FEATURED ARTICLE

‘Living stones’ reveal alternative petal identity programs within the core eudicots

Samuel F. Brockington1,2,*, Paula J. Rudall3, Michael W. Frohlich3, David G. Oppenheimer1, Pamela S. Soltis2 and Douglas E. Soltis1

1Department of Biology, University of Florida, Gainesville, FL 32611, USA, 2Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA, and 3Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK

Received 8 August 2011; revised 13 September 2011; accepted 19 September 2011; published online 23 November 2011.

*For correspondence (fax +44 (0)1223 333953; e-mail sb771@cam.ac.uk).

SUMMARY

Petals, defined as the showy laminar floral organs in the second floral whorl, have been shown to be under similar genetic control in distantly related core eudicot model organisms. On the basis of these findings, it is commonly assumed that the petal identity program regulated by B-class MADS-box gene homologs is invariant across the core eudicot clade. However, the core eudicots, which comprise >70% of angiosperm species, exhibit numerous instances of petal and sepal loss, transference of petal function between floral whorls, and recurrent petal evolution. In the face of these complex patterns of perianth evolution, the concept of a core eudicot petal identity program has not been tested. We therefore examined the petal identity program in the Caryophyllales, a core eudicot clade in which perianth differentiation into sepals and petals has evolved multiple times.

Specifically, we analyzed the expression patterns of B- and C-class MADS-box homologs for evidence of a conserved petal identity program between sepal-derived and stamen-derived petaloid organs in the ‘living stone’ family Aizoaceae. We found that neither sepal-derived nor stamen-derived petaloid organs exhibit gene expression patterns consistent with the core eudicot petal identity program. B-class gene homologs are not expressed during the development of sepal-derived petals and are not implicated in petal identity in stamen-derived petals, as their transient expression coincides with early expression of the C-class homolog. We therefore provide evidence for petal development that is independent of B-class genes and suggest that different genetic control of petal identity has evolved within this lineage of core eudicots. These findings call for a more comprehensive understanding of perianth variation and its genetic causes within the core eudicots—an endeavor that will have broader implications for the interpretation of perianth evolution across angiosperms.

Keywords: MADS-box, evolution of development, floral development, Caryophyllales, Aizoaceae, petal.

INTRODUCTION

Petals may be defined as showy laminar floral organs that are located in the second whorl of a differentiated perianth (Irish, 2009), where they function in the attraction of pollinators. Similarities in the genetic regulation of petals in the core eudicot clade (Gunneridae; Cantino et al., 2007), have been revealed by comparison between two model core eudicot taxa, the asterid Antirrhinum majus and the rosid Arabidopsis thaliana (Coen and Meyerowitz, 1991): (i) In the absence of the C-class MADS-box gene AGAMOUS (AG) or its orthologs, activity of B-class MADS-box genes is necessary for development of the petal in the second whorl of the flower (Jack et al., 1992; Mizukami and Ma, 1992; Bradley et al., 1993; Goto and Meyerowitz, 1994), (ii) persistent activity of B-class MADS-box genes through late stages of petal development is necessary to maintain the expression of characteristic petal features (Bowman et al., 1991; Sommer et al., 1991; Zachgo et al., 1995), and (iii) heterotopic expression of B-class MADS-box genes in the first-whorl sepals of the flower is sufficient to induce ectopic petal morphology (Coen and Meyerowitz, 1991; Krizek and Meyerowitz, 1996). Based on these observations, it has been proposed that a petal identity program regulated by B-class
MADS-box gene homologs had arisen at least by the time of the origin of the core eudicot clade (Kramer and Jaramillo, 2005).

