American Journal of Botany

American Journal of Botany 98(3): 397–403. 2011.

Reduce, reuse, and recycle: Developmental evolution of trait diversification[1]

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A major focus of evolutionary developmental (evo-devo) studies is to determine the genetic basis of variation in organismal form and function, both of which are fundamental to biological diversification. Pioneering work on metazoan and flowering plant systems has revealed conserved sets of genes that underlie the bauplan of organisms derived from a common ancestor. However, the extent to which variation in the developmental genetic toolkit mirrors variation at the phenotypic level is an active area of research. Here we explore evidence from the angiosperm evo-devo literature supporting the frugal use of genes and genetic pathways in the evolution of developmental patterning. In particular, these examples highlight the importance of genetic pleiotropy in different developmental modules, thus reducing the number of genes required in growth and development, and the reuse of particular genes in the parallel evolution of ecologically important traits.

Key words: *CRABS CLAW*; *CYCLOIDEA*; evo-devo; *FRUITFULL*; independent recruitment; KNOX1; parallelism; trait evolution.

Organisms show remarkable variation in phenotypic form and function, both of which are fundamental to biological diversification. A major focus of evolutionary developmental biology or "evo-devo" is to determine how and to what extent this phenotypic variation is reflected at the level of underlying developmental genetic pathways. Over recent years, tremendous advances in molecular phylogenetics have greatly facilitated evo-devo studies by allowing more precise reconstruction of character evolution, thus fostering identification of similar traits that are either derived from a common ancestor (homologs) or have evolved multiple times independently (analogs). For example, floral bilateral symmetry, an ecologically important trait related to plant pollination syndromes, evolved once and was lost multiple times within the plantain family (Plantaginaceae) (Reeves and Olmstead, 1998), whereas dehiscing fruits and branched trichomes evolved several times independently within the mustard family (Brassicaceae) (Beilstein et al., 2006). These different patterns of evolutionary lability likely reflect differences in selection and genetic constraint (reviewed in Langlade et al., 2005; Brakefield, 2006). In this review, we explore the basis of angiosperm (flowering plant) biodiversity in terms of the evolution of broadly conserved developmental genes, and attempt to discern whether phenotypic diversity is mirrored at the genetic level. Although we will focus on a few important angiosperm evo-devo studies, similar research is being done in metazoan animals and nonflowering plants (e.g., Rensing et al., 2008; Sakakibara et al., 2008; reviewed in Cañestro et al., 2007).

The developmental genetic toolkit—Ever since pioneering work in the 1990s on angiosperm MADS-box genes, it has been clear that distantly related organisms share a common set of conserved genes—often referred to as the developmental genetic toolkit—which have been repeatedly modified over evolutionary time to affect trait diversification (e.g., McGinnis et al., 1984; Scott and Weiner, 1984; Utset et al., 1987; Duboule and Dollé, 1989; Coen and Meyerowitz, 1991; Purugganan et al., 1995; reviewed in Carroll et al., 2005; Degnan et al., 2009). Indeed, despite evidence for extensive gene/genome duplications in different lineages that can expand the genetic toolkit (e.g., Tang et al., 2008, 2010), recent comparative genomic studies suggest that the generation of completely novel genes is rare (AGI, 2000; IRGSP, 2005; Paterson et al., 2009). Thus, the evolution of form predominantly occurs through the modification or co-option of existing genetic pathways to different or additional features, rather than to the de novo synthesis of genes and genetic pathways.

Modification of genetic pathways can occur through mutations within the protein-coding or *cis*-regulatory sequences (e.g., promoters) of genes, resulting in biochemical or developmental function diversification. For example, during Arabidopsis [*Arabidopsis thaliana* (L.) Heynh., Brassicaceae] and tomato (*Solanum lycopersicon* L., Solanaceae) development, the paralogous genes (i.e., derived from a duplication event), *TERMINAL FLOWER1/SELF PRUNING* (*TFL1/SP*) and *FLOWERING LOCUS T/SINGLE FLOWER TRUSS* (*FT/SFT*), are expressed concurrently in the same tissues but have antagonistic functions. Whereas *TFL1/SP* stimulates growth and development of apical meristems and leaf primordia, *FT/SFT* retards growth in these tissues, demonstrating that functional differences between these paralogs are due to differences in their amino acid sequences (Shannon and Meeks-Wagner, 1991; Ruiz-García et al., 1997; Samach et al., 2000; Wigge et al., 2005; Shalit et al., 2009). By

[1] Manuscript received 28 July 2010; revision accepted 22 November 2010.

The authors thank two anonymous reviewers for comments on a previous version of the manuscript. In situ hybridizations in *Schizanthus* sp. were carried out at the John Innes Centre in the laboratory of Enrico Coen. Their work is supported by grants from the Ministerio de Ciencia e Innovación (GEN2006-7788-E, BIO2008-00581 and CDS2007-00057 to P.C.), Fundación Ramón Areces, and the National Science Foundation (IOS-0616025 to L.C.H).

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doi:10.3732/ajb.1000279

contrast, expression of *APETALA3*-like (*AP3*-like) and *PISTILLATA*-like (*PI*-like) MADS-box genes in the second whorl of *Arabidopsis* flowers compared to the first and second whorls of tulip flowers (*Tulipa gesneriana* L., Liliaceae), likely underlies interspecific differences in first whorl morphology—sepals in *Arabidopsis* compared to petaloid tepals in tulip (Jack et al., 1992; Kanno et al., 2003). Since these genes are likely to function similarly to specify petal identity in the second whorl of both species, morphological differences in the first whorl are due to changes in the regulation of these orthologous genes (i.e., derived from speciation events), rather than to differences in their protein-coding regions.

The above examples highlight how flower developmental genetic pathways diversify through changes in gene regulation and protein function and the potential importance of gene duplication for developmental evolution. These mechanisms appear to underlie much of organismal diversification, but interestingly there does not seem to be an ever-expanding developmental genetic toolkit specifying novel and convergent phenotypes. Instead, growing evidence suggests extensive conservation through the *reduction, reutilization*, and *recycling* of developmental genetic programs. The following sections explore evidence for the reutilization of existing genetic pathways in different developmental modules (e.g., leaves and flowers) both within and between individuals, and the importance of independent cooption of the same genes or genetic pathways in the repeated evolution of similar traits.

