Control of Flower Size

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Publication Info
Published in *Journal of Experimental Botany*, Volume 64, Issue 6, 2013, pages 1427-1437.

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Flowering Newsletter Review

Control of flower size

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Received 31 October 2012; Revised 10 January 2013; Accepted 15 January 2013

Abstract
Flowers exhibit amazing morphological diversity in many traits, including their size. In addition to interspecific flower size differences, many species maintain significant variation in flower size within and among populations. Flower size variation can contribute to reproductive isolation of species and thus has clear evolutionary consequences. In this review we integrate information on flower size variation from both evolutionary and developmental biology perspectives. We examine the role of flower size in the context of mating system evolution. In addition, we describe what is currently known about the genetic basis of flower size based on quantitative trait locus (QTL) mapping in several different plant species and molecular genetic studies in model plants, primarily Arabidopsis thaliana. Work in Arabidopsis suggests that many independent pathways regulate floral organ growth via effects on cell proliferation and/or cell expansion.

Key words: Arabidopsis, floral evolution, floral organ growth, flower size, natural variation, selection.

Introduction
Flowers can vary dramatically in size with the gigantic flowers of Rafflesia arnoldii measuring almost one meter across compared with the tiny microscopic flowers of the genus Wolffia (Davis et al., 2008). Such extreme floral sizes may only be possible in plants with specialized life strategies (Davis et al., 2008; Endress, 2011). Flower size can also vary widely between related plants species with similar growth habits (Fig. 1) and even within species (Andersson, 2012; Delph et al., 2010; Hermann and Kuhlemeier, 2011; Mojica and Kelly, 2010; Spigler et al., 2011; Williams and Conner, 2001; Wu et al., 2008) with immediate consequences on reproductive success (Bradshaw et al., 1995; Goodwillie et al., 2006; Hodges et al., 2002; Schiestl and Schluter, 2009; Venail et al., 2010). Divergent selection on floral traits such as flower size imposed by variable abiotic and/or biotic conditions can drive population differentiation (Brunet, 2009; Galen, 1996) and could potentially contribute to reproductive isolation (Bradshaw et al., 1995; Hodges et al., 2002; Schiestl and Schluter, 2009; Venail et al., 2010). A recent review suggests that variation in floral morphology (including flower size) is a more important reproductive barrier than flower colour in the Orchidaceae (Schiestl and Schluter, 2009).

Ecologists and evolutionary biologists have extensively investigated the environmental causes and evolutionary consequences of floral trait variation in nonmodel organisms (Fenster et al., 2004; Galen, 2000; Gong and Huang, 2006).
2009; Stanton and Preston, 1988; Williams and Conner, 2001). Developmental biologists have identified the genetic basis of flower size in model species under controlled conditions (Sicard and Lenhard, 2011). Ultimately, integrating these approaches will enable a more thorough examination of the evolution of phenotypic variation, the co-evolutionary dynamics of plants and their pollinators, the tempo and mechanism of reproductive isolation and perhaps the genetic architecture of speciation (Bradshaw et al., 1995; Hodges et al., 2002; Langlade et al., 2005; Schiestl and Schluter, 2009; Venail et al., 2010). Furthermore, interdisciplinary investigations will enable researchers to test the mechanisms that maintain genetic variation in natural populations and examine how selection operates at the level of the gene (Anderson et al., 2011; Olson-Manning et al., 2012). Here we seek to review the evolution and developmental genetics of flower size variation, unifying disparate bodies of literature. To that end, we briefly discuss floral size in the context of mating system evolution, examine constraints on the evolution of flower size and explore studies that address the genetics of flower size via quantitative trait locus (QTL) mapping. We then focus on advancements that have been made possible through detailed genetic analyses of the model organism Arabidopsis thaliana in the laboratory and growth chamber.

