Integrative and Comparative Biology

Integrative and Comparative Biology, volume 57, number 6, pp. 1312–1321
doi:10.1093/icb/icx067

Society for Integrative and Comparative Biology

SYMPOSIUM

Changing MADS-Box Transcription Factor Protein–Protein Interactions as a Mechanism for Generating Floral Morphological Diversity

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From the symposium "Physical and Genetic Mechanisms for Evolutionary Novelty" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

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Synopsis Flowers display fantastic morphological diversity. Despite extreme variability in form, floral organ identity is specified by a core set of deeply conserved proteins—the floral MADS-box transcription factors. This indicates that while core gene function has been maintained, MADS-box transcription factors have evolved to regulate different downstream genes. Thus, the evolution of gene regulation downstream of the MADS-box transcription factors is likely central to the evolution of floral form. Gene regulation is determined by the combination of transcriptional regulators present at a particular *cis*-regulatory element at a particular time. Therefore, the interactions between transcription factors can be of profound importance in determining patterns of gene regulation. Here, after a short primer on flowers and floral morphology, I discuss the centrality of protein–protein interactions to MADS-box transcription factor function, and review the evidence that the evolution of MADS-box protein–protein interactions is a key driver in the evolution of gene regulation downstream of the MADS-box genes.

Floral diversity: variation within and between whorls

The flower is a collection of serial leaf homologs, specialized for sexual reproduction through pollination. Flowers are immensely diverse, but the overwhelming majority of flowers are comprised of some combination of four basic organ types. The canonical flower, represented in Fig. 1 by the dominant genetic model *Arabidopsis thaliana*, is composed of sepals, petals, stamens (male reproductive organs), and carpels (female reproductive organs). In most eudicots (like *A. thaliana*), and in most monocots (like the grasses), these four organ types are arranged in sequential whorls from the outside to the inside of the flower. The sepal and petal whorls together are termed the perianth (reviewed in Specht and Bartlett 2009).

Even in grasses like *Zea mays* (maize or corn), where floral morphology is highly derived, and specialized for wind pollination, the flower is composed of essentially the same organs. The outermost organs in grasses, which may or may not be homologous to sepals, are termed the palea and the lemma. The next whorl is composed of scale-like organs—the lodicules—that swell and force the flower open to allow cross-pollination (reviewed in Kellogg 2015; Schrager-Lavelle et al. 2017). Although lodicules are functionally and morphologically distinct from petals, genetic mutants and studies of gene expression show that petals and lodicules are clearly homologous (Ambrose et al. 2000; Nagasawa et al. 2003; Whipple et al. 2007; Bartlett et al. 2015). The stamens and carpels of grasses are even more clearly homologous to the stamens and carpels of eudicots. Although all four floral whorls initiate, maize flowers are unisexual at maturity (reviewed in Schrager-Lavelle et al. 2017). Therefore, the carpel whorl is no longer visible in the mature male maize flowers shown in Fig. 1.

These two distantly related extremes—*A. thaliana* and maize—illustrate that floral diversity is largely about variation on a theme. Most floral diversification, especially within families and genera, is because of variation in the number, size, shape, color, and arrangement of the four canonical organ types (Endress 1992).

