation coexisting with dynamic evolutionary change. The goal of this article is to outline some of the significant findings to date in the area of floral developmental evolution by discussing selected examples. This work demonstrates the importance of understanding gene lineage evolution, expanding analyses beyond the floral organ identity program, and combining multiple molecular approaches.

Evo-devo: Approaches and interpretation

Because the plant evo-devo field is somewhat younger than its metazoan cousin, we have the opportunity to learn from previous work on animals, which has provided important guidelines for project design and data interpretation. It remains the case that most developmental evolutionary studies are based on a "candidate gene" approach (Haag and True 2001), a technique that depends on model species to suggest promising genes that seem likely to be involved with the development of a given trait. The characteristics of a good candidate gene include a well-known developmental function that is clearly associated with the trait in question, a tissue-specific expression pattern, and a high degree of sequence conservation. The first of these conditions is fairly obvious, but the latter two are equally important. A distinct expression pattern is critical, since studies based on a candidate gene approach are often limited to comparative analysis of gene expression. For instance, a gene that plays a role in sepal development might seem like a good candidate for studying the evolution of these organs, but if the gene is constitutively expressed (as is sometimes the case with developmentally important loci), it will be uninformative for comparative studies. The third criterion, high sequence conservation, is critical in order to facilitate the identification of gene homologs in species outside the model. It is important to note that the only way to determine such genetic homology is through phylogenetic analysis of gene families (Theissen 2002).

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This brings us to the related issues of genetic homology and gene duplication, which have been important facets of candidate gene analysis since the early studies of the *Hox* genes in animals. Put simply, we know that genes duplicate over evolutionary time, both by genome duplication and through smaller events. Therefore, the phylogenetic relationships among genes are an important issue for comparative studies, particularly when the species involved are distantly related. Evo-devo researchers must concern themselves with gene duplication because the existence of duplicate copies can provide an opportunity for changes in gene function (Ohno 1970). When gene duplication occurs, the resultant gene copies are referred to as paralogs, while members of the same continuous genetic lineage separated only by speciation are called orthologs (figure 1).

There are three general models for the potential progression of functional evolution following gene duplication (reviewed in Prince and Pickett 2002, Zhang 2003). All of these are based on the fundamental assumption that equivalent paralogs with entirely redundant functions cannot be maintained over the course of time (Force et al. 1999). The first model, termed "pseudogenization," posits that following a duplication event, one of the paralogs will rapidly degrade and become nonfunctional (figure 2a). This is considered the most likely outcome of a gene duplication event, and it has been estimated that without selective pressure to maintain both duplicate copies, the average half-life of a gene duplicate is approximately four million years (Lynch and Conery 2000).

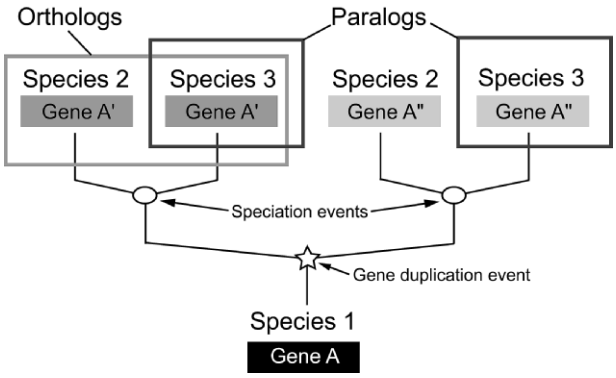

Figure 1. The effect of gene duplication on genetic relationships among homologs. In ancestral species 1, there is a single-copy gene A. If this locus is duplicated, it can give rise to two paralogous gene lineages, A′ and A″. When this event is followed by speciation to produce two sister species, 2 and 3, the genome of each descendent species can contain representatives of both gene lineages A′ and A″. Phylogenetic analysis of the genes will show that the A′ (or A″) loci in the different genomes are more closely related to each other than are the A′ and A″ loci in the same genome. The A′ and A″ within the genome of species 3 are paralogs, descended from a gene duplication event. The A′ loci in species 2 and 3 are orthologs, members of the same gene lineage that has been inherited in each species after a speciation event.