Petals have been lost and gained many times, and have been derived from either bracts (bracteopetals) or stamens (andropetals) (Kramer and Irish, 2000), and consequently occur in a homoplastic pattern across angiosperms. Furthermore, although petals are commonly found in the second whorl of the flower, floral organs outside of the second whorl can have a petal-like (or petaloid) appearance i.e. petal traits can have heterotopic expression in additional floral whors. The potential of homeotic petal identity programs to explain this heterotopic and homoplastic petal variation is widely cited (Bowman, 1997; Baum and Whitlock, 1999; Kramer and Jaramillo, 2005). Numerous studies have looked for a genetic signature consistent with this potential – a conserved petal identity program in all petals and petaloid organs, regardless of phylogenetic lineage, derivation, or position within the flower. Such analyses have focused largely on Ranunculales (Kramer and Irish, 1999, 2000), the monocots (Park et al., 2003, 2004; Whipple et al., 2007), Magnoliidae, and basal angiosperm lineages (Stellari et al., 2004; Kim et al., 2005). In contrast, relative to species diversity, few representatives of core eudicots have been studied for variation in petal identity (Irish, 2003); only one study in core eudicots has compared organ identity programs between taxa with significant perianth variation (Geuten et al., 2006). This emphasis on lineages outside the core eudicot clade is in part a consequence of efforts to include patterns of floral variability in these lineages afford opportunities to examine petal variation.

Several erroneous assumptions further encourage this phylogenetic bias. Core eudicot petals are commonly considered to be andropetals and are treated as homologous (Hiepko, 1965; Takhjian, 1991; Endress, 1994); conversely, bracteopetals are thought to be more common in lineages outside the core eudicots, such as the Magnoliidae (Walker and Walker, 1984). However, the assumed universality of andropetals in core eudicots is contradicted by aestivation patterns and the occurrence of sepaloid traits in petals of many core eudicots (Ronse De Craene, 2007, 2008). It is unclear whether there has been a single evolutionary origin of petals at the base of the core eudicots and/or multiple origins of petals (Ronse De Craene, 2007, 2008). Hence, historical homology of the core eudicot petal is uncertain. Furthermore, the rate of change in perianth evolution in core eudicots has been ignored by contemporary evo-devo approaches (Ronse De Craene, 2007, 2008); there are widespread examples of petaloidy in the sepals, loss of petals or sepals, and subsequent iterative regain of petals (Ronse De Craene, 2007, 2008; Brockington et al., 2009). These complex patterns of perianth variation in core eudicots have been inadequately explored. A stable core eudicot petal identity program has commonly been assumed (e.g. Kim et al., 2005), but rarely tested (see Geuten et al., 2006). Moreover, it is logical to look for evidence of heterotopy in petal evolution within core eudicots, after the likely fixation of the homeotic petal identity program, i.e., the divergence of the Rosidae and Asteridae; but as yet there is no genetic evidence for homeosis in the evolution of the core eudicot perianth. Finally, the core eudicots offer some of the clearest instances of derived andropetals (Ronse De Craene, 2008), but no genetic analyses of these recurrent andropetal origins have been conducted.

Given these uncertainties, the core eudicot clade Caryophyllales is an appropriate group to examine the genetics underlying iterative petal evolution in core eudicots. Previous analyses show that one whorl of perianth was lost early in the evolution of Caryophyllales (Figure 1; Brockington et al., 2009). The classical perspective is that petals were ancestrally absent in this clade (Figure 1), and that the organs that comprise the single-whorled perianth of many lineages of Caryophyllales are homologous to core eudicot sepals (Hofmann, 1994). Differentiation of the perianth into distinct petal and sepal whorls has occurred at least nine times in Caryophyllales, through either the recruitment of subtending bracts (e.g. Portulaca) or the gain of staminodial petals (e.g. Aizoaceae) (Brockington et al., 2009; Ronse De Craene, 2007; Figure 1). Thus, recurrent examples of a differentiated perianth in Caryophyllales fall into two categories with petaloid organs either sepal-derived or stamen-derived. Distinct evolutionary origins of a differentiated perianth, with contrasting petal derivations, provide the necessary variation and evolutionary replicates to assess the role of the canonical eudicot petal identity program in recurrent petal evolution.

Here we focus on the evolution of differentiated perianth in Aizoaceae, a clade of four subfamilies, in which Sesuvioidae and Aizooidae are successive sisters to Mesembryanthemoideae plus Ruschoideae (Klak et al., 2003; Figure 1). Two floral types occur across these four subfamilies. Sesuvioidae and Aizooidae exhibit an undifferentiated perianth comprising a single whorl of five tepals (Figure 1B), which are petaloid on their adaxial surfaces and sepaloid on their abaxial surfaces; this is the inferred ancestral condition for Aizoaceae. Mesembryanthemoideae and Ruschoideae display a differentiated perianth with an outer whorl of sepals (homologous to the tepals of Sesuvioidae/Aizooidae) and an inner whorl of putative andropetals (Hofmann, 1994; Ronse De Craene, 2007; Figure 1A). Therefore, a transfer of function has occurred from the adaxial tepal surface of Sesuvioidae and Aizooidae to the andropetals of Mesembryanthemoideae and Ruschoideae.