Advance Access publication July 8, 2017

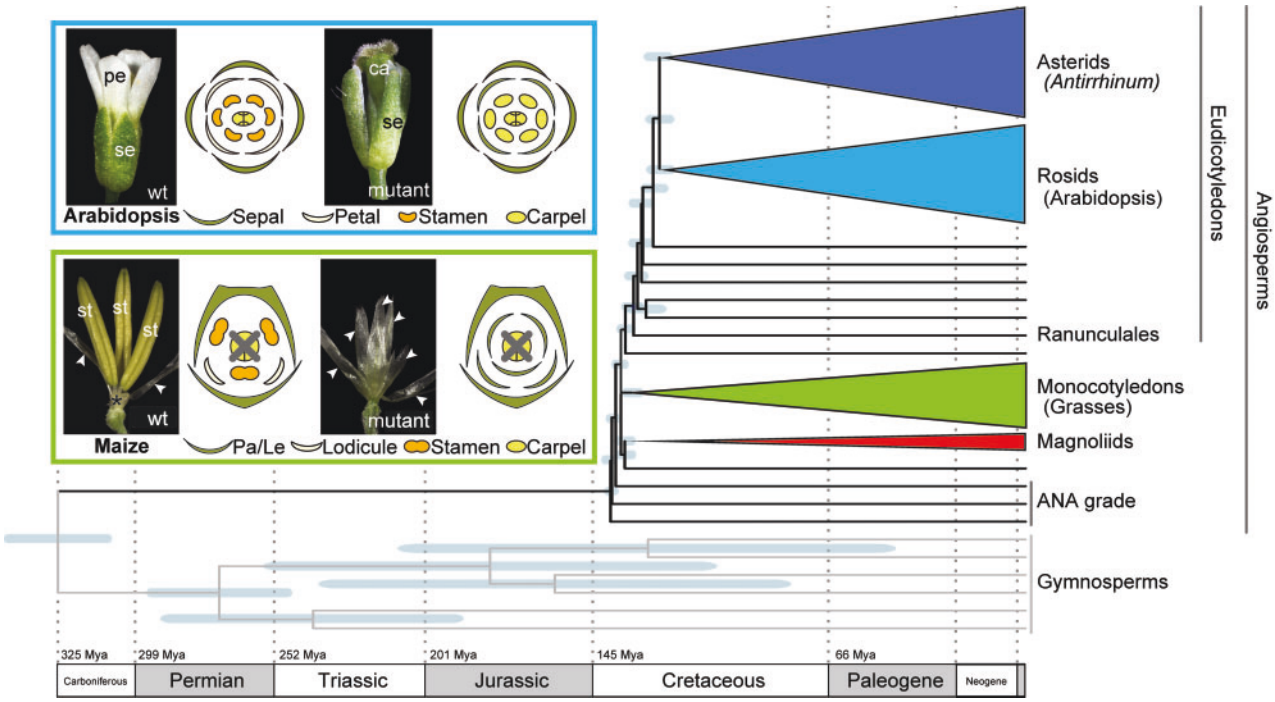

Fig. 1 The major lineages of flowering plants and the timing of flowering plant diversification. The topology shown, including dating and error estimates (shaded bars at nodes), is redrawn from Magallon et al. (2015). The "ANA grade" represents the orders Amborellales, Nymphaeales, and Austrobaileyales. Shown in the boxes to the right are representative flowers and MADS-box mutants from *A. thaliana* (upper box) and *Z. mays* (maize, lower box). Mutations in B-class MADS box genes in *A. thaliana* and maize result in the homeotic replacement of second whorl organs (petals in *A. thaliana*, lodicules in maize) in both species. ca = carpel; pa/le = palea/lemma; se = sepal; st = stamen. Arrowheads mark palea/lemma, asterisk marks a lodicule. Flower images are from Bartlett et al. (2015).

Studies of genetic mutants in *A. thaliana* and maize illustrate that the genes that control floral organ development are also deeply conserved. Mutations in orthologous transcription factor genes result in similar phenotypes in both species. For example, mutations in the MADS-box transcription factor genes *PISTILLATA* (*PI*) and *SUPERWOMAN1* result in the homeotic conversion of second whorl organs (petals or lodicules) and stamens in both *A. thaliana* and rice, respectively (Goto and Meyerowitz 1994; Krizek and Meyerowitz 1996; Nagasawa et al. 2003). Similarly, mutations in one of the *PI* orthologs in maize, *sterile tassel silky ear1 (sts1)* results in disrupted lodicule and stamen development (Fig. 1) (Bartlett et al. 2015). MADS-box gene mutants such as *pi*, both in *A. thaliana* and another eudicot, *Antirrhinum majus* (snapdragon), were used to derive the ABC model of floral development, which has proved to be a powerful framework for understanding the development and evolution of flowers (Krizek and Fletcher 2005; Thomson et al. 2017).

MADS-box proteins and the ABC model of floral development

The gene regulatory network that controls flower development is complex, and still incompletely understood, even in *A. thaliana* (Wellmer et al. 2014). However, the ABC model of flower development and its descendants offers an elegant framework to use to dissect this complexity. Discussing the full gene regulatory network that controls flower development is beyond the scope of this article, but the reader is directed to a number of excellent, recent reviews (Ó'Maoiléidigh et al. 2014; Thomson et al. 2017; Wils and Kaufmann 2017). In the original ABC model of floral development, based on the study of homeotic mutants, A-class transcription factor genes confer sepal identity, A- and B-class genes together confer petal identity, B- and C-class genes together confer stamen identity, and the C-class genes confer carpel identity. *PISTILLATA* and its orthologs all encode B-class MADS-box transcription factors. Later, the D- and E-class genes were identified in *A. thaliana*. The D-class genes specify ovule identity, while the E-class genes are required for floral organ identity in all four whorls. All of the ABC(DE) homeotic selector genes encode transcription factors, and all except for the A-class AP2-like genes encode MADS-box transcription factors (reviewed in Krizek and Fletcher 2005). A recent revision of the model incorporates evidence that A- and E-class genes regulate the transition to producing reproductive organs, and

groups C- and D-class function together in specifying female organ identity (Theissen et al. 2016).