Pleiotropy in inflorescence and flower development—One way to reduce the number of genetic programs required for phenotypic diversification is to reuse specific gene products in different protein complexes, thus altering their developmental functions in time and space within an individual. One of the best examples of this type of developmental pleiotropy is provided by the MADS-box transcription factors, including members of the well-studied *APETALA1/FRUITFULL* (*AP1/FUL*) subfamily that are restricted to the angiosperms (Litt and Irish, 2003).

Functional analyses across monocots and eudicots imply an ancestral function for *AP1/FUL* genes in meristem identity specification. However, the number and types of meristems in which these genes function have been altered following both duplication and speciation. *Arabidopsis* has three functionally characterized *AP1/FUL* genes—*AP1, FUL* and *CAULIFLOWER* (*CAL*)—derived from an ancient duplication event at the base of core eudicots and a more recent duplication event within Brassicaceae (Litt and Irish, 2003). In *Arabidopsis*, complexity of the developmental genetic toolkit is minimized through the recycling of *AP1* and *FUL* to specify different developmental trajectories (Fig. 1). During floral induction *FUL* is positively regulated in the shoot apical meristem (SAM), where its protein interacts with SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) to initiate the transition to inflorescence development (Hempel et al., 1997; Ferrándiz et al., 2000; Melzer et al., 2008). After the production of an inflorescence meristem, *AP1* and *CAL* are upregulated in emerging lateral meristems to specify floral identity (Irish and Sussex, 1990; Bowman et al., 1993; Kempin et al., 1995). This upregulation is achieved through the negative regulation of both *FUL* and another MADS-box gene *AGAMOUS* (*AG*), which is involved in floral organ production (Mandel and Yanofsky, 1995; Sridhar et al., 2006). The next phase of flower development is the production of floral organ primordia. At this stage *AP1* switches function from floral meristem to sepal/petal identity

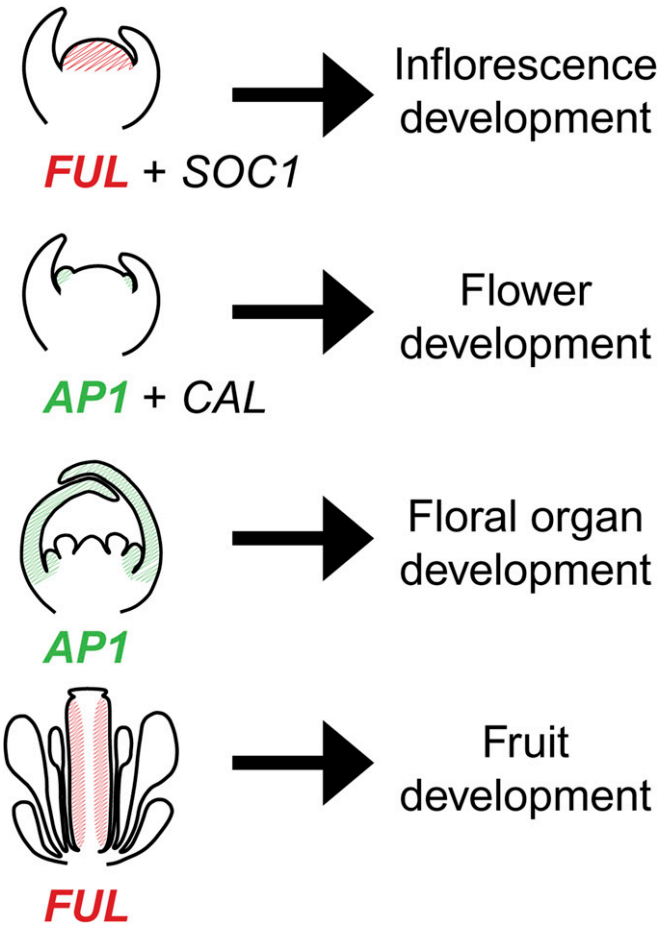

Fig. 1. Expression patterns of *Arabidopsis thaliana* (Brassicaceae) *APETALA1/FRUITFULL* (*AP1/FUL*) genes illustrating developmental pleiotropy and functional diversification following gene duplication. *FUL* (red) is expressed in the shoot apical meristem, where its protein product interacts with SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) to induce inflorescence development, and later in carpel valves to promote fruit elongation and differentiation. *AP1* (green) is expressed alongside its close paralog *CAULIFLOWER* (*CAL*) in floral meristems, where both genes specify floral meristem identity and later in sepals and petals to promote floral organ identity.

specification, partially through the downregulation of inflorescence meristem identity genes (e.g., *AGAMOUS-LIKE 24* [*AGL24*] and *SHORT VEGETATIVE PHASE* [*SVP*]) and concomitant upregulation of the floral organ identity gene *SEPALLATA3* (*SEP3*), the latter of which has been hypothesized to heterodimerize with AP1 in sepals and petals (Gregis et al., 2008, 2009; Liu et al., 2009; Kaufmann et al., 2010; Xu et al., 2010). Finally, following flower maturation, *FUL* is re-utilized in fruit production during which it controls elongation and differentiation of the carpel valve (Gu et al., 1998).

In addition to mediating flowering time, evidence suggests that *FUL* contributes to the annual herbaceous habit of *Arabidopsis*. Indeed, double *soc1:ful* mutants are very late flowering under long-day conditions and have characteristics of perennial plants, including secondary growth, inflorescence meristems that revert to vegetative meristems, and longer life cycles (Melzer et al., 2008). Because the perennial woody habit is likely ancestral to angiosperms, independent modifications in

the expression or function of *FUL*-like genes may be associated with repeated evolution of annual herbaceous taxa in different lineages (Melzer et al., 2008). Although this hypothesis awaits rigorous testing, these data suggest that the re-utilization of *FUL*-like genes plays a role in the independent evolution of the annual habit and that these genes are recycled during angiosperm ontogeny, both of which reduce the requirement of novel genes and genetic pathways.