We examined these differently derived petaloid organs for evidence of a shared core eudicot petal identity program. We isolated AP3, PI and AG homologs from all four subfamilies and observed in-situ expression patterns of these genes in two species representing the two distinct floral types:
Sesuvium portulacastrum (Sesuvioideae: petaloid tepals) and Delosperma napiforme (Ruschioideae: andropetals). Significantly, neither petal class exhibits gene expression patterns consistent with the classic core eudicot petal identity program. Thus, we provide evidence for core eudicot petal development that is independent of $AP3$- and $PI$- lineage MADS-box genes, and suggest that different genetic control of petal identity has evolved within the Caryophyllales in the context of its unusual floral evolutionary history.

RESULTS
Isolation and phylogenetic analysis of MADS-box genes from Aizoaceae
Orthologs of $AGAMOUS$ (AG), $PISTILLATA$ (PI), and $APETALA3$ (AP3) were isolated from representatives of all four subfamilies within Aizoaceae; Portulaca oleracea (Portulacaceae) and Antigonon leptopus (Polygonaceae) were also sampled as representatives of outgroups. Single copies of the AG, PI, AP3 were isolated from all four subfamilies (Figure 2). The degenerate nature of the polymerase chain reaction (PCR) also led to the isolation of numerous additional MADS lineages, including representatives of the TM6 lineage (a paralogous clade to the AP3 and PI lineages) from all four subfamilies (Figure 2). Identities of all isolated AG, PI, AP3, and TM6 genes were confirmed by their phylogenetic positions within subclades containing previously identified genes from model eudicots. A single PI locus and two copies of AP3 were isolated from Antigonon, and an AG ortholog was isolated from Portulaca. The detection of two copies of AP3 in Antigonon is consistent with the two copies found in Rumex, suggesting a possible Polygonaceae-specific duplication in the AP3 lineage (Figure 2). Accession numbers for published sequences used in this phylogenetic analysis together with sequences submitted by this study are listed in Supplementary Information online (Table S1).

Floral development in Sesuvium portulacastrum and Delosperma napiforme
In Sesuvium portulacastrum, the five tepal primordia are initiated in a 2/5 sequence (Figure 3C). The primordia are crescent-shaped with a broad base of insertion (Figure 3C). Androecial primordia are initiated in somewhat chaotic fascicles (Figure 3D). The gynoecium develops with three carpels (Figure 3D). Outer stamens grow and subsequently hide the inner stamens and developing carpels (Figure 3E). The tepal primordia differentiate into upper and lower domains early in development (Figure 3G). In S. portulacastrum, an adaxial cross-zone gives rise to a ligule that marks the boundary between the upper and lower domains (digitally colored green and purple, respectively, in Figure 3G–I), allowing the two domains to be tracked through the ontogeny of the tepal. Early in development, the unifacial upper domain represents the bulk of the tepal (Figure 3F). However, subsequent differential development ensures that the bifacial lower domain forms the bulk of the mature tepal (Figure 3H–I). Thus, the petaloid lamina of the tepal is derived from the lower domain while a distal unifacial tip represents the upper domain (Figure 3J).
These ontogenetic data illustrating derivation of the petaloid portion (lamina) of the tepal from the lower domain can be compared with data from the vegetative leaves of the same species, in which the leaf sheath is derived from the lower domain, below the cross-zone, and the lamina is derived from the upper domain (Figure 3L). Thus, the tepal lamina corresponds to the leaf sheath and the reduced unifacial distal tip of the tepal to the leaf blade (Figure 3J). This close homology between the lamina of the tepal and the leaf sheath is strongly reflected in the gross morphology (compare images in Figure 3J,K,M,N).