### Mating system evolution and selection on flower size

Flower size is a key ecological trait as it influences mating system evolution and reproductive success (Goodwillie et al., 2010; Sargent et al., 2007). In outcrossing plants, floral traits, including flower size, are thought to co-evolve with pollinators. To attract pollinators, sex allocation theory predicts that outcrossing species should invest more resources in floral display than self-pollinating species (Goodwillie et al., 2010). The origin of selfing from an outcrossing ancestor has occurred independently many times during angiosperm evolution and is often associated with characteristic changes in floral morphology that include reductions in flower size (reviewed in Sicard and Lenhard, 2011). Species that self-pollinate autonomously tend to have smaller flowers than both outcrossers and selfing species that require pollinator visitation (Goodwillie et al., 2010). This pattern holds even within species when populations vary in mating system (reviewed in Goodwillie et al., 2010). Small-flowered genotypes capable of autonomous selfing can have a fitness advantage over larger outcrossing genotypes when pollinators are rare (Elle and Carney, 2003). Indeed, reproductive assurance can offset the fitness costs of self-fertilization, resulting in populations with mixed mating systems (Kalisz et al., 2004).

Flower size is often correlated with other floral traits that increase pollinator visitation rates (Fenster et al., 2006). For example, large flowers generally contain more nectar rewards and are more conspicuous than smaller flowers (Blair et al., 2002; Fenster et al., 2006). Thus, pollinators tend to be more attracted to larger than smaller flowers both within and between plant species, and pollinator behaviour can impose strong directional selection favouring large flowers in outcrossing plants (e.g., Bell, 1985; Conner and Rush, 1996; Dudash et al., 2011; Elle and Carney, 2003; Galen, 1996; Glaethtli and Barrett, 2008; Harder and Johnson, 2009; Kingsolver et al., 2001; Mojica and Kelly, 2010; Parachnowitsch and Kessler, 2010; Sandring and Ågren, 2009; Schemske and Ågren, 1995; Stanton and Preston, 1988; Venail et al., 2010). Experimental manipulations of flowers provide powerful support for pollinator-mediated selection on flower size and other floral characteristics through both male (pollen transfer) and female (fruit and seed set) components of reproductive success (Dudash et al., 2011; Fenster et al., 2004; Galen and Cuba, 2001; Parachnowitsch and Kessler, 2010; Sandring and Ågren, 2009).

Nevertheless, floral size evolution is not necessarily a direct response to selection exerted by pollinators. For one, large flowers can be disadvantageous for female fitness under stressful conditions such as drought (Galen, 2000). Consistent directional selection should deplete populations of variation in ecologically relevant traits, yet natural populations maintain substantial genetic variation for flower size despite pollinator-mediated selection for larger flower size (Mojica and Kelly, 2010; Mojica et al., 2012; Stanton and Preston, 1988; Williams and Conner, 2001). The maintenance of genetic variation in flower size could result from genetic correlations with other traits, environmental trade-offs, selection operating at earlier life history stages, or antagonistic selection imposed by floral enemies (Campbell, 2009; Galen, 2000; Mojica and Kelly, 2010; Navarro and Medel, 2009; Parachnowitsch and Caruso, 2008).

When reproductive success is used as the fitness component, the pattern of directional selection for larger flowers holds in a diverse array of species (reviewed in Kingsolver et al., 2001), including the ecological model Mimulus guttatus (Phrymaceae) (Mojica and Kelly, 2010). However, viability selection early in the life history of M. guttatus reverses the overall direction of selection on flower size (Mojica and Kelly, 2010). Despite their fecundity advantage, large-flowered genotypes have a greater propensity to die before flowering than small-flowered genotypes; by integrating viability and fecundity components of fitness, Mojica and Kelly (2010) found that natural selection actually favours small-flowered genotypes. Thus, the genetic response to selection imposed by pollinators can be constrained by selection occurring at other life history stages.