The power and utility of the ABC(DE) model for evo-devo researchers is derived from the deep conservation of core regulators—particularly B- and C-class genes—across the flowering plants (e.g., Dreni et al. 2011; Wang et al. 2015; Otani et al. 2016) (earlier work reviewed in Di Stilio (2011) and Ferrario et al. (2004)). Even in the grasses, where organ homologies have been the subject of debate, B-class genes control inner whorl perianth (petals in the eudicots, lodicules in the grasses) and stamen development in a manner largely consistent with the established ABC(DE) model (Ambrose et al. 2000; Nagasawa et al. 2003; Bartlett et al. 2015). Similarly, compromised C-class genes result in compromised stamen and carpel identity in both rice and maize flowers (Mena et al. 1995; Yamaguchi et al. 2006; Dreni et al. 2011). This deep conservation of B- and C-class MADS-box gene function in morphologically divergent flowers indicates that regulatory evolution, downstream of the MADS-box transcription factors, has been instrumental in the divergence of floral morphology. Although *cis*-regulatory changes are no doubt important in the evolution of gene regulation downstream of the MADS-box genes, protein–protein interactions are critical for MADS-box transcription factor function. The diversification of MADS-box protein–protein interactions, in conjunction with *cis*-regulatory change, may have been a critical driver of the evolution of gene regulation downstream of the ABCDE genes.

Protein–protein interactions and MADS-box function

Protein–protein interactions are key for MADS-box protein function. MADS-box transcription factors must dimerize to bind DNA, and floral MADS-box proteins may function *in vivo* as part of tetramers or "floral quartets" (dimers of DNA-binding dimers) (Pellegrini et al. 1995; Theissen and Saedler 2001). The floral quartet model provides a mechanistic explanation for the ABCDE model, and proposes that the composition of MADS-box protein tetramers is instructive in determining floral organ identity (Fig. 2). For example, tetramers (floral quartets) of B-, C-, and E-class proteins may function to specify stamen identity, while tetramers of C- and E-class proteins may specify carpel identity (Theissen and Saedler 2001; Theissen et al. 2016). The floral quartet model provides an appealing explanation for why floral organs are homeotically replaced in single gene mutants, while single genes are necessary, but not sufficient to convert leaves into floral organs. In other words, while the petals and stamens of B-class mutants are homeotically transformed into sepals and carpels, respectively, the overexpression of just B-class genes is insufficient to turn leaves into petals or stamens. C-, B-, and E-class genes must all be overexpressed in leaves to convert them into stamen-like organs (Honma and Goto 2001). A growing body of evidence supports the floral quartet model, although the overwhelming majority of functional data is from *A. thaliana* (reviewed in Theissen et al. 2016). Testing the functional significance of floral quartets outside of *A.thaliana* remains a critical goal in floral evo-devo. In most species, it is still unclear whether floral quartets assemble, even *in vitro*. This knowledge gap exists in part because the necessary experiments can be technically challenging, and often require tools only available in model systems. However, as more model systems are developed (Chang et al. 2016), and as game-changing tools like next-generation sequencing come on line, this has the capacity to change for the better.

The precise composition of a particular kind of floral quartet is predicted to influence gene regulation and organ morphology. For example, a BCE quartet might always specify stamen identity, but stamen morphology might be determined by which B-, C-, and/or E-class proteins make up a particular quartet (Fig. 2(B)). This has been proposed in orchids, where the precise identity of B- and E-class proteins in floral quartets may determine perianth organ morphology (see below, Mondragon-Palomino and Theissen 2008; Hsu et al. 2015). Indeed, MADS-box paralogs and protein–protein interaction network architecture is largely maintained following whole genome duplication events (Veron et al. 2007; Zhang et al. 2016). When new MADS-box proteins arise through gene and genome duplication, they tend to interact with the same complement of protein classes as their ancestors did (Veron et al. 2007). While completely new interaction networks and floral quartets have emerged, more often the same general classes of quartets are predicted to form following a duplication event. However, the precise identity of paralogous proteins in these quartets varies over space and time in floral development and evolution, perhaps contributing to the evolution of floral organ form (Veron et al. 2007; Zhang et al. 2016).