In *D. napiforme*, the five sepals also arise in a 2/5 arrangement (data not shown). However, removal of the sepals reveals that five carpel primordia comprising the gynoecium are visible prior to the emergence of the five primary androecial primordia, which alternate with the carpel primordia (Figure 3P). Further primordia arise outside of these inner androecial primordia in a centrifugal direction (Figure 2Q). Only the innermost primordia develop into fertile stamens (Figure 3R); in a mature flower approximately 10 fertile stamens form a single ring around the gynoecium (Figure 3T). Outer primordia develop into sterile staminodes and become increasingly petaloid in a centrifugal direction (Figure 3R), resulting in many white, showy petals in the mature flower that contrast with the green sepals in the mature flower (Figure 3O).

The initiation of several whorls of petal primordia in a centrifugal pattern provides an opportunity to simultaneously examine gene expression in several petals at different stages of development (Figure 3R,S).

### Expression patterns of AGAMOUS, PISTILLATA, APETALA3 orthologs

In *S. portulacastrum*, *SpPI* expression is restricted to the androecium and is absent from the developing carpels and tepals (Figure 4A–D). Expression in the androecium persists throughout later stages of stamen differentiation and is present in both filament and differentiated anther (Figure 4G,H). *SpPI* expression is absent from developing ovules. Similarly, *SpAP3* expression in *S. portulacastrum* is restricted to the androecium and is absent from the developing carpels and tepals (Figure 4E–H) but is strong in developing ovules (Figure 4I). Expression in the androecium persists throughout later stages of stamen differentiation and is present in both filament and differentiated anther (Figure 4G,H). *SpAG* is absent for the tepals expressed early in the development of the androecium and gynoecium (Figure 4J) and persists until late stages of stamen and carpel differentiation (Figure 4K,L). *SpAG* is also strongly expressed in developing ovules (Figure 4L).

In *D. napiforme*, *DnPI* expression is restricted to the androecial primordia that give rise to fertile stamens and staminodes (Figure 5A–F). Expression is initially strong in
all androecial primordial (Figure 5A,B). In fertile stamens, expression is maintained throughout differentiation of the filament and anther (Figure 5C,D). Expression remains strong in the developing filaments and anthers, particularly in the locule wall (Figure 5E). Sections that simultaneously capture petals at varying stages of development reveal temporal variation in expression of DnPl. In primordia that will give rise to outer petals (the andropetals), DnPl expression is initially strong (Figure 5C), but expression becomes restricted to the distal tip of the petals during early development (compare oldest petal P1 with younger outer petal P3 in Figure 5C,E,F). Expression is lost or very weak by the time the staminodes reach approximately 300–400 µm in length. DnAP3 is very similar to DnPl expression — it is also mainly restricted to androecial primordia giving rise to fertile stamens and petals. Expression is similarly strong in all androecial primordia (Figure 5G,H). In fertile stamens, expression is maintained throughout differentiation of the filaments and anthers (Figure 5H,I). In contrast, in primordia giving rise to petals, DnAP3 expression is initially strong but weakens in development of petals (Figure 5I) and becomes restricted in a distal direction (Figure 5H,I), in a comparable manner to DnPl. Expression is lost by the time the petals reach approximately 300–400 µm in length (Figure 5I). DnAP3 is strongly expressed in the ovules (Figure 5J). DnAG is expressed in the meristem prior to the emergence of androecial and gynoecial primordia (Figure 5K).
is strong in the developing carpel and in all organs of the androecium: it is strongly expressed in the primordia that eventually form sterile petals as well as in primordia that give rise to fertile stamens (Figure 5L, M). In petals, DnAG is expressed throughout the organs until after they have reached approximately 100 µm in length (Figure 5N, O). After this point of petal development, expression of DnAG appears to become restricted in a distal direction until expression is restricted to the tip of the maturing sterile staminode (Figure 5N, O). Later in development, this restricted expression is lost (Figure 5N, O). DnAG is strongly expressed in the ovary wall and the developing anthers and ovules (Figure 5F).

**DISCUSSION**

Homeotic transformation of organ identity, mediated by the expression of the canonical petal identity program, has the potential to explain the homoplastic occurrence of petaloid organs, and variation in the spatial expression of petaloid traits (Bowman, 1997; Baum and Whitlock, 1999; Kramer and Jaramillo, 2005). However, the challenge of defining perianth homology at multiple hierarchical levels (Jaramillo and Kramer, 2007) (i.e., positional, historical, morphological, and genetic correspondence) makes such potential difficult to evaluate. Caryophyllales are a useful clade in which to assess the evolutionary role of a homeotic petal identity program, precisely because we can confidently articulate homology at several levels. This is exemplified in Aizoaceae, which exhibit an unequivocal origin of andropetals and a clear transference of function between petaloid organs of distinct historical derivation.