Repeated evolution of floral bilateral symmetry—Within the angiosperms, bilaterally symmetrical (monosymmetric) flowers have evolved multiple times from radially symmetrical (polysymmetric) ancestors (Fig. 2), and these shifts in symmetry are strongly correlated with increased pollinator specialization and higher speciation rates (Donoghue et al., 1998; Ree and Donoghue, 1999; Endress, 2001; Sargent, 2004; Knapp, 2010). Recent studies in multiple flowering plant lineages have greatly advanced our understanding of how independent evolutionary transitions to bilateral flower symmetry is established at the developmental genetic level. Strikingly, these studies strongly support a common genetic basis for bilaterally symmetrical flowers, through parallel evolutionary shifts in class II TCP *CYCLOIDEA* (*CYC*)-like gene expression (reviewed in Preston and Hileman, 2009; Hileman and Cubas, 2009; Rosin and Kramer, 2009). In other words, flowering plants appear to reuse the same elements of the developmental genetic toolkit to establish independent transitions to bilateral flower symmetry.

The importance of *CYC*-like genes for specifying a dorsiventral floral axis was first identified in the model organism snapdragon (Fig. 2C, *Antirrhinum majus* L., Plantaginaceae). In snapdragon, floral bilateral symmetry is established through the differentiation of the dorsal, lateral, and ventral petals and stamens and is partly mediated by the action of two recently duplicated dorsal identity genes, *CYC* and *DICHOTOMA* (*DICH*) (Luo et al., 1996, 1999). Both genes are expressed in the dorsal region of the flower during early to late stages of development, with double *cyc:dich* mutant flowers being fully radially symmetrical due to the loss of dorsal identity (Luo et al., 1996, 1999). By contrast, in radially symmetrical flowers of the distantly related model species *Arabidopsis*, the *CYC/DICH* ortholog *TCP1* is expressed dorsally only in very early stages of flower development (Cubas et al., 2001). Thus, since class II TCP genes generally function as regulators of cell proliferation and expansion (Doebley et al., 1997; Crawford et al., 2004; reviewed in Preston and Hileman, 2009; Martín-Trillo and Cubas, 2010), the early dorsal expression of *TCP1* in *Arabidopsis* suggests that *CYC* and *DICH* were recruited for specifying dorsal identity in snapdragon flowers through temporal shifts in their expression, rather than modifications to their biochemical function (Cubas et al., 2001).

In addition to snapdragon, recruitment of *CYC*-like genes to establish dorsal identity in lineages with independently derived bilaterally symmetrical flowers has been functionally demonstrated for candytuft (Fig. 2D, *Iberis amara* L., Brassicaceae), and in the legumes pea and lotus (Fig. 2E, *Pisum sativum* L. and *Lotus japonicus* L., Leguminoseae) (Feng et al., 2006; Busch and Zachgo, 2007; Wang et al., 2008). Importantly, phylogenetic analyses support the independent evolution of floral bilateral symmetry in the Plantaginaceae, Brassicaceae, and Leguminoseae. As predicted, mutations in *CYC*-like genes of these species result in radially symmetrical flowers due to the loss of dorsal identity. Interestingly, whereas *CYC*-like genes promote increased petal size in snapdragon and legumes, orthologs in candytuft function to reduce petal size (Luo et al., 1996; Busch and Zachgo, 2007; Wang et al., 2008). In addition to functional studies, a general role for *CYC*-like genes in establishing independently derived floral bilateral symmetry is supported by asymmetric patterns of *CYC*-like gene expression in late stage bilaterally, but not radially, symmetrical flowers within Malpighiaceae (Zhang et al., 2010).

Independently derived patterns of stamen abortion—Intra- and interfloral variation in stamen number and length is common within the angiosperms (Fig. 3), and is thought to increase individual fitness by partitioning function between stamens (e.g., feeding stamens vs. pollinating stamens), maximizing pollen placement (e.g., dorsal staminodes vs. ventral stamens), and ensuring reproduction through a mixed mating strategy (e.g., didynamous stamens or dicliny) (Endress, 1999; Escaravage et al., 2001; Kalisz et al., 2006; Friedman and Barrett, 2009). In snapdragon, the dorsal stamen arrests early in development to leave a residual staminode, whereas the two lateral stamens develop to be shorter than the two ventral stamens. Similar to petals, the resulting bilateral symmetry in the stamen whorl is controlled by the action of *CYC*, which inhibits cell division along the dorsilateral axis (Luo et al., 1996, 1999; reviewed in Kalisz et al., 2006).

Recent evo-devo studies are starting to reveal a role for *CYC*-like gene expression evolution in interspecific patterns of stamen number variation (reviewed in Hileman and Cubas, 2009). First, in the close relative of snapdragon, desert ghostflower [*Mohavea confertiflora* (Benth.) A. Heller, Plantaginaceae], which has staminodes in both the dorsal and lateral positions,

Fig. 2. Independent recruitment of *CYC*-like genes in the evolution of flower bilateral symmetry across core eudicots. (A) Example of a radially symmetrical rosid flower from sulphur cinquefoil (*Potentilla recta* L., Rosaceae) with multiple lines of symmetry (dashed lines). (B) Illustration of a typical core eudicot flower with strong bilateral symmetry along the dorsiventral axis due partly to the action of CYC protein function in the dorsal region (zigzag). (C–E) Functional studies have shown that *CYC* was recruited multiple times in the independent origin of (C) snapdragon (*Antirrhinum majus* L.; Plantaginaceae; asterid I), (D) candytuft (*Iberis amara* L.; Brassicaceae; rosid II), and (E) pea (*Pisum sativum* L.; Leguminoseae; rosid I) flower bilateral symmetry.

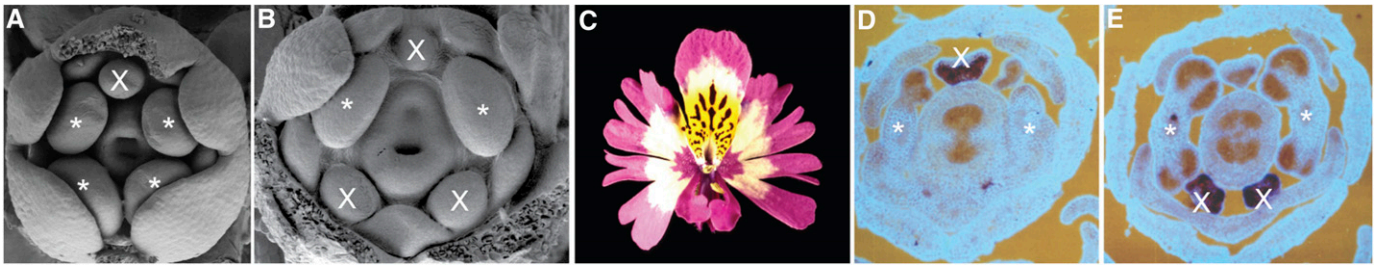

Fig. 3. Patterns of stamen abortion and *CYCLOIDEA* (*CYC*) expression in exemplary asterids. (A) Scanning electron micrograph (SEM) showing typical Plantaginaceae flower morphology in *Collinsia heterophylla* Buist ex Graham, with two ventral and two lateral stamens (*), and a single dorsal staminode (X). (B) SEM showing the divergent flower morphology of *Gratiola officinalis* L. (Plantaginaceae), with two lateral stamens and two ventral and one dorsal staminode. (C) *Schizanthus* Ruiz & Pav. flower showing strong dorsiventral bilateral symmetry. (D, E) Transverse section through a *Schizanthus* flower showing *CYC* expression in the (D) dorsal and (E) ventral staminodes.