Changes in MADS-box protein–protein interactions, including potential changes in floral quartet assembly, can have significant effects on plant development and on protein function. For example, in *Thalictrum thalictroides* (Ranunculaceae, eudicot), disrupted protein–protein interactions between C- and E-class proteins provide an explanation for a C-class mutant phenotype, where stamens and carpels are homeotically replaced by perianth organs

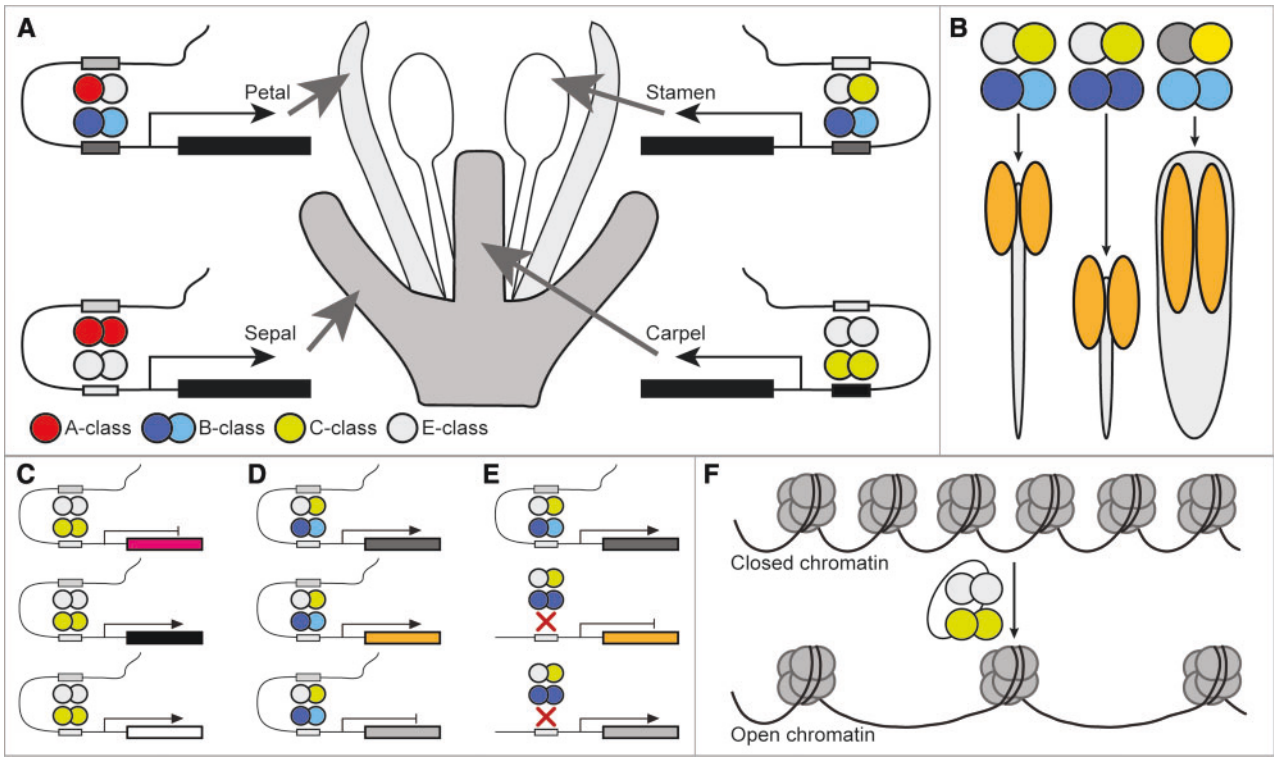

Fig. 2 MADS-box protein–protein interactions in flower development and evolution. (**A**) The floral quartet model proposes that tetramers of floral MADS-box proteins specify organ identity in *A. thaliana* flowers. Redrawn from Theissen and Saedler (2001). (**B**) Variation in the precise identity of proteins in quartets might impact organ morphology within an organ series. For example, variation in which B-, C-, and E-class proteins make up a floral quartet might modify stamen morphology. (**C** and **D**) The composition of floral quartets may directly impact the set of genes turned on and off in the specification of organ identity. (**E**) Transcription factor protein–protein interactions might negatively regulate gene expression by sequestering proteins in inactive complexes that cannot bind DNA (indicated with a red cross), resulting in altered gene expression patterns. (**F**) MADS-box proteins interact with chromatin remodeling factors, potentially allowing effector transcription factors access to DNA in the development of organ identity.