The petaloid tepals of Sesuvioideae and Aizoioideae fulfill criteria of sepal-derived petals (Ronse De Craene, 2008). The tepal primordia are crescent-shaped, have a broad base at
insertion, are supplied by three vascular traces (compared with one trace in stamens), and have a spiral sequence of initiation (Figure 2C). They also differentiate into upper and lower domains (Figure 2F–I), which represents a common morphogenetic feature in the leaves of many angiosperm species (Kaplan, 1973). The green, chlorophyllous sepals of

---

Figure 5. Expression patterns of MADS-box homologs in Delosperma napiforme (A–P). Sepal (Se), Androecium (A), Gynoecium (G), Stamen (St), Filament (Fl), Anther (At), Petal (P), Ovary (Ov). (A) DnPI expression in emerging and developing androecial primordial, absent from gynoecium. (C) enlarged from (B) Expression maintained in anthers and filament; expression in innermost petals (P1 and P2) but weaker than in outermost petal (P3). (E) enlarged from (D) Expression of DnPI weak in P1; restricted to the distal tip of P2 (black arrow) and strong in P3; DnPI expression absent in developing ovules. (F) Expression of DnPI lost in P1 and P2 and restricted to the tip of the outermost petal P3 (black arrow). (G) DnAP3 expression in emerging and developing androecial primordia. (H) DnAP3 expression in developing placenta, expression maintained in anthers and filament; expression in petal 1 weaker than in petals 2 and 3. (I) Expression strong in locule walls, absent in petal 1, expression (black arrows) restricted to distal tip of petal 2, stronger in petal 3. (J) Strong expression of DnAP3 in the ovules. (K) DnAG expression in the gynoecium and androecium primordia. (L, M) DnAG expression in gynoecium and all fertile and sterile members of the androecium. (N) DnAG expression strong in anthers and filaments of inner stamens, and ovules; absent from developing petal 1; expression restricted to the tip of petal 2 (black arrow), strong expression in emerging petal 3 (black arrow). (O) Expression of DnAG in anthers; absent now in petals 1 and 2; expression restricted to distal tip of petal 3 (black arrow). (P) Strong expression of DnAG in developing ovules.
Mesembryanthemoideae and Ruschioideae are clearly homologous with the petaloid tepals and exhibit similar patterns of initiation, insertion, vasculature, and differentiation into upper and lower domains (Payer, 1857). The petals of Mesembryanthemoideae and Ruschioideae fulfill the criteria of stamen-derived petals. They possess a singular vascular trace, a narrow point of insertion, and both petals and stamens develop from primordia that are initiated centrifugally (Hofmann, 1994). Members of Aizooidae (e.g., *Gunniiopsis*) exhibit centrifugal androecial initiation but all primordia develop into fertile stamens (Hofmann, 1994); positionally homologous primordia can therefore develop into either petals or stamens. Finally, organs are present that are intermediate between stamens and petals, as floral organs developing closest to the fertile stamens are increasingly filamentous while outermost organs are increasingly laminar.

Our morphological data suggest an interesting correspondence between the petaloid lamina and leaf sheath. The tepals differentiate into an upper and lower zone early in development: differentiation is a well-established first stage in the morphogenesis of the leaves of many species of angiosperms (Kaplan, 1973). Tracking the expansion of these two zones during development reveals that the petaloid lamina of the tepal is derived from the lower zone and thus corresponds to the leaf sheath in the vegetative context there is also correspondence between the delicate petaloid margins (e.g., *Hypertelis*). The correspondence between hyaline margins and petal tissue implies that petal tissues may be formed by different developmental mechanisms depending on the historical derivation of the petaloid organ.