there has been an expansion of *CYC*-like gene expression into the lateral region of the stamen whorl (Hileman et al., 2003). Second, in *Opithandra* B. L. Burtt (Gesnericaceae) and *Schizanthus* Ruiz & Pav. (Solanaceae) (Fig. 3C–E), *CYC*-like gene expression has expanded into the ventral region, correlating with derived patterns of stamen abortion in both the dorsal and ventral regions (Song et al., 2009). Third, the maize (*Zea mays* L., Poaceae) *CYC*-like homolog *TEOSINTE BRANCHED1* (*TB1*) is strongly expressed in the stamen whorl of female, but not male, florets correlating with patterns of stamen abortion (Hubbard et al., 2002). Although there are noteworthy exceptions to this correlation—including the lack of *CYC*-like expression in ventral staminodes of *Gratiola* L. and *Veronica* L. (Plantaginaceae) (Preston et al., 2009) (Fig. 3B)—these results suggest an important role for recurrent evolutionary shifts in *CYC*-like gene expression for the independent patterning of stamen development across angiosperms.

Parallel recruitment of KNOXI genes in leaf shape evolution—Angiosperm leaves show a great diversity in shape, largely resulting from differences in the number, arrangement, and shape of blades or blade units (leaflets) on the main leaf axis. Simple leaves (e.g., *Arabidopsis*, snapdragon and maize, Fig. 4A, B) are borne in one piece, can be either entire or serrated along the leaf margin, and are thought to be the ancestral angiosperm leaf type. By contrast, compound (complex or dissected) leaves (e.g., tomato and giant starfruit [*Averrhoa carambola* L., Oxalidaceae], Fig. 4C) are derived from several leaflets that dissect the leaf at the main axis. Across angiosperms, compound leaves have evolved independently multiple times (Bharathan et al., 2002; reviewed in Blein et al., 2010). Regardless of form—simple or compound—leaf initials emerge similarly from groups of differentiated cells that subtend a zone of uncommitted (indeterminate) cells within the SAM. Indeterminancy in the central zone of the SAM is maintained primarily by a group of related genes within the KNOX1 family of homeobox genes and their upstream regulators *CUP-SHAPED COTYLEDON* (*CUC*) and *CUC2* (Aida et al., 1999; Takada et al., 2001; Long et al., 1996; Williams, 1998; Hibara et al., 2003; reviewed in Langdale, 1994).

One of the best-characterized KNOX1 genes, *KNOTTED 1* (*KN1*), is expressed in both vegetative and floral meristematic cells of maize, but *KN1* transcripts are undetectable in regions of lateral organ formation (Smith et al., 1992; Jackson et al., 1994). Ectopic expression of *KN1* in simple maize leaves causes cell proliferation around the lateral leaf veins. Furthermore, constitutive expression of *KN1* causes ectopic shoot formation in simple leaves of tobacco (*Nicotiana tabacum* L., Solanaceae) and *Arabidopsis*. These data from maize, tobacco, and *Arabidopsis* suggest that expression of *KN1* homologs during the development of leaves may contribute to compound leaf development. This expression evolution may be due to changes in the *cis*-regulatory elements of *KN1* genes and/or to variation in their upstream regulators (e.g., *CUC1* and *CUC2*).

Comparative studies have revealed a role for differential regulation of *KN1* orthologs in the independent origins of simple and compound leaves across angiosperms, some Leguminoseae being notable exceptions (Champagne et al., 2007; Chen et al., 2010). In the simple leaf primordia of *Amborella* Baill. (Amborellaceae, basal dicot), grasses (Poaceae, monocot), and *Arabidopsis* (rosid), *KN1*-like gene expression is repressed throughout leaf development, whereas in the complex leaf primordia of *Lepidium perfoliatum* L. (Brassicaceae, rosid) and tomato (asterid) *KN1*-like transcripts are abundantly expressed (Bharathan et al., 2002). The picture emerging from studies focusing on the role of *KN1*-like genes in diverse angiosperms is that multiple shifts in *KN1*-like expression likely underlie parallel evolution of compound leaves. It is important to note that simple leaves can develop from complex primordia and vice versa and that *KN1*-like gene expression strongly correlates with young, but not necessarily adult, leaf morphology (Bharathan et al., 2002). This correlation between *KN1*-like gene expression and young leaf morphology highlights the importance of careful morphological work in evo-devo studies.

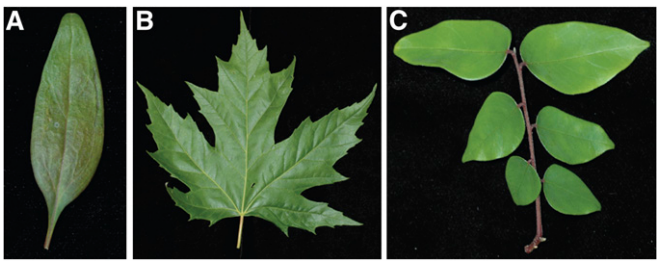

Fig. 4. Variation in angiosperm leaf complexity can largely be explained by evolution of *KN1* expression. (A) Simple snapdragon (*Antirrhinum majus* L., Plantaginaceae) leaf. (B) Simple dissected maple (*Acer* L., Sapindaceae) leaf. (C) Compound leaf of the giant starfruit (*Averrhoa carambola* L.; Oxalidaceae).