(Galimba et al. 2012). In *Eschscholzia californica* (California poppy, Ranunculaceae, eudicot), a B-class mutant phenotype may be explained by differences in protein–protein interactions between B-class, and C- and E-class proteins (Smaczniak et al. 2012). In snapdragon (*A. majus*), the C-class MADS-box proteins PLENA and FARINELLI specify stamen and carpel development, with PLENA playing a larger role (Davies et al. 1999; Causier et al. 2005). This divergence in function may in part be due to an amino acid insertion in FARINELLI that prevents it from interacting with certain E-class proteins (Airoldi et al. 2010). In the monocot order Poales, B-class MADS-box proteins from maize (Poaceae) and from a non-grass relative (*Joinvillea ascendens*, Joinvilleacae) differ in their protein–protein interaction profiles, and in their effects on floral development when expressed in *A. thaliana*. The B-class protein from maize (STS1) cannot bind DNA as part of a homodimer, while its *J. ascendens* ortholog can homodimerize and bind DNA. When ectopically expressed in *A. thaliana* sepals, the *J. ascendens* protein caused the complete transformation of sepals into petals, while the maize protein had much milder effects on floral form. Given that the predicted DNA-binding domains of the *J. ascendens* and maize proteins are identical, differences in protein–protein interactions offer a plausible explanation for the observed differences in protein function in *A. thaliana* (Bartlett et al. 2016). These data indicate that shifting MADS-box protein–protein interactions can have profound effects on protein function and, in turn, on floral morphology.

MADS-box protein–protein interactions and morphological evolution

MADS-box protein–protein interactions have been invoked as major players in a number of key macroevolutionary events in angiosperm history, including in the origin of the flower itself. The closest relatives of the flowering plants, the gymnosperms (Fig. 1), have separate male and female "cones"—branching systems that produce pollen and ovules,

respectively. A key angiosperm innovation was the production of the bisexual flower—a branching system that bears male and female reproductive structures on the same axis. The evolution of MADS-box protein–protein interactions has been hypothesized as central in the evolution of the bisexual flower (Wang et al. 2010; Ruelens et al. 2017). While the gymnosperms have B-, C- and E-class MADS-box proteins, B- and C-class proteins can interact directly to form quartets (Wang et al. 2010). This is in contrast to the angiosperms, where B- and C-class proteins only form quartets in conjunction with E-class proteins (Theissen and Saedler 2001; Smaczniak et al. 2012). Indeed, ancestral sequence resurrection indicates that BCE quartets evolved along the lineage leading to the flowering plants (Ruelens et al. 2017). The evolution of novel BCE quartets, coupled to MADS-box gene expression variation along the floral axis, may have been instrumental in the evolution of the bisexual angiosperm flower (Wang et al. 2010; Ruelens et al. 2017).

Changing B-class MADS-box protein–protein interactions have also been connected to later macroevolutionary shifts in floral morphology. In particular, the evolution of B-class protein–protein interactions has been connected to the evolution of the characteristic organization of the eudicot flower, and the canalization of floral development (Lenser et al. 2009; Melzer et al. 2014; Winter et al. 2002). The *A. thaliana* B-class proteins APETALA3 and PISTILLATA bind DNA as obligate heterodimers with one-another (Riechmann et al. 1996). This obligate heterodimerization is unusual for floral MADS-box proteins, most of which can bind DNA both as homodimers and heterodimers with other MADS-box proteins (de Folter et al. 2005). Coupled to obligate heterodimerization, AP3 and PI also upregulate their own expression in an autoregulatory feedback loop (Hill et al. 1998). The obligate heterodimerization of AP3 and PI, in conjunction with positive autoregulation, may confer developmental robustness. Specifically, modeling experiments show that obligate B-class heterodimers need fewer activatory molecules than homodimers to turn on and to remain on. In addition, B-class proteins that can both hetero- and homodimerize are more likely to activate the B-class program in the wrong place and/or time than obligate heterodimers (Lenser et al. 2009). While B-class dimerization patterns (homodimerization vs. obligate heterodimerization) can be relatively labile in early-diverging lineages and in the monocots, obligate heterodimerization is almost universal in the core eudicots, where flower development is extremely canalized (Melzer et al. 2014). Organs of mixed identity are rare in wild type eudicot flowers, and this may be because B-class protein–protein interactions ensure that the B-class program is very rarely activated in the wrong time or place. The evolution of obligate B-class heterodimerization and autoregulation may have contributed to the precise organization of eudicot flowers into discrete organ classes (Lenser et al. 2009).