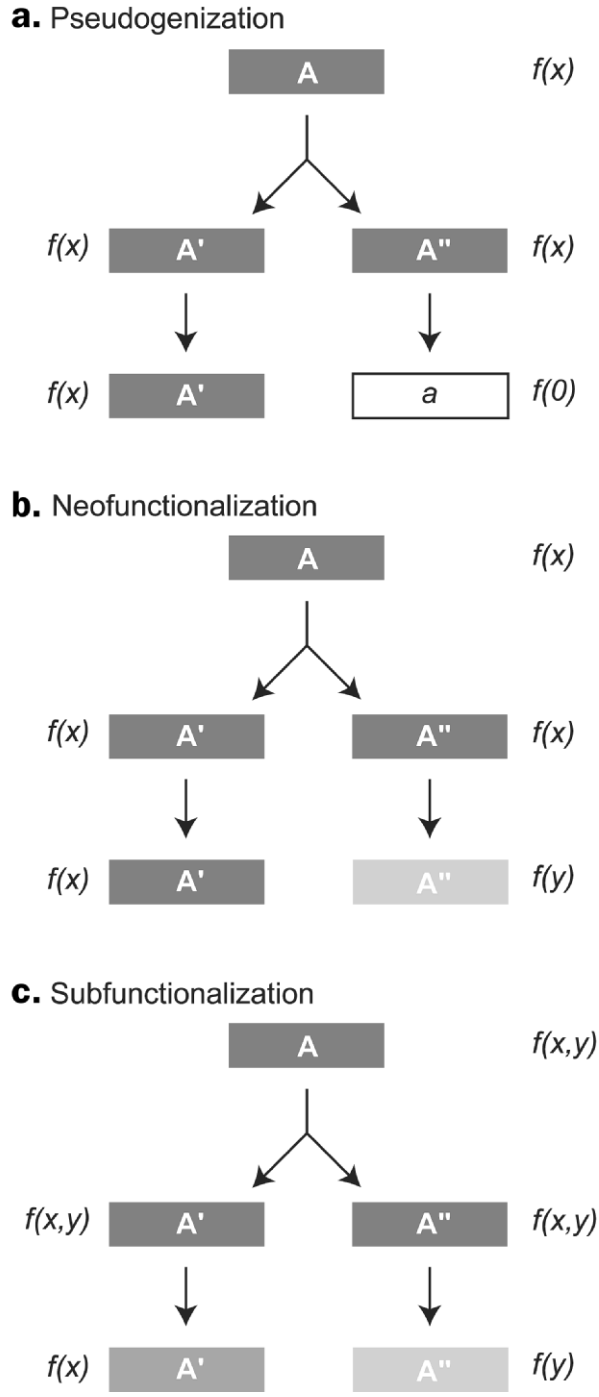

Figure 2. The three main models for functional evolution following gene duplication. (a) Pseudogenization posits that one of the paralogous copies will become nonfunctional (a pseudogene) through degenerative mutations. (b) Neofunctionalization describes a scenario whereby both copies are retained as a result of the acquisition of novel function by one of the two paralogs. (c) In subfunctionalization, the multiple functions of the ancestral gene become parsed out between the two paralogs, such that they both must be retained to preserve all of the ancestral roles.

The classic alternative to such paralog loss is neofunctionalization, in which one of the two copies maintains the ancestral function while the second acquires a new function, resulting in the selective maintenance of both copies (figure 2b; Ohno 1970). More recently, a third model has been proposed; this model, called subfunctionalization (figure 2c), is based on the modern understanding of the complexity of gene functional repertoires (Force et al. 1999, Hughes 1999). Under this scenario, resultant gene copies do not necessarily acquire any novel functions. Instead, over the course of time, the multiple functional roles of the single ancestral gene become parsed out—subdivided—between the paralogs so that both copies must be maintained to conserve the original repertoire of functions.

These models are not mutually exclusive, and theoretical approaches have demonstrated that subfunctionalization can serve as a transitional phase to neofunctionalization (He and Zhang 2005, Rastogi and Liberles 2005). In addition, targeted mutagenesis experiments in many model systems have found evidence for maintained genetic redundancy. Thus it appears that, despite the fundamental assumption regarding redundancy mentioned earlier, genetic redundancy can be sustained under certain conditions (Nowak et al. 1997, Krakauer and Nowak 1999). These issues related to gene duplication are particularly relevant in plants, which tend to retain paralogs at a higher rate than metazoa do (Shiu et al. 2005).