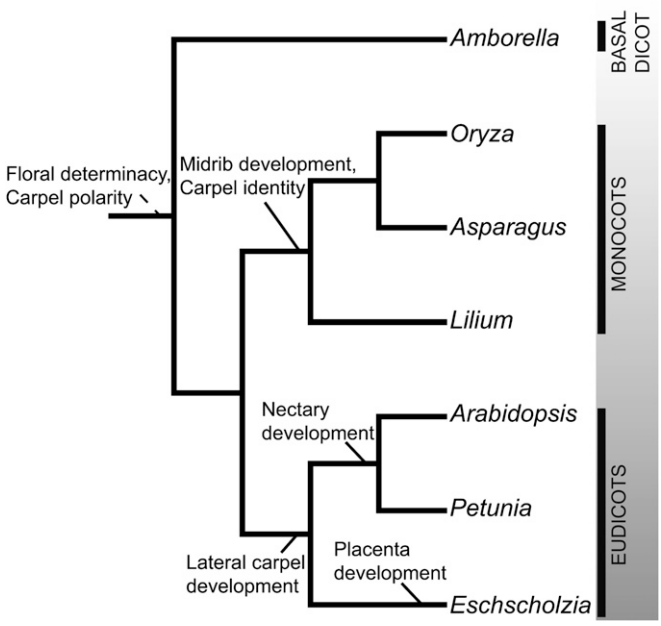

Fig. 5. Major hypotheses of *CRABS CLAW* (*CRC*) functional evolution across the angiosperms based on gene expression, interspecific genetic complementation tests, and mutant analyses (for references, see section *Reuse of CRABS CLAW (CRC) during angiosperm diversification*). Dotted line at the basal node represents uncertainty due to lack of functional analyses in *Amborella* Baill. and other basal dicots.

Reuse of CRABS CLAW (CRC) during angiosperm diversification—The independent recruitment of *CRC*-like YABBY family transcription factors in carpel, leaf, and nectary development exemplifies the frugal use of genes in plant evolution and development (Fig. 5). Indeed, not only is *CRC* used at different stages during ontogeny (i.e., within an individual), but it has also been recruited multiple times independently to perform similar functions in different lineages of angiosperms (Lee et al., 2005b; Orashakova et al., 2009). *CRC* was first identified through mutant screens of *Arabidopsis* plants that showed defects in carpel development (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). Mutants occasionally develop supernumerary carpels that are shorter and wider than normal and that are unfused at the apex. These mutant phenotypes suggest a role for *CRC* both in establishing floral determinancy and in carpel patterning (Alvarez and Smyth, 1999; Eshed et al., 1999). A second role of *Arabidopsis CRC* is in the development of floral nectaries that develop at the base of the stamens; nectaries are entirely absent in *crc* mutants (Bowman and Smyth, 1999). Simultaneous expression of *CRC* in nectaries and carpels is partly regulated by floral homeotic protein complexes that bind to the MADS-box binding site in the *CRC* promoter (Lee et al., 2005a). However, the fact that gynoecium- and nectary-specific *CRC* expression is regulated by different *cis*-regulatory elements suggests that these distinct functions may have evolved independently (Lee et al., 2005a).

Comparative expression and functional analyses in monocots, early-diverging dicots, and eudicots, support an ancestral role for *CRC*-like genes in floral meristem determinancy and carpel polarity differentiation of angiosperms (Fourquin et al., 2005, 2007; Nakayama et al., 2010) (Fig. 5). However, although CRC function in nectary development is likely ancestral to core eudicots, this function appears to have been recycled multiple times in developmentally distinct tissues. For example, petunia [*Petunia hybrida* (Hook.) Vilm., Solanaceae] nectaries are associated with the base of the ovary wall, and are therefore only serially homologous to nectaries of *Arabidopsis*. Regardless, the petunia *CRC* ortholog is strongly expressed in growing nectary tissue, and gene silencing causes loss of nectary development (Lee et al., 2005b). By contrast, *CRC*-like genes have not been demonstrated to function in nectary development in monocots. Instead, evidence from lily (*Lilium longiflorum* Thunb., Liliaceae) and rice (*Oryza sativa* L., Poaceae) suggests neo-functionalization of *CRC* in monocot leaf midrib development and carpel identity (Ishikawa et al., 2009; Wang et al., 2009) (Fig. 5). Thus, data suggest that *CRC*-like genes have been reutilized and neo-functionalized multiple times, independent of gene duplication, within the angiosperms.

Concluding remarks—The examples outlined above illustrate the frugal use of genes and genetic pathways in different developmental modules during the ontogeny of individual plants and during the evolutionary diversification of form and function—both of which are critical components of biodiversity. In the case of angiosperm *AP1/FUL* genes, recycling of gene function during ontogeny has occurred partly through gene duplication, allowing expansion of the genetic toolkit without the de novo synthesis of genes (Rosin and Kramer, 2009). Therefore, to reuse developmental genetic programs within ontogeny and over evolutionary diversification is not always to reduce total gene number. By contrast, developmental pleiotropy in *AP1/FUL* and *CRC* genes has fostered reduction in the genetic toolkit through the reuse of specific genes within different modules of an individual. Similarly, repeated diversification of *CYC*, *CRC*, *KN1*, and *AP1/FUL* genes has resulted in the independent evolution of flower bilateral symmetry, nectaries, compound leaves, and possibly the annual habit, across the angiosperms, respectively. These examples highlight the ability of evolutionary forces to reduce the number of tools within the genetic toolkit while simultaneously increasing biodiversity, and together resolve the apparent mismatch between diversity at the phenotypic and genetic levels.

LITERATURE CITED

AIDA, M., T. ISHIDA, AND M. TAKAKA. 1999. Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: Interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* 126: 1563–1570.

AGI [ARABIDOPSIS GENOME INITIATIVE]. 2000. Analysis of the genome of the flowering plant *Arabidopsis thaliana. Nature* 408: 796–815.

ALVAREZ, J., AND D. R. SMYTH. 1999. *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS. Development* 126: 2377–2386.

BEILSTEIN, M. A., I. A. AL-SHEHBAZ, AND E. A. KELLOGG. 2006. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607–619.

BHARATHAN, G., T. E. GOLIBER, C. MOORE, S. KESSLER, T. PHAM, AND N. R. SINHA. 2002. Homologies in leaf form inferred from *KNOXI* gene expression during development. *Nature* 296: 1858–1860.