Although intriguing, not all the available data supports the hypothesis that obligate B-class heterodimerization is instrumental in the canalization of floral development. In the grasses, floral development is also highly canalized, and there are strict boundaries between floral organ identities (reviewed in Kellogg 2015). However, B-class dimerization is relatively labile in the grasses and their relatives (Bartlett et al. 2016). In addition, structural analyses have revealed that the interaction surfaces of MADS-box proteins are poorly conserved, and highly sensitive to sequence variation, allowing for particularly rapid evolution of protein–protein interactions within the protein family (Silva et al. 2016). More fine-grained assessment of MADS-box protein–protein interactions might reveal far more lability in all plant lineages, including the eudicots, than has been uncovered thus far. To this point, most investigations into variation in MADS-box protein–protein interactions have been at very large evolutionary scales—at the level of orders, or entire angiosperm clades (e.g., Winter et al. 2002; Melzer et al. 2014). Given their capacity for rapid evolution, more investigations into variation in MADS-box protein–protein interactions at smaller taxonomic scales—within species, genera, or families—is certainly warranted, and will likely provide a rich dataset to explore in functional studies of MADS-box protein function. Even if the correlation between obligate B-class heterodimerization and canalized eudicot floral development is not causative, the Lenser et al. (2009) modeling experiments clearly illustrate how differential protein–protein interactions may contribute to the dynamics of organ identity specification. Variation in MADS-box protein–protein interactions could have profound effects on how, when, and where organ identity programs are activated.

MADS-box gene duplications, coupled to the evolution of protein–protein interactions, have been invoked to explain the evolution of the orchid flower (Mondragon-Palomino and Theissen 2008; Hsu et al. 2015). In many monocots there is often very little differentiation between the two perianth whorls. Thus, the perianth organs are often termed tepals in monocots. Orchids have three inner whorl tepals, one of which is highly specialized for mediating plant-pollinator interactions. This specialized tepal is called the labellum. Orchid labella are highly variable between species, and morphological specialization of the labellum is a

significant contributor to morphological diversification of orchid flowers more broadly (Endress 1994). While there is a single *PI*-like gene in orchids, there are multiple *AP3*-like gene lineages in all surveyed orchid genera (Mondragon-Palomino and Theissen 2008). The expression patterns of these *AP3*-like genes are correlated with tepal identity: the labellum versus the other tepals express distinct sets of *AP3*-like genes. These expression patterns suggest that the complement of B-class genes in an orchid tepal determines its identity as a labellum or as an unelaborated tepal (Mondragon-Palomino and Theissen 2008). Protein–protein interaction networks and RNAi knockdown experiments support and extend this hypothesis, and indicate that the composition of orchid MADS-box complexes may be a key determinant of orchid tepal identity. In particular, the precise identity of the AP3-like (B-class) and AGL6-like (potential orchid E-class) proteins in predicted B-class/E-class floral quartets may determine whether a perianth organ develops as a labellum or as an ordinary tepal (Hsu et al. 2015).

There is evidence that lability in MADS-box protein–protein interactions may have functional consequences in a number of deeply-divergent lineages. In the rosid genus *Medicago* (Fabaceae), variation in MADS-box protein–protein interaction strength is strongly correlated with the evolution of a novel fruit phenotype (Fourquin et al. 2013). In the early-diverging eudicot *T. thalictroides* (Ranunculaceae), changing MADS-box protein–protein interactions may be associated with the subfunctionalization of one C-class MADS-box protein to specifying ovule identity (Galimba and Di Stilio 2015). In oil palm (*Elaeis guinneensis*, Arecaceae, a monocot), a MADS box gene, *SHELL*, underlies the development of thick- versus thin-walled fruit, a character trait of critical importance in oil palm breeding. *SHELL* is a homolog of the D-class *A. thaliana* gene, *SEEDSTICK*. Two separate mutant *shell* alleles arose spontaneously, one of which has been traced to a single ancestral palm tree. Each of these alleles encodes a protein with a single amino acid change in the MADS domain, which mediates DNA binding and dimerization (a separate amino acid change in each allele, two residues apart from one-another). Both of these amino acid changes affect protein–protein interactions *in vitro*, providing a plausible explanation for how allelic variation in the *SHELL* gene might contribute to phenotypic variation in oil palm fruits (Singh et al. 2013).

In all of these examples, there is a correlation between variable protein–protein interactions and variation in fruit or flower form. However, directly connecting changing protein–protein interactions to changing gene function remains challenging. To directly test how protein–protein interactions might affect plant form, one needs both evolutionary variability in protein–protein interactions, and the ability to manipulate these interactions *in planta*. In the grasses, there is MADS-box protein–protein interaction variability, and some MADS-box protein–protein interactions can be manipulated (Bartlett et al. 2016). Coupled to the availability of multiple genetic model systems in the grass family (Chang et al. 2016), this opens the door for directly testing the connection between changing protein–protein interactions and changing plant form.