However, given the concept of a conserved petal identity program within core eudicots, and a hypothesis of heterotopy in petal evolution, we expected similarities in MADS-box gene expression between petaloid tepals and petaloid stamens in members of Aizoaceae. Contrary to this expectation, *SpAP3* and *SpPI* are not expressed at any point in the development of the petaloid tepal (in *S. portulacastrum*) with expression of these genes restricted to the stamens while *DnAP3* and *DnPI* are transiently expressed in the petaloid staminodes (in *D. napiforme*). Similarly, *SpAG* is not expressed in petaloid tepals while *DnAG* is transiently expressed in petaloid staminodes. Therefore, at the level of *AP3*, *PI* and *AG* homolog gene expression, we find no evidence for homology between the petaloid tepals and petaloid staminodes of *S. portulacastrum* and *D. napiforme*, (see Figure 6) and by inference to other members of Aizoaceae with similar floral types.

These data demonstrate an absence of *AP3* and *PI* homologs at the transcriptional level in a petaloid organ within a core eudicot lineage. Petal identity and expression of *AP3/PI* homologs have been similarly decoupled in angiosperm taxa outside of the core eudicots. For example, in the magnoliid *Aristolochia*, *AP3* and *PI* homologs are expressed only late in the differentiation of the perianth (Jaramillo and Kramer, 2004); *AP3* and *PI* homologs are not expressed in the outermost petaloid perianth whorl in flowers of the monocot *Asparagus* (Park et al., 2003, 2004); and *AqvP1* is required for petal identity in the second whorl but not the petaloid first whorl in the basal eudicot *Aquilegia* (Kramer et al., 2007). The absence of *SpAP3* and *SpPI* activity in the petaloid tepals of *Sesuvium portulacastrum* extends these observations to the core eudicots, and contradicts early hypotheses (Kramer and Irish, 1999, 2000) that all petaloid organs in core eudicots might share a common *AP3*- and *PI*-dependent petal identity pathway.

Figure 6. Summary of gene expression patterns found in a core eudicot flower such as Arabidopsis versus the two floral types of Aizoaceae; petaloid tepal floral type (e.g. *Sesuvium*) and andropetal floral type (e.g. *Delosperma*). Se, Sepal; Pe, Petal; Te, Tepal; St, Stamen; Ca, Carpel. A: A-class, B: B-class, C: C-class, Asterix*: transient early gene expression.
The distal restriction and subsequent loss of DnAP3 and DnPI expression within the petaloid staminodes is similar to that reported for some petaloid organs in Ranunculales (Kramer and Irish, 1999). Constant expression until late stages of petal development is necessary for the maintenance of petal identity in A. thaliana and A. majus (Bowman et al., 1991; Sommer et al., 1991; Zachgo et al., 1995). Given inconstant expression of B-class MADS-box homologs, it was suggested that the petal identity program differs within Ranunculales, either because of alternative petal evolution in some taxa, or because Ranunculales arose prior to the fixation of a petal identity program. Following this logic, the detection of a similar inconstant pattern of gene expression within the core eudicots argues against an ancestral petal identity program that has simply been turned back on in the petaloid staminodes of Aizoaceae.

In model organisms (e.g. Arabidopsis) the ability of AP3 and PI homologs to specify petal identity is contingent on the absence of the C-class MADS-box gene homologs (Jack et al., 1992; Mizukami and Ma, 1992; Bradley et al., 1993; Goto and Meyerowitz, 1994). Here, DnAG is expressed concurrently with the B-class MADS-box homologs and exhibits identical early expression, distal restriction, and loss in the petaloid staminodes. This co-expression of DnAP3 and DnPI with DnAG suggests that DnAP3 and DnPI expression is not implicated in petal identity. Rather, coincident early loss of B- and C-class MADS-box gene expression in the development of petaloid staminodes more likely signifies deactivation of the stamen developmental program. These data emphasize the importance of observing AG homolog expression when interpreting variable patterns of B-class gene expression.