Another factor that complicates the candidate gene approach is a phenomenon termed "developmental system drift" (True and Haag 2001). Developmental system drift results when the genetic pathways underlying an otherwise conserved, homologous morphological feature diverge over time. This process has been uncovered in both animal and plant systems (True and Haag 2001, Jaramillo and Kramer 2007), and it underscores the importance of avoiding blanket assumptions about the conservation of genetic pathways. Just as morphological conservation does not guarantee genetic conservation, there are also cases in which clearly homoplastic (independently derived) morphologies are controlled by similar genetic programs (Hodin 2000, Gilbert and Bolker 2001). This situation may result from the independent cooption of a certain genetic pathway for similar developmental roles. For instance, in the case of compound leaves, a genetic program that promotes meristematic growth has been recruited many times independently to produce degrees of indeterminacy in otherwise determinate lateral organs (Bharathan et al. 2002, Hay and Tsiantis 2006). This trend reflects what has been referred to as the "smallness of the genetic toolbox"—evolution's tendency to take advantage of existing genetic modules, even in the course of evolving novel features (Gilbert and Bolker 2001). All of these issues must enter into the consideration of floral diversification.

The basics of floral development

The genetic programs controlling floral development have been the focus of intense molecular studies for approximately 20 years, primarily in the two model systems *Arabidopsis thaliana* and *Antirrhinum majus* (snapdragon). The first pathway to be well characterized was the so-called ABC model, which controls the establishment of floral organ identity (reviewed in Jack 2004, Krizek and Fletcher 2005). This model outlines the existence of three gene classes, termed A, B, and C, which function in overlapping domains to create unique combinatorial codes for each of the four major organ types: The A function alone encodes sepal identity; A + B function, petal identity; B + C function, stamen identity; and C function alone, carpel identity (figure 3a). A fourth class of organ identity genes, the E class, was recently identified on the basis of their genetic sequence similarity rather than primary mutant phenotypes. The E-class loci act broadly across the floral meristem to facilitate the function of the other three classes.

The ABCE genetic program is a homeotic program, meaning that elimination or overexpression of gene function results in transformations in organ identity. For instance, loss of B gene function causes transformation of the petals into sepals and stamens into carpels, while overexpression of the B-class genes turns the sepals into petals and carpels into stamens. All of the originally characterized members of these gene classes have now been mapped and sequenced, and all but one are representatives of the type II MADS-box family of transcription factors, the exception being the A-class gene *APETALA2.* The rest of the loci are, in the A class, *APETALA1;* in the B class, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*); in the

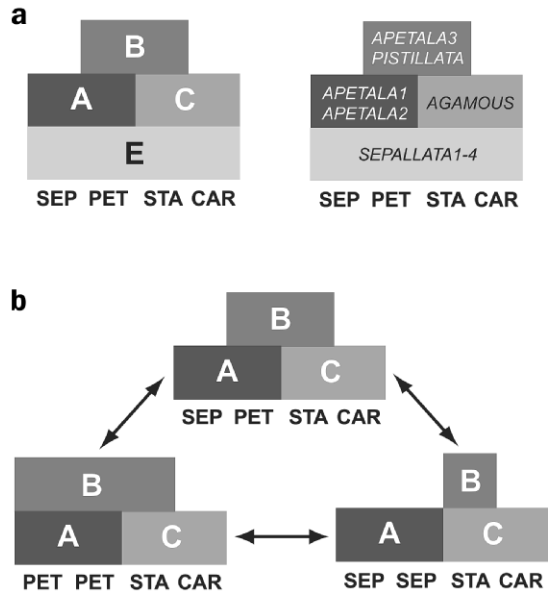

Figure 3. The ABCE model and potential variants of ABC gene expression. (a) A basic schematic of the ABCE model (left) and the corresponding ABCE loci from* Arabidopsis *(right). (b) Variations on the ABC model that could be achieved through shifts in the expression domain of the B-class genes. Such shifts could produce homeotic changes in morphology (Bowman 1997, Albert et al. 1998). Abbreviations: CAR, carpel; PET, petal; SEP, sepal; and STA, stamen.

C class, *AGAMOUS*; and, in the E class, the four *SEPALLATA1–4* genes. The protein products of these genes function in combination as dimers that may further interact with one another to form higher-order protein complexes. In evolutionary terms, many aspects of what is now termed the ABCE model are deeply conserved across the angiosperms, most notably the stamen and carpel identity pathways (Becker and Theissen 2003). The situation for sepal and petal identity is much more complicated, however, possibly reflecting the fact that these organ types may have evolved independently in different angiosperm lineages (Kramer and Jaramillo 2005, Litt 2007).