BLEIN, T., A. HASSON, AND P. LAUFS. 2010. Leaf development: What it needs to be complex. *Current Opinion in Plant Biology* 13: 75–82.

BOWMAN, J. L., J. ALVAREZ, D. WEIGEL, E. M. MEYEROWITZ, AND D. R. SMYTH. 1993. Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* 119: 721–743.

BOWMAN, J. L., AND D. R. SMYTH. 1999. *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126: 2387–2396.

BRAKEFIELD, P. M. 2006. Evo-devo and constraints on selection. *Trends in Ecology & Evolution* 21: 362–368.

BUSCH, A., AND S. ZACHGO. 2007. Control of corolla monosymmetry in the Brassicaceae *Iberis amara. Proceedings of the National Academy of Sciences, USA* 104: 16714–16719.

CAÑESTRO, C., H. YOKOI, AND J. H. POSTLETHWAIT. 2007. Evolutionary developmental biology and genomics. *Nature Reviews Genetics* 8: 932–942.

CARROLL, S. B., J. K. GRENIER, AND S. D. WEATHERBEE. 2005. From DNA to diversity: Molecular genetics and the evolution of animal design, 2nd ed. Blackwell, Malden, Massachusetts, USA.

CHAMPAGNE, C. E., T. E. GOLIBER, M. F. WOJCIECHOWSKI, R. W. MEI, B. T. TOWNSLEY, K. WANG, M. M. PAZ, ET AL. 2007. Compound leaf development and evolution in legumes. *Plant Cell* 19: 3369–3378.

CHEN, J., J. YU, L. GE, H. WANG, A. BERBEL, Y. LIU, Y. CHEN, ET AL. 2010. Control of dissected leaf morphology by a Cys(2)His(2) zinc finger transcription factor in the model legume *Medicago truncatula. Proceedings of the National Academy of Sciences, USA* 107: 10754–10759.

COEN, E., AND E. M. MEYEROWITZ. 1991. The war of the whorls: Genetic interactions controlling flower development. *Nature* 353: 31–37.

CRAWFORD, B. C. W., U. NATH, R. CARPENTER, AND E. S. COEN. 2004. *CINCINATTA* controls both cell differentiation and growth in petal lobes and leaves of *Antirrhinum. Plant Physiology* 135: 244–253.

CUBAS, P., E. COEN, AND J. M. M. ZAPATER. 2001. Ancient asymmetries in the evolution of flowers. *Current Biology* 11: 1050–1052.

DEGNAN, B. M., M. VERVOORT, C. LARROUX, AND G. S. RICHARDS. 2009. Early evolution of metazoan transcription factors. *Current Opinion in Genetics & Development* 19: 591–599.

DOEBLEY, J., A. STEC, AND L. HUBBARD. 1997. The evolution of apical dominance in maize. *Nature* 386: 485–488.

DONOGHUE, M. J., R. H. REE, AND D. A. BAUM. 1998. Phylogeny and the evolution of flower symmetry in the Asteridae. *Trends in Plant Science* 3: 311–317.

DUBOULE, D., AND P. DOLLÉ. 1989. The structural and functional organization of the murine *HOX* gene family resembles that of *Drosophila* homeotic genes. *EMBO Journal* 8: 1497–1505.

ENDRESS, P. K. 1999. Symmetry in flowers: Diversity and evolution. *International Journal of Plant Sciences* 160: S3–S23.

ENDRESS, P. K. 2001. Evolution of flower symmetry. *Current Opinion in Plant Biology* 4: 86–91.

ESCARAVAGE, N., E. FLUBACKER, A. PORNON, B. DOCHE, AND I. TILL-BOTTRAUD. 2001. Stamen dimorphism in *Rhododendron ferrugineum* (Ericaceae): Development and function. *American Journal of Botany* 88: 68–75.

ESHED, Y., S. F. BAUM, AND J. L. BOWMAN. 1999. Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis. Cell* 99: 199–209.

FENG, X. Y., Z. ZHAO, Z. X. TIAN, S. L. XU, Y. H. LUO, Z. G. CAI, Y. M. WANG, ET AL. 2006. Control of petal shape and floral zygomorphy in *Lotus japonicus. Proceedings of the National Academy of Sciences, USA* 103: 4970–4975.

FERRÁNDIZ, C., Q. GU, R. MARTIENSSEN, AND M. F. YANOFSKY. 2000. Redundant regulation of meristem identity and plant architecture by *FRUITFULL, APETALA1* and *CAULIFLOWER. Development* 127: 725–734.

FOURQUIN, C., M. VINAUGER-DOUARD, P. CHAMBRIER, A. BERNE-DEDIEU, AND C. P. SCUTT. 2007. Functional conservation between *CRABS CLAW* orthologues from widely diverged angiosperms. *Annals of Botany* 100: 651–657.

FOURQUIN, C., M. VINAUGER-DOUARD, B. FOGLIANI, C. DUMAS, AND C. P. SCUTT. 2005. Evidence that CRABS CLAW and TOUSLED have conserved their roles in carpel development since the ancestor of extant angiosperms. *Proceedings of the National Academy of Sciences, USA* 102: 4649–4654.

FRIEDMAN, J., AND S. C. H. BARRETT. 2009. Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* 103: 1515–1527.

GREGIS, V., A. SESSA, L. COLOMBO, AND M. M. KATER. 2008. *AGAMOUS-LIKE24* and *SHORT VEGETATIVE PHASE* determine floral meristem identity in *Arabidopsis. Plant Journal* 56: 891–902.

GREGIS, V., A. SESSA, C. DORCA-FORNELL, AND M. M. KATER. 2009. The Arabidopsis floral meristem identity genes *AP1, AGL24* and *SVP* directly repress class B and C floral homeotic genes. *Plant Journal* 60: 626–637.

GU, Q., C. FERRÁNDIZ, M. F. YANOFSKY, AND R. MARTIENSSEN. 1998. The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* 125: 1509–1517.

HEMPEL, F. D., D. WEIGEL, M. A. MANDEL, G. DITTA, P. C. ZAMBRYSKI, L. J. FELDMAN, AND M. F. YANOFSKY. 1997. Floral determination and expression of floral regulatory genes in *Arabidopsis. Development* 124: 3845–3853.