These two unique patterns of gene expression in petaloid organs of core eudicot taxa (see Figure 6) can be simply explained in an evolutionary context. Early in the evolution of the Caryophyllales, one whorl of perianth was lost such that the uniseriate perianth of the Sesuvioideae/Aizooideae type predominates. Assuming loss of the petal whorl, the tepal whorl in a simple perianth of Caryophyllales (as occurs in Sesuvium portulacastrum) is historically homologous with the sepal whorl of other core eudicots (Hoffmann, 1994). We suggest that the absence of SpAP3 and SpPI in the petaloid tepals of Sesuvium portulacastrum is a result of their derivation from core eudicot sepal organs, which also do not require B-class MADS-box gene activity. The subsequent return to the petaloid condition was consequently achieved independently of the AP3- and PI-dependent pathway. Numerous lineages across the Caryophyllales possess petaloid organs that are historically homologous with the tepals of Sesuvioideae and Aizooideae. Consequently, the sepal-derived interpretation of these gene expression patterns predicts further cases of petal development independent of AP3 and PI homologs; for example, in the tepals of Molluginaceae, Nyctaginaceae, Hypertelis, and the inner perianth whorls of the Portulacineae.

Across the Caryophyllales, multiple origins of a differentiated perianth entail the recruitment of staminodes to function as petals (Brockington et al., 2009). Although an ancestral petal identity program might be iteratively recruited in this pattern of perianth evolution, our data do not support this model for the recurrent evolution of petals in the Caryophyllales. The absence of AP3 and PI homolog expression in petaloid tepals and sepals in Aizoaceae implies that an AP3- and PI-dependent petal identity pathway was not retained by deployment to the uniseriate perianth. Without such spatial redeployment, it is unclear how an AP3- and PI-dependent petal identity pathway could have been maintained following petal loss. It is more likely that loss of petals resulted in loss of a former petal identity pathway dependent on AP3 and PI homologs; this loss is then reflected in the gene expression patterns of andropetals in Aizoaceae, which are novel floral organs exhibiting a unique developmental genetic program compared with the petals of other core eudicots.

Considerable data have now been collated on the expression patterns of AP3 and PI homologs across angiosperms, but a collective interpretation of these data is not easy. Some authors have proposed a correlation between the expression of AP3 and PI homologs and petaloidy through ‘sliding’ (Bowman, 1997; Kramer et al., 2003) or ‘fading borders’ (Solits et al., 2007) while others have explored the link between variation in gene expression patterns and ‘complexity’ in petal morphology (Kramer and Jaramillo, 2005). More recently it has been suggested that AP3 and PI homologs are conserved primarily to specify regional domains in the flower rather than organ identity per se (Drea et al., 2007; Whipple et al., 2007). Finally, in our analysis we propose that observed gene expression patterns in Aizoaceae are best explained through the distinct historical derivation of the petaloid organs in the context of an unusual evolutionary history. These models are not mutually exclusive as each may hold for different floral structures, taxonomic groups, phylogenetic levels, and episodes of evolutionary history. Nevertheless, in the absence of evidence for heterotopy at the level of MADS-box genes, one future challenge lies in determining which genes are responsible for the specification of novel petals in the Caryophyllales.

EXPERIMENTAL PROCEDURES

Plant material

Aptenia cordifolia, Delosperma napiforme, and Tetragonia tetragonooides were grown in a greenhouse at the University of Florida, USA. Flowering material of Sesuvium portulacastrum was collected from Cedar Key, Florida, USA. Voucheried specimens are deposited at the herbarium at the Florida Museum of Natural History (FLAS).
Isolation of MADS-box genes from Aizoaceae

Total RNA was extracted from floral buds of various stages of development using the RNeasy extraction kit (Qiagen, http://www.qiagen.com/). cDNA was synthesized using Superscript II (Invitrogen, http://www.invitrogen.com/) according to the manufacturer’s instructions. Amplification of target genes was carried out using degenerate primers and degenerate PCR thermocycling conditions from previously published studies, (B-class genes; Kramer et al., 1998) and, C-class; Stellari, Jaramillo and Kramer, 2004). PCR bands over 500 bp in size were excised from the agarose gel and purified using the Geneclean II Kit (QiBioGene, http://www.qbiogene.com/). Purified DNAs were cloned using the TOPO TA Cloning Kit (Invitrogen). In total, 50–100 clones were screened from each PCR band. Plasmid DNAs were amplified using the Templiphi Cycle (Amersham, http://www5.amershambiosciences.com/) and sequenced were generated on an ABI 3730 XL DNA sequencer (Applied Biosystems Inc., http://www.appliedbiosystems.com/).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Accession numbers for submitted and previously published sequences included in the phylogenetic analyses. Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

REFERENCES


Alternative petal identity in ‘Living stones’ 203