If pre-dispersal seed predators and nectar robbers diminish plant fecundity, selection exerted by these natural enemies can counteract selection imposed by pollinators, further constraining floral trait evolution (Irwin et al., 2001; Navarro and Medel, 2009; Parachnowitsch and Caruso, 2008). Pre-dispersal seed predators rely on the activities of pollinators to produce seeds and can be attracted to the same floral traits as pollinators, decreasing the fitness of plants that invest in attractive flowers (Parachnowitsch and Caruso, 2008). Natural enemies can exert selection on floral traits, including flower shape, size and phenology (Galen and Cuba, 2001; Irwin et al., 2001; Parachnowitsch and Caruso, 2008). However, in a recent
review, Parachnowitsch and Kessler (2010) found no difference in selection on floral traits (including flower size) in the presence and absence of seed predators, suggesting that seed predators are not strong agents of selection on flower size. This result should be treated cautiously, as few studies have manipulated natural enemies to test their effects on floral trait evolution (Parachnowitsch and Kessler, 2010). To understand the evolution of flower size and other traits in natural populations, it will probably be necessary to investigate the interactions of different agents of selection at multiple life history stages and across growing seasons (Brody et al., 2008; Brunet and Holmquist, 2009; Galen, 2000; Galen and Cuba, 2001; Irwin, 2006; Mojica and Kelly, 2010).

**Quantitative trait loci and the genetic basis of flower size**

Quantitative genetics studies of flower size have revealed how natural selection operates at the level of the QTL and have begun to dissect the genetic basis of this trait in model organisms, natural populations of non-model species, as well as cultivated species and their wild relatives (Bouck et al., 2007; Bradshaw et al., 1995; Feng et al., 2009; Frary et al., 2004; Galliot et al., 2006; Goodwillie et al., 2006; Hodges et al., 2002; Juenger et al., 2000, 2005; Kelly and Mojica, 2011; Meagher et al., 2005; Mojica et al., 2012; Scoville et al., 2011; Spigler et al., 2011). For example, Mojica and colleagues (2012) found that alleles that promote large flowers in *M. guttatus* increase fecundity while depressing viability, consistent with earlier genotypic selection analyses conducted at the organismal level (Mojica and Kelly, 2010). Furthermore, epistatic interactions among QTLs can substantially influence segregating variation within a single population (Kelly and Mojica, 2011) and between species (Frary et al., 2004). Similar to other quantitative traits, continuous variation in flower size is most likely to be polygenic (Galliot et al., 2006; Meagher et al., 2005), but QTL of major effect on flower size variation have also been uncovered (Bouck et al., 2007; Scoville et al., 2011; Venail et al., 2010). Finally, some flower size QTL appear to be maintained at intermediate frequencies in natural populations by balancing selection (Scoville et al., 2011).

Co-localization of QTL for integrated aspects of floral organ size such as petal width and length as well as QTL underlying the size of multiple floral organs have been reported (Bouck et al., 2007; Fishman et al., 2002; Goodwillie et al., 2006; Juenger, 2000). However, work in *Lycopersicum* suggests that distinct genes regulate the size of sepals and petals (Frary et al., 2004). In addition, several studies document co-localization of flower size QTL with QTL for other floral and life history characteristics (Bouck et al., 2007; Fishman et al., 2002; Goodwillie et al., 2006; Hermann and Kuhlemeier, 2011), including traits associated with sexual dimorphism and male sterility on a proto-sex chromosome in *Fragaria virginiana* (Spigler et al., 2011) and sex-determining loci in *Silene latifolia* (Delph et al., 2010). Co-localization could result from pleiotropy or tightly linked causal genes, either of which could produce genetic correlations that constrain floral trait evolution, such as the trade-off between flower size and the number of flowers (Delph et al., 2004; Goodwillie et al., 2010; Sargent et al., 2007; Spigler et al., 2011). Future endeavours that identify causal loci underlying key QTL will help to elucidate the genetic architecture and basis of trait correlations, sexual dimorphism and perhaps even reproductive isolation (Delph et al., 2010; Goodwillie et al., 2006; Hodges et al., 2002; Schiechl and Schluter, 2009; Spigler et al., 2011).