HIBARA, K.-I., S. TAKADA, AND M. TASAKA. 2003. *CUC1* gene activates the expression of SAM-related genes to induce adventitious shoot formation. *Plant Journal* 36: 687–696.

HILEMAN, L. C., AND P. CUBAS. 2009. An expanded evolutionary role for flower symmetry genes. *Journal of Biology* 8: 1–4.

HILEMAN, L. C., E. M. KRAMER, AND D. A. BAUM. 2003. Differential regulation of symmetry genes and the evolution of floral morphologies. *Proceedings of the National Academy of Sciences, USA* 100: 12814–12819.

HUBBARD, L., P. MCSTEEN, J. DOEBLEY, AND S. HAKE. 2002. Expression patterns and mutant phenotype of *teosinte branched1* correlate with growth suppression in maize and teosinte. *Genetics* 162: 1927–1935.

IRGSP [INTERNATIONAL RICE GENOME SEQUENCING PROJECT]. 2005. The map-based sequence of the rice genome. *Nature* 436: 793–800.

IRISH, V. F., AND I. M. SUSSEX. 1990. Function of the *apetala-1* gene during *Arabidopsis* floral development. *Plant Cell* 2: 741–753.

ISHIKAWA, M., Y. OHMORI, W. TANAKA, C. HIRABAYASHI, K. MURAI, Y. OGIHARA, T. YAMAGUCHI, AND H. Y. HIRANO. 2009. The spatial expression patterns of *DROOPING LEAF* orthologs suggest a conserved function in grasses. *Genes & Genetic Systems* 84: 137–146.

JACK, T., L. L. BROCKMAN, AND E. M. MEYEROWITZ. 1992. The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell* 68: 683–697.

JACKSON, D., B. VEIT, AND S. HAKE. 1994. Expression of maize *Knotted1*-related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* 120: 405–413.

KALISZ, S., R. H. REE, AND R. D. SARGENT. 2006. Linking floral symmetry genes to breeding system evolution. *Trends in Plant Science* 11: 568–573.

KANNO, A., H. SAEKI, T. KAMEYA, H. SAEDLER, AND G. THEISSEN. 2003. Heterotopic expression of class B floral homeotic genes supports a modified ABC model for tulip (*Tulipa gesneriana*). *Plant Molecular Biology* 52: 831–841.

KAUFMANN, K., F. WELLMER, J. M. MUIÑO, T. FERRIER, S. E. WUEST, V. KUMAR, A. SERRANO-MISLATA, ET AL. 2010. Orchestration of floral initiation by APETALA1. *Science* 328: 85–89.

KEMPIN, S. A., B. SAVIDGE, AND M. F. YANOFKSY. 1995. Molecular basis of the cauliflower phenotype in *Arabidopsis. Science* 267: 522–525.

KNAPP, S. 2010. On 'various contrivances': Pollination, phylogeny and flower form in the Solanaceae. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 365: 449–460.

LANGDALE, J. A. 1994. More knots untied. *Current Biology* 4: 529–531.

LANGLADE, N. B., X. FENG, T. DRANSFIELD, L. COPSEY, A. I. HANNA, C. THÉBAUD, A. BANGHAM, ET AL. 2005. Evolution through genetically controlled allometry space. *Proceedings of the National Academy of Sciences, USA* 102: 10221–10226.

LEE, J.-Y., S. F. BAUM, J. ALVAREZ, A. PATEL, D. H. CHITWOOD, AND J. L. BOWMAN. 2005a. Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. *Plant Cell* 17: 25–36.

LEE, J.-Y., S. F. BAUM, S.-H. OH, C.-Z. JIANG, J.-C. CHEN, AND J. L. BOWMAN. 2005b. Recruitment of *CRABS CLAW* to promote nectary development within the eudicot clade. *Development* 132: 5021–5032.

LITT, A., AND V. F. IRISH. 2003. Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: Implications for the evolution of floral development. *Genetics* 165: 821–833.

LIU, C., W. XI, L. SHEN, C. TAN, AND H. YU. 2009. Regulation of floral patterning by flowering time genes. *Developmental Cell* 16: 711–722.

LONG, J. A., E. I. MOAN, J. I. MEDFORD, AND M. K. BARTON. 1996. A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis. Nature* 379: 66–69.

LUO, D., R. CARPENTER, L. COPSEY, C. VINCENT, J. CLARK, AND E. COEN. 1999. Control of organ asymmetry in flowers of *Antirrhinum. Cell* 99: 367–376.

LUO, D., R. CARPENTER, C. VINCENT, L. COPSEY, AND E. COEN. 1996. Origin of floral symmetry in *Antirrhinum. Nature* 383: 794–799.

MANDEL, M. A., AND M. F. YANOFSKY. 1995. The Arabidopsis *AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1. Plant Cell* 7: 1763–1771.

MARTÍN-TRILLO, M., AND P. CUBAS. 2010. TCP genes: A family snapshot ten years later. *Trends in Plant Science* 15: 31–39.

MCGINNIS, W., M. S. LEVINE, E. HAFEN, A. KUROIWA, AND W. J. GEHRING. 1984. Conserved DNA sequence in homeotic genes of the *Drosophila* Antennapedia and bithorax complexes. *Nature* 308: 428–433.

MELZER, S., F. LENS, J. GENNEN, S. VANNESTE, A. ROHDE, AND T. BEECKMAN. 2008. Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana. Nature Genetics* 40: 1489–1492.

NAKAYAMA, H., T. YAMAGUCHI, AND H. TSUKAYA. 2010. Expression patterns of *AaDL*, a *CRABS CLAW* ortholog in *Asparagus asparagoides* (Asparagaceae), demonstrate a stepwise evolution of *CRC/DL* subfamily of YABBY genes. *American Journal of Botany* 97: 591–600.

ORASHAKOVA, S., M. LANGE, S. LANGE, S. WEGE, AND A. BECKER. 2009. The *CRABS CLAW* ortholog from California poppy (*Eschscholzia californica*, Papaveraceae), *EcCRC*, is involved in floral termination, gynoecium differentiation and ovule initiation. *Plant Journal* 58: 682–693.

PATERSON, A. H., J. E. BOWERS, R. RUGGMANN, I. DUBCHAK, J. GRIMWOOD, H. GUNDLACH, G. HABERER, ET AL. 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature* 457: 551–556.