Although one might get the impression that variation in the ABCE model is the primary determinant of floral diversity, given all the attention paid to it, this would be inaccurate. The ABCE model has become central to floral evo-devo studies because the major genetic players show relatively high sequence conservation and their expression patterns correlate fairly well with their functional domains (Becker and Theissen 2003). In other words, they make very convenient candidate genes. However, any survey of floral diversity will reveal that much of the morphological variation does not occur in aspects of floral organ identity but in other features, such as the arrangement of the organs within the meristem (phyllotaxy), the number of organs of each type (merosity), the elaboration of the floral organs (e.g., organ fusion), and floral symmetry (Endress 1994). All of these morphological characteristics are controlled by genes acting either in parallel or genetically downstream of the ABCE loci, a fact that has major implications for floral evo-devo studies (Kramer 2005). For example, analysis of the B- or C-class genes cannot be informative for comparative studies of stamen number, since stamens will express these homologs regardless of how many of them there are.

Unfortunately, good candidate genes are lacking for many of these morphological features. The one notable exception is floral symmetry, which has been studied in detail in the bilaterally symmetric (zygomorphic) flowers of *Antirrhinum* (figure 4; Cubas 2004). Several classes of mutations have been described in *Antirrhinum* that result in loss of zygomorphy, causing the flowers to become more radially symmetric (actinomorphic). The first of these to be characterized were a pair of closely related paralogs, *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*), representatives of the TCP family of transcription factors. These loci are essential for establishing the distinct dorsal (upper) and lateral regions of the *Antirrhinum* flower (figure 4). Subsequently, another key player, the gene *DIVARICATA*, was identified as a MYB transcription factor that is required for ventral meristem identity and acts antagonistically with *CYC/DICH* (Corley et al. 2005). As it turns out, homologs of *CYC/DICH* have become key candidate genes for comparative studies of floral symmetry for some of the same reasons that the MADS-box genes have been targeted: They show relatively high sequence conservation, and their distinct expression patterns are correlated with their functional domains (Cubas 2002).

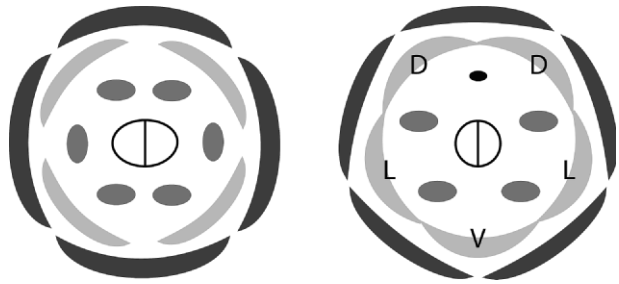

Figure 4. Floral diagrams for* Arabidopsis *(left) and* Antirrhinum *(right). In each flower there are four concentric whorls of different floral organs, starting with the outermost: sepals, petals, stamens, and carpels. The flower of* Arabidopsis *has two planes of symmetry (disymmetric), while that of* Antirrhinum *has only one (zygomorphic). There are three distinct types of petals in* Antirrhinum*, dorsal (D), lateral (L), and ventral (V). In the stamen whorl of* Antirrhinum*, the dorsal stamen is aborted.

Evolutionary change in floral organ identity: The roles of homeosis and gene duplication

From the time the original ABC model was described, it has been the focus of intense evolutionary study and speculation (Bowman et al. 1991, Doyle 1994, Bowman 1997). In addition to the features that make type II MADS-box genes such useful candidate loci, the homeotic nature of this genetic program has been seen as a powerful evolutionary mechanism for the origin of the angiosperms and for certain aspects of their diversification (Bowman 1997, Albert et al. 1998). For example, one of the long-standing questions in seed plant evolution was how an ancestor with separate male and female reproductive axes (e.g., pinecones) made the transition to an angiosperm-like plant with hermaphroditic axes (flowers). We now know that homologs of the B- and C-class genes are expressed in the reproductive structures of gymnosperms much as the corresponding genes are expressed in angiosperms, with C gene homologs expressed in both male and female organs but B gene homologs only in male tissues (reviewed in Theissen et al. 2000). Therefore, hermaphroditic axes could have evolved through the simple restriction of B gene expression to part of a conelike structure, yielding both male and female organs on the same axis (Theissen et al. 2002, Baum and Hileman 2006).

Within the angiosperms, other morphological features could be explained by homeosis, primarily in the sterile floral organs, collectively termed the perianth. These organs show enormous diversity in their morphology. In some cases, such as *Magnolia,* the perianth is undifferentiated and composed entirely of petaloid organs, which are called tepals. In other cases, there are two distinct organ types, the outer sepals and inner petals; the sepals generally play a protective role and are photosynthetic, while the petals function in pollinator attraction and are bright and showy. Evolutionary transitions between these two types are quite common, and

in some instances the perianth may even lose the appearance of petaloidy altogether (Endress 1994, Zanis et al. 2003).