The mechanistic basis of protein–protein interaction-driven evolution

At a molecular level, there are a number of ways in which variation in MADS-box protein–protein interactions can affect protein function and downstream gene expression. One potential consequence of variable protein–protein interactions is that distinct protein complexes may be recruited to distinct *cis*-regulatory elements, and thus regulate distinct genes (Fig. 2(A)) (Lynch and Wagner 2008; Tuch et al. 2008). For example, a quartet of B-, C-, and E-class proteins might regulate the set of genes necessary for stamen development (Fig. 2(C)), while a quartet of C- and E-class proteins might regulate the set of genes necessary for carpel development (Fig. 2(D)) (Theissen and Saedler 2001; Theissen et al. 2016). The precise composition of a particular MADS-box protein complex might also have an impact on downstream gene regulation and, in turn, organ form. For example, BCE complexes may always specify stamen identity, but stamen morphology might be determined by the precise identity of the B-, C-, or E-class proteins in a particular complex (Fig. 2(B)). The primary models for understanding how variation in transcription factor protein–protein interactions might result in variation in gene expression propose that a new interaction might recruit a transcription factor (α) to the site of its new interactor (β). Over time, the interaction between α and β might become stronger, or a new binding site for α might evolve, modifying downstream gene expression (Lynch and Wagner 2008; Tuch et al. 2008). Indeed, transcriptional re-wiring of mating-type switching in yeast may have proceeded along a similar path (Baker et al. 2012).

Changing protein–protein interactions can also *directly* influence the DNA-binding specificity of transcription factors. For example, evolutionary modification of the interaction between LEAFY protein monomers, and possibly between LEAFY proteins and other interactors, has contributed to the evolution of new LFY binding specificities (Sayou et al. 2014; Silva et al. 2016). In maize, the bHLH transcription factors R and C interact in the regulation of anthocyanin

biosynthesis. Variable dimerization of R and C directly determines which DNA sequences R can bind, and thus which genes R activates (Kong et al. 2012). Similarly, variable protein–protein interactions can modify the DNA-binding specificities of Hox proteins in *Drosophila melanogaster* (Slattery et al. 2011). Protein–protein interactions can also increase the DNA-binding affinity of transcription factors to their binding sites, as has been shown for the *A. thaliana* E-class MADS-box protein SEP3 (Jetha et al. 2014). Thus, changing protein–protein interactions can have profound effects on the DNA-binding specificities of transcription factors, either by recruitment to the *cis*-regulatory elements bound by the new interactor, or by the direct modification of the protein/DNA interaction.

While there are some downstream genes uniquely regulated by each class of MADS-box proteins, there is extensive overlap between the genome-wide targets of floral MADS-box genes, and the floral MADS-box genes regulate each other in a complicated network (reviewed in Yan et al. 2016). In the *A. thaliana* C-class mutant *agamous* (*ag*), stamens and carpels are homeotically replaced with petals and sepals, respectively. Yet, comparisons of genome-wide MADS-box function between *ag* mutant and wild type flowers revealed surprisingly similar patterns of DNA binding in mutant versus wild type plants (Kaufmann et al. 2009). Alongside some uniquely regulated genes, quantitative differences in how genes are regulated, including quantitative levels of transcription factor binding at particular genomic locations, may be critical in determining organ identities (Kaufmann et al. 2009; Pajoro et al. 2014).

One way in which gene regulation might be affected in a quantitative manner is through transcription factors acting as negative regulators of other transcription factors through protein–protein interactions (Fig. 2(E)). This is illustrated most potently by the LITTLE ZIPPER (ZPR) proteins, which are thought to regulate the HD-ZIPIII proteins in shoot apical meristem and leaf development. The ZPR proteins are similar to HD-ZIPIII proteins, but lack DNA binding domains. The ZPR proteins interact with HD-ZIPIIIs, preventing the HD-ZIPIII proteins from dimerizing with other HD-ZIPIIIs, and reducing the concentration of active DNA-binding HD-ZIPIII dimers in the system (reviewed in Wenkel et al. 2007; Seo et al. 2011). Similar variability in active versus inactive transcription factor complexes, mediated through protein–protein interactions, has been proposed to underlie natural variation in leaf architecture in tomato (Kimura et al. 2008), and the regulation of flowering time in the grass *Brachypodium distachyon* (Qin et al. 2017). A system of post-translational regulation mediated by protein–protein interactions has been proposed for regulating the CUP-SHAPED COTYLEDON (CUC) proteins in *A. thaliana* and its relative *Cardamine hirsuta* (Rubio-Somoza et al. 2014). The CUC proteins are key regulators of leaf development, and their activity in the leaf margin results in the development of serrated and dissected leaves (Bilsborough et al. 2011). TCP transcription factors interact with CUC transcription factors, and early in development, the TCP–CUC interaction may prevent the CUC proteins from binding DNA and regulating their target genes. This early repression of CUC activity is proposed to result in juvenile leaves with smoother margins than adult leaves (Rubio-Somoza et al. 2014). An analogous mechanism may be acting in the tomato-relative *Physalis*, where the negative regulation of one MADS-box protein by another MADS-box protein may be because of protein–protein interactions (Zhao et al. 2013).