PRESTON, J. C., AND L. C. HILEMAN. 2009. Developmental genetics of floral symmetry evolution. *Trends in Plant Science* 14: 147–154.

PRESTON, J. C., M. A. KOST, AND L. C. HILEMAN. 2009. Conservation and diversification of the symmetry developmental program among close relatives of snapdragon with divergent floral morphologies. *New Phytologist* 182: 751–762.

PURUGGANAN, M. D., S. D. ROUNSLEY, R. J. SCHMIDT, AND M. F. YANOFSKY. 1995. Molecular evolution of flower development: Diversification of the plant MADS-box regulatory gene family. *Genetics* 140: 345–356.

REE, R. H., AND M. J. DONOGHUE. 1999. Inferring rates of change in flower symmetry in asterid angiosperms. *Systematic Biology* 48: 633–641.

REEVES, P. A., AND R. G. OLMSTEAD. 1998. Evolution of novel morphological and reproductive traits in a clade containing *Antirrhinum majus* (Scrophulariaceae). *American Journal of Botany* 85: 1047–1056.

RENSING, S. A., D. LANG, A. D. ZIMMER, A. TERRY, A. SALAMOV, H. SHAPIRO, T. NISHIYAMA, ET AL. 2008. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* 319: 64–69.

ROSIN, F. M., AND E. M. KRAMER. 2009. Old dogs, new tricks: Regulatory evolution in conserved genetic modules leads to morphologies in plants. *Developmental Biology* 332: 25–35.

RUIZ-GARCÍA, L., F. MADUEÑO, M. WILKINSON, G. HAUGHN, J. SALINAS, AND J. M. MARTÍNEZ-ZAPATER. 1997. Different roles of flowering-time genes in the activation of floral initiation genes in *Arabidopsis. Plant Cell* 9: 1921–1934.

SAKAKIBARA, K., T. NISHIYAMA, H. DEGUCHI, AND M. HASEBE. 2008. Class 1 KNOX genes are not involved in shoot development in the moss *Physcomitrella patens* but do function in sporophyte development. *Evolution & Development* 10: 555–566.

SAMACH, A., H. ONOUCHI, S. E. GOLD, G. S. DITTA, Z. SCHWARZ-SOMMER, M. F. YANOFSKY, AND G. COUPLAND. 2000. Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis. Science* 288: 1613–1616.

SARGENT. R. D. 2004. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London, B, Biological Sciences* 271: 603–608.

SCOTT. M. P., AND A. J. WEINER. 1984. Structural relationships among genes that control development: Sequence homology between *Antennapedia, Ultrabithorax*, and *fushi tarazu* loci of *Drosophila. Proceedings of the National Academy of Sciences, USA* 81: 4115-4119.

SHALIT, A., A. ROZMAN, A. GOLDSHMIDT, J. P. ALVAREZ, J. L. BOWMAN, Y. ESHED, AND E. LIFSCHITZ. 2009. The flowering hormone florigen functions as a general systemic regulator of growth and termination. *Proceedings of the National Academy of Sciences, USA* 106: 8392–8397.

SHANNON, S., AND D. R. MEEKS-WAGNER. 1991. A mutation in the Arabidopsis *TFL1* gene affects inflorescence meristem development. *Plant Cell* 3: 877–892.

SMITH, L. G., B. GREENE, B. VEIT, AND S. HAKE. 1992. A dominant mutation in the maize homeobox gene, *Knotted-1*, causes ectopic expression in leaf cells with altered fates. *Development* 116: 21–30.

SONG, C. F., Q. B. LIN, R. H. LIANG, AND Y. Z. WANG. 2009. Expressions of ECE-CYC2 clade genes relating to abortion of both dorsal and ventral stamens in *Opithandra* (Gesneriaceae). *BMC Evolutionary Biology* 9: 244–255.

SRIDHAR, V. V., A. SURENDRARAO, AND Z. LIU. 2006. APETALA1 and SEPALLATA3 interact with SEUSS to mediate transcription repression during flower development. *Development* 133: 3159–3166.

TAKADA, S., K. HIBARA, T. ISHIDA, AND M. TASAKA. 2001. *The CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development* 128: 1127–1135.

TANG, H., J. E. BOWERS, X. WANG, AND A. H. PATERSON. 2010. Angiosperm genome comparisons reveal early polyploidy in the monocot lineage. *Proceedings of the National Academy of Sciences, USA* 107: 472–477.

TANG, H., X. WANG, J. E. BOWERS, R. MING, M. ALAM, AND A. H. PATERSON. 2008. Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. *Genome Research* 18: 1944–1954.

UTSET, M. F., A. AWGULEWITSCH, F. H. RUDDLE, AND W. MCGINNIS. 1987. Region-specific expression of two mouse homeo box genes. *Science* 235: 1379–1382.

WANG, A. J., J. F., TANG, D. Y. LI, C. Y. CHEN, X. Y. ZHAO, L. AND H. ZHU. 2009. Isolation and functional analysis of *LiYAB1*, a *YABBY* family gene, from lily (*Lilium longiflorum*). *Journal of Plant Physiology* 166: 988–995.

WANG, Z., Y. H. LUO, X. LI, L. P. WANG, S. L. XU, J. YANG, L. WENG, ET AL. 2008. Genetic control of floral zygomorphy in pea (*Pisum sativum* L.). *Proceedings of the National Academy of Sciences, USA* 105: 10414–10419.

WIGGE, P. A., M. C. KIM, K. E. JAEGER, W. BUSCH, M. SCHMID, J. U. LOHMANN, AND D. WEIGEL. 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis. Science* 309: 1056–1059.

WILLIAMS, R. W. 1998. Plant homeobox genes: Many functions stem from a common motif. *BioEssays* 20: 280–282.

XU, M., T. HU, S. M. MCKIM, J. MURMU, G. W. HAUGHN, AND S. R. HEPWORTH. 2010. Arabidopsis BLADE-ON-PETIOLE1 and 2 promote floral meristem fate and determinacy in a previously undefined pathway targeting *APETALA1* and *AGAMOUS-LIKE24. Plant Journal* 63: 974–989.

ZHANG, W., E. M. KRAMER, AND C. C. DAVIS. 2010. Floral symmetry genes and the origin and maintenance of zygomorphy in a plant–pollinator mutualism. *Proceedings of the National Academy of Sciences, USA* 107: 6388–6393.