It is reasonable to ask whether these shifts between completely petaloid perianths and differentiated perianths of sepals and petals have involved correlated shifts in the B gene domain (figure 3b). This question has been investigated in several different angiosperm lineages, and, perhaps not surprisingly, variable answers have been recovered. In the monocots and magnoliid dicots, which commonly have petaloid tepals, the expression of *AP3* and *PI* homologs has been detected in all of the morphologically similar petaloid organs, although there is one intriguing exception in *Asparagus* (reviewed in Kramer and Jaramillo 2005, Jaramillo and Kramer 2007). More complex cases include taxa in which the perianth is composed of only one whorl of petaloid sepals and taxa in which there are two whorls of petaloid organs that are morphologically distinct (figure 5a, 5b). Analyses of B homolog expression in such flowers have not revealed the persistent gene expression that is normally associated with a role in organ identity (Jaramillo and Kramer 2004, Kramer et al. 2007). Overall, these studies suggest that homeotic shifts may have occurred over the course of evolution, but there are also instances in which truly novel forms of petaloidy have evolved without the input of the B-class genes.

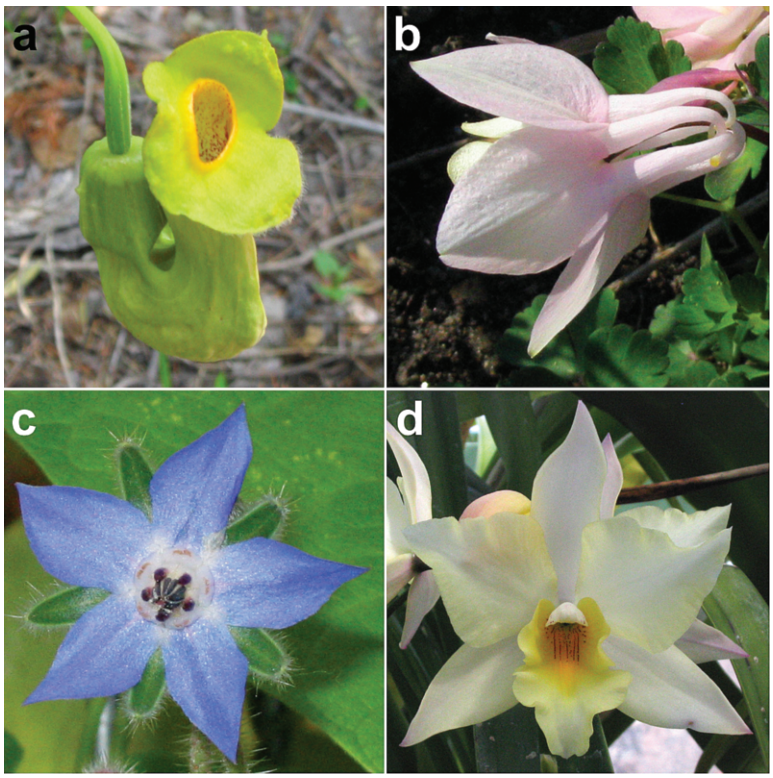

Figure 5. (a) The flowers of* Aristolochia *possess only one whorl of petaloid organs, which are considered to be modified sepals. (b)* Aquilegia *has two whorls of petaloid organs that are morphologically very different from one another. (c) This flower of* Solanum *exhibits perfect radial symmetry, or actinomorphy. (d) In contrast, orchids such as this* Laelia *hybrid have flowers with strong bilateral symmetry and are termed zygomorphic. Photographs: Elena M. Kramer.

Within this context of shifting gene expression patterns, there is also evidence for a multitude of independent gene duplication events (Becker and Theissen 2003, Kramer and Zimmer 2006). It can be quite difficult, however, to determine what significance such events have had for morphological evolution. The presence of gene paralogs may seem to correlate with novel aspects of morphology, but since paralogs can be retained as a result of either functional conservation (via subfunctionalization) or innovation (neofunctionalization), such correlation is a poor proxy for direct functional studies. Luckily, the classical floral genetic models, as well as newer systems, are shedding light on the significance of gene duplication events.

For example, genetic studies are providing critical evidence on functional evolution within the *AP3* gene lineage. This lineage is of particular interest because of a duplication event that occurred close to the base of the core eudicot clade (figure 6), which includes 70% of all angiosperm species (Magallon et al. 1999). At some point before the diversification of this group, the ancestral *AP3* lineage, known as the paleo*AP3* lineage, duplicated to give rise to two paralogous lineages, termed eu*AP3* and *TM6* (reviewed in Kramer and Zimmer 2006). It has been shown that in the eu*AP3* lineage, the gene sequence underwent a surprising degree of remodeling that altered several highly conserved protein motifs. In contrast, the *TM6* orthologs retain most of the ancestral paleo*AP3* sequence characteristics.