**Arabidopsis flower size control**

Although *A. thaliana* is a selfing plant with relatively small flowers, we believe that studies of this model species can contribute to a general understanding of the genetic basis of flower size. Most close relatives of *Arabidopsis* in the crucifer (Brassicaceae) family are self-incompatible (SI), and selfing in *Arabidopsis* is thought to have arisen relatively recently, approximately 1 million years ago (Tang et al., 2007). Introduction of the male and female specificity determinants of self-incompatibility from SI *Arabidopsis lyrata* or *Capsella grandiflora* into *Arabidopsis* confers self-incompatibility (reviewed in Rea et al., 2010). Using this transgenic SI *A. thaliana* model, several genes have been identified that influence both the self-incompatibility response and carpel morphology, specifically enhanced elongation of the carpel resulting in stigma exertion (Tantikanjana and Nasrallah, 2012; Tantikanjana et al., 2009). Thus, factors involved in the coordinated evolution of selfing and flower size appear to be present within the *Arabidopsis* genome.

Furthermore, *Arabidopsis* ecotypes possess significant genetic variation in flower size (Juenger et al., 2000, 2005). Juenger et al. (2000) detected 18 QTL affecting at least one aspect of flower size using *Arabidopsis* recombinant inbred lines; several of these QTL mapped to regions containing known regulators of organ size. In addition, several studies investigating the function of *Arabidopsis* genes in other plants suggest conserved functions in regulating flower size. For example, *Antirrhinum majus* flowers downregulated for the growth-promoting gene AINTEGUMENTA (*Am-ANT*) produce smaller floral organs, while the larger flowers of *forsmola* (*fo*) mutants are associated with increased expression of *Am-ANT* (Delgado-Benarroch et al., 2009; Kim et al., 2011).

Genetic studies, primarily in *Arabidopsis*, suggest that many different pathways act independently to determine flower size, and that plant hormones and transcriptional regulation play important roles in these pathways (Fig. 2) (reviewed in Breuninger and Lenhard, 2010; Weiss et al., 2005). Many of the identified size regulators control the growth of both vegetative (leaves) and reproductive (flowers) lateral organs that are formed on the flanks of the dome-shaped shoot apical meristem. Several excellent reviews on the genetic basis of lateral organ size in general and leaves in particular have been published recently (Gonzalez et al., 2012; Johnson and Lenhard, 2011; Powell and Lenhard, 2012). Here we focus on the genes that control floral organ size.
Typical eudicot flowers are composed of four types of floral organ – sepals, petals, stamens and carpels – with the size of each organ dependent on both the number and size of the constituent cells. Founder cells give rise to floral organ primordia at precise positions within the flower primordium. Growth of these primordia into mature floral organs is thought to consist of two partially overlapping phases (Fig. 2). Initial growth is a result of cell proliferation with cells growing in size with the synthesis of new cytoplasmic material and then dividing. Later, cell proliferation often becomes restricted to particular regions within a developing organ. During the second growth phase, increases in floral organ size are largely a result of cell expansion due to increases in the size of the plant vacuole. Extremely large cell sizes present in some floral organs are often a result of endoreduplication, in which cells undergo multiple rounds of mitosis but do not divide, resulting in polyploid cells (reviewed in Sugimoto-Shirasu and Roberts, 2003). In Arabidopsis, endoreduplication occurs in epidermal cells of sepals but has not been observed in other floral organs (Galbraith et al., 1991; Roeder et al., 2010). However in some species petal epidermal cells undergo endoreduplication, resulting in the production of very large cells (Kudo and Kimura, 2001; Lee et al., 2004). Examination of Arabidopsis mutants has revealed that changes in the rate and/or duration of either the cell proliferation or cell expansion phases of growth can be responsible for alterations in floral organ size (reviewed in Powell and Lenhard, 2012).

Fig. 2. Pathways regulating floral organ size in Arabidopsis. Top pictures show different stages of Arabidopsis flower development from the time of sepal initiation (leftmost) to flower opening (rightmost). Bars representing the cell-proliferation and cell-elongation phases of growth are shown below the corresponding stages of flower development. The known factors and pathways regulating cell proliferation and/or cell expansion are summarized. Arrows indicate positive interactions while bars represent negative interactions. ca, carpel; pe, petal; se, sepal; st, stamen; for gene and factor names see text.