Similarly, the production of active versus inactive MADS-box protein complexes may regulate temperature-dependent flowering in *A. thaliana*. The *A. thaliana* MADS-box protein FLOWERING LOCUS M (FLM) represses flowering under cold ambient temperatures (Balasubramanian et al. 2006). *FLM* has multiple splice variants that vary in concentration according to ambient temperature (Posé et al. 2013; Sureshkumar et al. 2016). The protein encoded by one variant, FLM-β, forms DNA-binding heterodimers with another MADS-box protein, SHORT VEGETATIVE PHASE (SVP), to repress the transcription of floral activators and delay flowering (Lee et al. 2013; Posé et al. 2013). A second variant, *FLM-*δ, encodes a protein that also forms heterodimers with SVP, but FLM-δ/SVP heterodimers do not bind DNA *in vitro*. *FLM-*β expression levels decrease with increasing temperatures, while *FLM-*δ expression increases with increasing temperatures (Posé et al. 2013). In addition, SVP is degraded at warmer temperatures (Lee et al. 2013). These data have been integrated into a model where, at warmer temperatures, the concentration of active FLM-β/SVP complexes is reduced in favor of inactive FLM-δ/SVP complexes, resulting in accelerated flowering (Posé et al. 2013). While variation in *FLM-*β transcript levels is associated with natural variation in flowering time in *A. thaliana* (Lutz et al. 2017; Lutz et al. 2015), the *FLM-*δ transcript is one of many alternative *FLM* transcripts produced at warmer temperatures that may not be biologically active, and are degraded by nonsense mediated decay (Sureshkumar et al. 2016). Although the attractive model based on active versus inactive MADS-complexes may not be as critical as alternative splicing and nonsense mediated decay in the regulation of flowering, dosage-dependent regulation of flowering by MADS-complexes is emerging as a common theme (Sheldon et al. 1999; Lee et al. 2013; Posé et al. 2013; Rosloski et al. 2013; Airoldi et al. 2015;

Lutz et al. 2015, 2017). It is thus conceivable that intraspecific variation in MADS-box protein–protein interactions could affect variation in flowering time.

MADS-box proteins do not only interact with other MADS-box proteins. Dissection of MADS-containing protein complexes has revealed that MADS-box proteins can be found associated both with other transcription factor classes and with chromatin remodeling factors (Smaczniak et al. 2012). These results are supported by MADS-box ChIP-Seq data, where, for example, the *A. thaliana* E-class protein SEPALLATA3 is found associated with binding sites for other transcription factor classes (Kaufmann et al. 2009). In addition, analysis of genome-wide DNA-binding by the A-class protein AP1 and the E-class protein SEP3 has revealed that binding of AP1 and SEP3 is preceded by a marked increase in chromatin accessibility (Pajoro et al. 2014). These data have been integrated into a model for MADS-box function where floral quartets recruit chromatin remodeling factors and, in turn, "effector transcription factors" that go on to realize organ identity (Fig. 2(F)). In the absence of effector transcription factors, transcriptional corepressor complexes might be engaged, thus keeping transcription turned off (Yan et al. 2016). Thus, the protein–protein interaction network of MADS-box proteins extends beyond transcription factors, and may be critical in reshaping downstream gene regulation through modifying chromatin accessibility.

Conclusions

Variation in MADS-box protein–protein interactions may be a potent driver of floral developmental evolution and provides a mechanism for linking floral development and morphological evolution. While much progress has been made, MADS-box function in non-model systems remains mysterious. Even within *A. thaliana*, the mechanisms by which the MADS-box transcription factors act as developmental switches in the specification of organ identity are still unclear. Moving forward, more fine-grained analyses of MADS-box protein–protein interaction networks in additional families and orders of plants have the potential to reveal not only how floral morphological diversity arose, but also how transcription factor evolution contributes to the evolution of morphology more broadly.

Acknowledgments

I would like to thank three anonymous reviewers for suggestions that helped improve the manuscript considerably. I would also like to apologize to those authors whose work I have inadvertently omitted or could not review at length due to space limitations.

Funding

This work was supported by National Science Foundation [IOS-1652380].

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