The *Arabidopsis* B gene *AP3* is actually an ortholog of the divergent eu*AP3* lineage, which raises several questions. First and foremost, how significant was the *AP3* duplication event for functional evolution within this lineage? Is the sequence divergence of the eu*AP3* lineage correlated with important changes in the functional repertoire of these genes, or is it simply an indicator of developmental system drift? One of the first steps in answering these questions is to determine the exact functions of eu*AP3, TM6,* and paleo*AP3* lineage members. Much work has already been done on eu*AP3* orthologs, which are known to promote petal and stamen identity in several core eudicot taxa. Unfortunately, however, the *TM6* ortholog has been lost from the *Arabidopsis* genome and is also unknown from *Antirrhinum.* Several genetically tractable members of the core eudicot family Solanaceae do possess *TM6* orthologs, however, allowing endogenous functional studies. This work has demonstrated that while the eu*AP3* orthologs of *Petunia* and tomato contribute to petal and stamen identity, the *TM6* orthologs function only in the identity and development of the stamens (de Martino et al. 2006, Rijpkema et al. 2006).

The interpretation of these results is still somewhat difficult. It is unclear whether the stamen-specific function of these

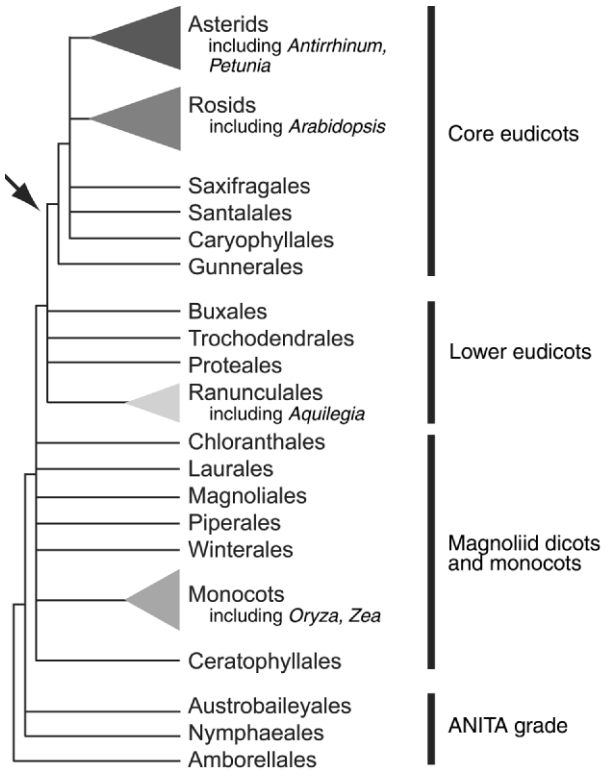

***Figure 6. Simplified phylogeny of the angiosperms based on recent molecular analyses (Soltis and Soltis 2004). Arrow indicates the relative position of the eu*AP3/TM6 *gene duplication. Acronym: ANITA,* Amborella, *Nymphaeales, Illiciales,* Trimenia*, and* Austrobaileya.**

TM6 loci is a general feature of the orthologous lineage or whether it reflects a more recent subfunctionalization event. Moreover, without functional data from paleo*AP3* lineage members, it is not possible to determine whether *TM6* function in the core eudicots is a useful proxy for the functional repertoire of the ancestral paleo*AP3* lineage. These questions are being addressed through functional studies outside the core eudicots, particularly in the grasses and lower eudicots. In both *Zea mays* (corn) and *Oryza sativa* (rice), it has been shown that paleo*AP3* lineage members are essential to the identity of stamens as well as organs thought to be derived from petals (Ambrose et al. 2000, Nagasawa et al. 2003). Similarly, in the emerging model system *Aquilegia* (columbine), B gene function clearly encompasses petal and stamen identity (Kramer et al. 2007). Although there are alternative interpretations of these data (see Kramer and Jaramillo 2005), it appears that *AP3* lineage members function in both petal and stamen identity before and after the eu*AP3*/*TM6* duplication. This suggests that while the duplication event may be significant in terms of biochemical function, it is not necessarily associated with major changes in the genes' developmental functions.