**Regulation of cell proliferation in floral organs**

One mechanism controlling final flower size involves the timing of cell proliferation arrest within developing floral organ primordia. Extending the period in which cells are competent to undergo cell division can result in larger floral organs as seen in Arabidopsis plants constitutively expressing the auxin-inducible gene AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE (ARGOS) or the gene encoding the AP2/ERF type transcription factor AINTEGUMENTA (ANT) (Hu et al., 2003; Krizek, 1999; Mizukami and Fischer, 2000). Conversely, floral organs reach a smaller final size in plants lacking ARGOS or ANT function (Elliott et al., 1996; Hu et al., 2003; Klucher et al., 1996; Krizek, 1999; Mizukami and Fischer, 2000). ARGOS and ANT appear to act in a common auxin pathway regulating growth with ANT acting downstream of ARGOS (Hu et al., 2003). ANT may act by regulating the expression of cell-cycle genes such as CYCLIN.
growth may be coordinated in self-fertilizing plants through endoplasmic reticulum-localization with ARGOS are ARGOS-LIKE (ARL) and ORGAN SIZE RELATED1 (OSR1) (Feng et al., 2011). ARL promotes organ growth through effects on cell expansion (Hu et al., 2006). OSR1 primarily affects cell proliferation via maintenance of ANT expression in maturing lateral organs but also promotes cell expansion independently of ANT (Feng et al., 2011). Despite having overlapping functions in organ growth, ARGOS, ARL, and OSR1 are regulated by different hormones, suggesting that these genes may integrate distinct signals during organ growth (Fig. 2) (Feng et al., 2011; Hu et al., 2003, 2006). At least two transcription factors of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) family, which share high sequence similarity within the DNA-binding AP2 repeat region of ANT, can act redundantly with ANT to regulate floral organ growth. ant ail6 double mutants make smaller sepals (Krizek, 2009); conversely, misexpression of AIL5 and AIL6 can result in the production of larger floral organs (Krizek and Eaddy, 2012; Nole-Wilson et al., 2005).

*Arabidopsis* KLUH (KLU/CYP78A5), a cytochrome P450 monoxygenase, promotes floral growth by preventing the premature arrest of cell proliferation within developing floral organs (Anastasiou et al., 2007). klu mutants produce smaller floral organs with fewer cells while overexpression of KLU results in larger flowers with more cells. Because KLU expression during petal development does not match the spatial patterns of cell proliferation, KLU is thought to function non-cell-autonomously through generation of a novel mobile growth signal (Anastasiou et al., 2007). A KLU-derived signal appears to move both within a flower and between flowers to regulate organ growth at the whole flower or even whole inflorescence level (Eriksson et al., 2010). In this way, floral organ growth may be coordinated in self-fertilizing *Arabidopsis* to promote reproductive success.

The plant hormone cytokinin also affects the duration of cell division within developing floral organs. Mutations in the genes for two cytokinin degrading enzymes in Arabidopsis, cytokinin oxidase/dehydrogenase CKX3 and CKX5, result in larger floral organs due to the presence of more cells (Bartrina et al., 2011). In transgenic petunia, expression of a cytokinin biosynthetic gene under the control of a flower-specific promoter results in larger flowers primarily due to increased cell number (Verdonk et al., 2008). These results indicate that cytokinin promotes floral organ growth but the downstream effectors in this pathway have not been identified. While high cytokinin levels promote floral organ growth, no effect on flower size was observed in Arabidopsis plants in which cytokinin levels were reduced, even though these plants produce smaller leaves than the wild-type (Holst et al., 2011).

Cell division within floral organs is also promoted by GROWTH-REGULATING FACTORS (GRFs) and GRF-INTERACTING FACTORs (GIFs), which function as transcription factors and co-activators, respectively, that physically interact (Kim et al., 2003; Kim and Kende, 2004). Mutations in these genes result in smaller petals owing to reduced numbers of cells (Horiguchi et al., 2005; Kim and Kende, 2004; Lee et al., 2009). These proteins appear to have partly overlapping functions in floral organ growth as higher-order mutants generally show more severe defects. Kinematic analyses of leaf growth in gif single and higher-order mutants indicates that GIFs regulate both the rate and duration of cell proliferation but once again this has not been examined in floral organs (Lee et al., 2009).

Several genes have been identified that restrict the duration of the cell proliferation phase of floral organ growth. These include the *Arabidopsis* genes BIG BROTHER (BB), which encodes an E3 ubiquitin-ligase, as well as DAI1 and DARI, which encode putative ubiquitin receptors (Disch et al., 2006; Li et al., 2008). Mutations in these genes result in larger floral organs while increased expression of these genes results in floral organs that reach a smaller final size. The identification of these proteins suggests that the ubiquitin-proteasome protein-degradation pathway plays a role in organ size control and that BB and DAI act via proteolysis of growth-promoting factors, but no substrates of BB activity have been identified.

Members of the TCP (TEOSINTE BRANCHED/CYCLOIDEA/PCF) transcription factor family regulate growth within developing plant organs (reviewed in Martin-Trillo and Cubas, 2009). There are two major groups of TCP genes with class I genes acting as promoters of leaf growth and class II genes repressing leaf growth. Mutations in class II genes result in larger leaves that have a crinkled appearance resulting from altered cell proliferation patterns during leaf development (Nath et al., 2003; Schommer et al., 2008). While the class II Antirrhinum gene *CINCINNATA* (CIN) restricts growth in leaves, it promotes cell division and growth of the petal lobe as well as the differentiation of conical cells on the epidermal surface (Crawford et al., 2004). Thus, some TCP genes can have opposite effects on growth in different tissues. In contrast to CIN, Arabidopsis TCP4 represses petal growth. This role was revealed by the isolation of a loss of function mutation in miR319a129, which downregulates five TCP genes (TCP2, TCP3, TCP4, TCP10 and TCP24) in flowers (Nag et al., 2009). The narrow-petal phenotype of miR319a129 was partly suppressed by expression of a tcp4 allele containing a mutation in the miRNA-binding site complementary to the miR319a129 mutation. The cellular basis for the narrow-petal phenotype has not been reported but may result from a reduced number of cells based on the known involvement of TCP genes in cell proliferation.

**Regulation of cell expansion in floral organs**

Besides the previously mentioned organ growth promoter ARL, several other factors are known to regulate floral organ size primarily by affecting cell size. Two of these factors are...
components of Mediator, a multiprotein complex involved in transcription regulation that acts as an adapter between transcription factors bound to regulatory elements and the general transcription machinery. MED25 acts to restrict floral organ growth via effects primarily on cell expansion but with some effects on cell proliferation (Xu and Li, 2011). Increased cell growth in med25 mutants may be due to increased expression of several expansin genes (Xu and Li, 2011) that mediate cell wall loosening during cell expansion (Cosgrove, 2000). Petunia plants downregulated for the expansin gene PhEXP1A produce flowers with smaller petal limbs due to smaller cells while overexpression of PhEXP1A leads to larger petal limbs as a result of larger cells (Zenoni et al., 2004, 2011).

While MED25 is a repressor of floral organ growth, two other Mediator subunits – MED8 and STRUWWELPETER (SWP)/MED14 – promote growth (Aufran et al., 2002; Xu and Li, 2012). MED8 regulates organ growth via cell expansion while SWP regulates cell proliferation during early stages of organogenesis. It is possible that distinct Mediator complexes regulate the transcription of different sets of growth-regulatory genes in response to different signals (Xu and Li, 2012).

Floral organ-specific regulators of growth

Few factors that regulate growth in a specific floral organ have been identified. BIGPETAL (BPE), a basic helix-loop-helix (bHLH) transcription factor, restricts the expansion of petal cells (Szecsi et al., 2006). BPE undergoes alternate splicing to generate two transcripts: a ubiquitously expressed BPEub and a petal-specific BPEp transcript. Both transcripts encode proteins containing the bHLH domain but with distinct C-terminal regions that appear to be functionally important. The C-terminal domain of BPEp interacts with AUXIN RESPONSE FACTORS (ARF8) and mutations in ARF8 also result in larger petals (Varaud et al., 2011). The increased size of arf8 petals appears to result from both increases in cell size and cell number (Varaud et al., 2011). bpe arf8 double mutants produce petals larger than either single mutant alone; this does not result from further increases in cell size but an increased number of cells. Thus BPEp and ARF8 may work in distinct pathways early in petal development to limit the period of cell proliferation but later work together to limit cell expansion (Varaud et al., 2011).