This is not to say that all gene duplication events are similarly conservative. In *Aquilegia,* there are independent duplications in the paleo*AP3* lineage that may have contributed to the evolution of a novel organ type (Kramer et al. 2007), and similar phenomena have been uncovered in orchids (Tsai et al. 2004). This gives us a picture of general conservation in the ABCE program, with variation introduced through shifts in gene expression or through gene duplication events.

Floral symmetry as a model for independent co-option events

The evolution of zygomorphic flowers from actinomorphic ancestors has clearly occurred many times independently across the angiosperms (Endress 2001). This would seem to make it very difficult to study zygomorphy from a comparative standpoint, since novel loci might have been recruited in each of these evolutionary events. Interestingly, researchers have found that this is not necessarily the case. As discussed above, the TCP genes *CYC* and *DICH* were first described as controlling floral symmetry in *An. majus* (reviewed in Cubas 2004). More generally, members of the TCP family play common roles in controlling rates of cell division in many different developmental contexts. In fact, a homolog of *CYC* in *Z. mays,* the gene *TEOSINTE BRANCHED1,* is expressed on the dorsal side of axillary meristems and regulates their outgrowth and identity (reviewed in Cubas et al. 1999). Even in *Arabidopsis,* which is generally considered to have actinomorphic flowers (although they are more accurately described as disymmetric; figure 4), a *CYC*-like gene called *TCP1* is expressed on the dorsal side of vegetative and floral meristems (Cubas 2004).

These findings seem to suggest that representatives of the *CYC* lineage of TCP genes are commonly expressed on the dorsal side of meristems, possibly providing orientation even in the case of actinomorphic flowers. If this was the case, *CYC*-like genes would be uniquely suited to function in later developmental aspects of floral symmetry. Although researchers have identified *CYC* homologs from a wide range of core eudicots (Cubas 2004), it was only recently that this possibility was functionally tested. Working with the legumes, a rosid core eudicot group that evolved zygomorphy independently from the asterid *Antirrhinum,* researchers have found that a *CYC* homolog of *Lotus japonicus* controls floral symmetry (Feng et al. 2006). Consistent with the incorporation of *CYC*-like genes into separate genetic programs promoting zygomorphy, there are differences in the details of gene function between the two divergent models. Further dissection of the *CYC* homolog pathways in both *Antirrhinum* and *Lotus* will provide insight into how evolution has co-opted members of this lineage for the production of convergent floral features.

As with the floral MADS-box genes, shifts in gene expression (Hileman et al. 2003, Citerne et al. 2006) and gene duplication events (Howarth and Donoghue 2006) have contributed to the role of *CYC* homologs in the evolution of floral morphology. What remains to be seen is how common the recruitment of *CYC*-like genes to function in zygomorphy has been across all angiosperms. In *Antirrhinum,* the *CYC* genetic pathway acts in parallel with the floral organ identity pathway (reviewed in Kramer 2005). This means that, for

instance, in the petal whorl, it is solely the differential expression of *CYC/DICH* that promotes the distinct dorsal, lateral, and ventral petal types; there is no variation in B gene expression. Work with several monocots, however, seems to suggest that the establishment of zygomorphy in these taxa may occur upstream of the floral organ identity genes. This is indicated by the fact that B and C gene homologs show bilateral symmetry in their expression patterns in the zygomorphic flowers of orchids and rice (Tsai et al. 2004, Yamaguchi et al. 2006), something that has not been observed in the core eudicots. These results do not necessarily mean that *CYC*-like genes are not playing any role, just that whatever pathway controls zygomorphy in these taxa is integrated into the floral developmental program differently in monocots and eudicots. Overall, *CYC* homologs stand as a classic example of the smallness of the genetic toolbox and the tendency of evolution to reuse genetic mechanisms that are conveniently at hand.

Moving beyond the candidate gene approach

As discussed above, there are many aspects of floral diversity for which there are no good candidate loci. These include features that are present in models such as *Arabidopsis* but have so far been recalcitrant to genetic analysis (e.g., control of floral merosity or phyllotaxy), as well as those that simply are not represented in current model species. In the former case, continued work with existing model species will ultimately provide the necessary information. In the latter case, however, researchers can never understand the genetic basis of the development and evolution of, for instance, nectar spurs or fleshy fruits in taxa that simply do not possess these structures. Luckily, the ever-growing set of genetic and genomic tools, combined with the decreasing cost of DNA sequencing, is allowing the rapid development of new model genetic systems. These include many members of the core eudicots, such as *Mimulus, Gerbera, Helianthus* (sunflower), and *Populus* (poplar), as well as the lower eudicots *Aquilegia* and *Papaver* (poppy) and the monocots *Phalaenopsis* (an orchid) and *Setaria* (foxtail millet). Several of these taxa, most notably *Mimulus, Helianthus,* and *Aquilegia,* represent recent adaptive radiations that may help researchers understand the genetic changes underlying such evolutionary processes (Rieseberg et al. 1995, Hodges 1997, Bradshaw et al. 1998).