Floral organ identity proteins and the regulation of floral organ growth

Primordia on the flanks of the Arabidopsis reproductive-shoot apical meristem adopt a floral fate due to the activity of a transcription factor called LEAFY (LFY) (Weigel et al., 1992). Within a flower primordium, LFY acts in combination with other factors to establish the spatially restricted expression patterns of four classes of floral organ identity genes (also called floral homeotic genes) that specify the distinct identities of floral organ primordia (reviewed in Siriwardana and Lamb, 2012). The ABCE model describes the distinct combination of floral organ identity gene activities that specify sepal (A+E), petal (A+B+E), stamen (B+C+E) and carpel (C+E) identities in each whorl of the flower (reviewed in Krizek and Fletcher, 2005). The class A gene APETALA1 (AP1), class B genes APETALA3 (AP3) and PISTILLATA (PI), class C gene AGAMOUS (AG) and class E SEPALLATA genes (SEPI–4) encode MADS domain transcription factors while the class A gene APETALA2 (AP2) encodes an AP2/ERF transcription factor. These transcription factors are expressed throughout floral organ development and identification of their regulatory targets reveals that these proteins control distinct processes during floral organogenesis (Gomez-Mena et al., 2005; Ito et al., 2004, 2007; Wuest et al., 2012).

Genome-wide approaches such as chromatin immunoprecipitation in combination with high-throughput sequencing (ChIP-Seq) have identified many floral organ size regulators as targets of LFY and the floral organ identity proteins AP1, AP3, PI and SEP3 (Table 1). In addition to specifying a floral fate, LFY appears to regulate early growth of the flower primordium by directly binding to growth-regulatory targets (Moyroud et al., 2011; Winter et al., 2011). LFY also activates expression of the floral homeotic genes whose proteins themselves regulate floral organ size factors during flower development (Kaufmann et al., 2009, 2010; Moyroud et al., 2011; Winter et al., 2011; Wuest et al., 2012). The identification of target genes that encode factors regulating both cell proliferation and cell expansion is consistent with the floral homeotic proteins controlling growth during both early and late stages of flower development. Genetic support for this role in organ growth is provided by Antirrhinum compacta (co) mutants that produce smaller petals due to reduction in class B activity during late stages of petal development (Manchado-Rojo et al., 2012). Growth within floral organ primordia is thus tightly coupled with the establishment of organ identity and the elaboration of floral form (reviewed in Dornelas et al., 2010).

Conclusions and future directions

Flower size is an important trait that affects mating system evolution and fitness. Within a species, variation in flower size and other floral traits can promote reproductive isolation and ultimately speciation. Although pollinators often prefer larger flowers, the evolution of flower size can be constrained by selection imposed by natural enemies, selection that occurs at earlier plant life history stages, and/or genetic trade-offs. Identifying the complex suite of abiotic and biotic agents of selection that sculpt floral evolution remains challenging. Another important future goal will be to elucidate the genetic basis of flower size variation in natural plant populations. QTL cloning in model and non-model species as well as testing of candidate genes identified in Arabidopsis will contribute towards achieving this goal. Such studies may reveal genes that enable population divergence and influence plant–pollinator interactions. Furthermore, they will begin
to indicate the degree to which studies in *Arabidopsis* contribute to a general understanding of the genetic control of flower size. While numerous *Arabidopsis* genes regulating floral organ size have been identified through molecular genetic studies, many questions remain about the pathways in which these proteins function. A number of these growth-regulatory factors encode transcription factors, but few targets of these proteins function. Identification of such targets will be helpful in revealing the molecular and cellular mechanisms by which these proteins control growth in flowers.

**Acknowledgements**

Work in the Krizek lab is supported by National Science Foundation (NSF) grant IOS 0922367. Work in the Anderson lab is supported by start-up funds from the University of South Carolina.

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