While it is true that candidate gene–based research is driving much of the current work in the emerging models, there are alternatives that can identify novel loci. One of these, called quantitative trait locus (QTL) analysis, involves the characterization of genes that are responsible for natural morphological differences between populations or species (Ehrenreich and Purugganan 2006). This technique has allowed the identification of genomic regions controlling diverse traits such as nectar volume (Galliot et al. 2006); the production of closed, self-fertilizing flowers (Fishman et al. 2002); and nectar spur length (Hodges et al. 2002). The ultimate goal of these studies is to identify the specific gene associated with a given QTL. Although this requires a large amount of genomic information, it has been achieved in established models such as *Arabidopsis* and *Z. mays* (Doebley 2004, Ehrenreich and Purugganan 2006) and is becoming more feasible in the new models. In addition, many researchers are taking advantage of the floral diversity that exists within the close phylogenetic vicinity of existing genetic models. For instance, major loci responsible for shifts in floral color among relatives of *Antirrhinum* have been identified using hybrid crosses between the naturally occurring species and known mutants of *Antirrhinum* (Schwinn et al. 2006, Whibley et al. 2006).

As a complement to QTL studies, traditional molecular genetic techniques such as mutagenesis screens and comparative expression using microarrays hold great promise for the identification of novel loci controlling unique morphological features. For basal angiosperms, which are often woody, long-lived, and recalcitrant to genetic approaches, sequencing of complementary DNA libraries to yield expressed sequence tags (ESTs) is proving to be another useful means of identifying candidate gene homologs (Albert et al. 2005). Ultimately, these approaches can be paired with functional studies using stable transformation or transient knockdowns of gene function (e.g., virus-induced gene silencing; Burch-Smith et al. 2004) to achieve a better understanding of the genetic basis for floral diversification.

Conclusions

The diversity in floral morphology is likely to have been facilitated by the existence of multiple parallel genetic pathways that can evolve independently of one another. These modular systems include the genetic programs controlling floral organ identity, floral symmetry, organ polarity, phyllotaxy, and merosity (Kramer 2005). The current focus of evo-devo studies on floral organ identity and floral symmetry will certainly expand dramatically in coming years as model species provide more information on the genetic control of additional programs. One point that requires particular attention is the question of how the range of floral organ morphology is generated downstream of the otherwise highly conserved ABCE program. Current data highlight the importance of gene duplication as a source of variation in aspects of the floral program, while shifts in gene expression appear to be a major factor in morphological evolution. We have every reason to believe that these trends will hold for other pathways, as they do for MADS-box genes and *CYC*-like genes. As a complement to these candidate gene–based approaches, QTL and EST-driven studies have the potential to provide new insights into the development of unique morphologies that are not represented in the major model systems. Through this combination of approaches, research in floral evo-devo is seeking to elucidate the genetic basis for the enormous diversity in floral morphology and to understand how this variation has contributed to the radiation of the angiosperms.

Acknowledgments

I would like to thank the three anonymous reviewers for their suggestions. Some of the work discussed here was supported by NSF grant IBN-0319103.

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doi:10.1641/B570605
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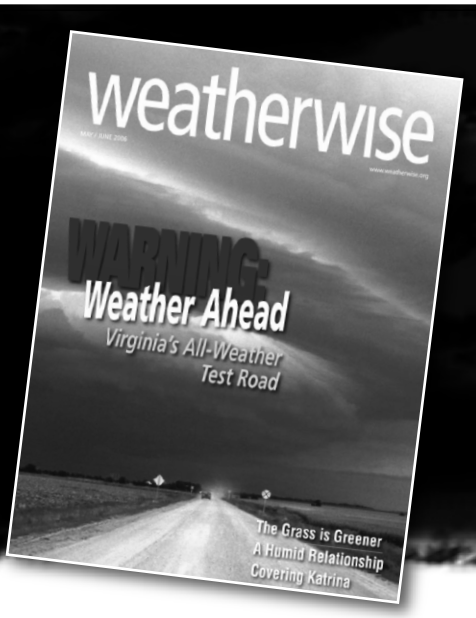

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