

VIEWPOINT: PART OF A SPECIAL ISSUE ON FLOWER DEVELOPMENT

Current trends and future directions in flower development research

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Flowers, the reproductive structures of the approximately 400 000 extant species of flowering plants, exist in a tremendous range of forms and sizes, mainly due to developmental differences involving the number, arrangement, size and form of the floral organs of which they consist. However, this tremendous diversity is underpinned by a surprisingly robust basic floral structure in which a central group of carpels forms on an axis of determinate growth, almost invariably surrounded by two successive zones containing stamens and perianth organs, respectively. Over the last 25 years, remarkable progress has been achieved in describing the molecular mechanisms that control almost all aspects of flower development, from the phase change that initiates flowering to the final production of fruits and seeds. However, this work has been performed almost exclusively in a small number of eudicot model species, chief among which is *Arabidopsis thaliana*. Studies of flower development must now be extended to a much wider phylogenetic range of flowering plants and, indeed, to their closest living relatives, the gymnosperms. Studies of further, more wide-ranging models should provide insights that, for various reasons, cannot be obtained by studying the major existing models alone. The use of further models should also help to explain how the first flowering plants evolved from an unknown, although presumably gymnosperm-like ancestor, and rapidly diversified to become the largest major plant group and to dominate the terrestrial flora. The benefits for society of a thorough understanding of flower development are self-evident, as human life depends to a large extent on flowering plants and on the fruits and seeds they produce. In this preface to the Special Issue, we introduce eleven articles on flower development, representing work in both established and further models, including gymnosperms. We also present some of our own views on current trends and future directions of the flower development field.

Key words: Flower development, floral structure, inflorescence architecture, molecular control, angiosperm evolution, *Arabidopsis thaliana*, CRABS CLAW, MADS-box, RAV, REM, TERMINAL FLOWER, WOX.

INTRODUCTION

What is a flower ...

Flowers are the sexual reproductive structures, usually arranged as a group of lateral organs that arise from a short axis of determinate growth, of the flowering plants or angiosperms. Flowers differ in numerous respects from the reproductive structures of the remaining seed plants, the gymnosperms, although exceptions can be found for most of the features generally ascribed to flowers, making an all-encompassing definition of the flower difficult to produce. The flowers of most angiosperm species contain both male and female reproductive organs and, with a very small number of exceptions, the female organs in such bisexual species occupy a central position in the flower, surrounded by the male organs. By contrast, gymnosperm reproductive axes are functionally unisexual (although a few gymnosperms, such as *Welwitschia*, contain non-functional organs of one sex in cones of the opposite sex). Most flowers contain a perianth of sterile organs surrounding their reproductive organs and this may contain a single type or several types of floral organ, including: tepals, sepals, petals, nectar spurs or such highly specialized organs as the paleas and lemmas of grass flowers. Perianth organs often play roles in protecting

the flower bud and/or in the attraction of animal pollinators. Importantly, the ovules of flowering plants are enclosed within a unique type of female reproductive organ, the carpel, whereas the ovules in most gymnosperms arise as naked structures, often in the axil of a cone-scale or fertile, leaf-like organ. The presence of the carpel in angiosperms forces pollen tubes to grow, on their way to reach the ovules, through specialized female tissues, or in some cases through a secretion-filled aperture or canal (Endress and Igersheim, 2000). Carpel tissues provide pollen tube guidance mechanisms to ensure efficient fertilization and in many angiosperms these tissues also enable the selection of compatible pollen, thereby preventing very wide hybridizations and/or self-fertilization. Carpels may arise separately or be fused together in a syncarpous gynoecium (Armbruster *et al.*, 2002). The syncarpous arrangement typically provides a compitum (a common route of pollen growth through which any pollen grain may attain any ovule) and in some cases also provides a larger landing platform for pollinating insects. Syncarpy also enables the generation of larger and more elaborate fruits. Only flowering plants produce fruits, which are derived by the post-fertilization development of ovary wall tissues in the carpel or gynoecium and represent an enormous source of developmental biodiversity.

... And why study its development?

Studies of flower development form a major area of modern plant science. Flowers, and the fruits and seeds they produce, are of key economic interest in many angiosperms, while a vast amount of fascinating biology also occurs in these structures, making them of enormous fundamental interest for plant scientists. Evolutionary aspects of flowers also provide a rich area of study as, while the underlying structure of the flower has been remarkably conserved through evolution, the number, form and arrangement of floral organs has diversified extensively to produce a tremendous range of form among the approximately 400 000 species of angiosperms alive today. A further fascinating aspect of the evolution of the angiosperms concerns their apparently abrupt Cretaceous origin from an unknown but presumably gymnosperm-like ancestor: an event that Charles Darwin famously described as an 'abominable mystery' (Friedman, 2009).

It is not possible, in a short viewpoint article such as this, to fully list the range of subject areas that contribute to 'flower development'. However, in the broadest terms, these include the stimuli and mechanisms that induce flowering, the control of inflorescence structure, the patterning of the floral meristem, the development of individual floral organ types, the development of male and female gametophytes, sex determination and other topics related to plant breeding systems, floral symmetry and other topics related to plant–pollinator interactions, and finally the development of fruits and seeds. All of these subject areas include evolutionary aspects, due in part to the considerable diversity of flower structure to be found throughout the angiosperms. From a technical point of view, studies of flower development may involve the complete range of methods open to modern biology: *in vivo* and *in vitro* experimentation, field work, paleontology, macroscopic and microscopic observation at all levels, genetics, genomics, proteomics, molecular biology, biochemistry, biophysics, bioinformatics and phylogenetics.

This Special Issue brings together both primary and review papers from an eclectic mix of research on a number of the above-listed topics. Below, we provide a brief introduction to the work presented and also contribute some personal views on the directions the field of flower development is taking.

FROM THE FIRST FLOWERS TO THE LAST FRUITS: THE WORK PRESENTED IN THIS ISSUE

How it all started

To throw some light on the mysterious origin of the flower, Gramzow *et al.* (2014) analyse the angiosperms' closest living relatives, the gymnosperms. Gymnosperm genomes are notoriously large, but three of these are now fully sequenced, making it possible for the first time to make definitive lists of flower-related gene orthologues in gymnosperms. Gramzow *et al.* focus particularly on the MADS-box family of transcription factors, which plays a central role in flower development. They use sophisticated phylogenetic and bioinformatics-based approaches to reconstruct the minimal set of MADS-box genes and proteins present in the most recent common ancestors of the living gymnosperms and the living seed plants (angiosperms + gymnosperms). Their work thus provides a starting point to understand the genomic changes that contributed to the origin of the flower through elaboration of the

MADS-box family in the angiosperm stem lineage, after its separation from that of the living gymnosperms. Melzer *et al.* (2014) take a complementary approach by analysing the intermolecular interactions of MADS-box proteins in both early- and later-emerging angiosperm groups. Their work thus helps to explain how, at the biochemical level, MADS-box proteins functioned in the first flowering plants and how these ancestral functions became canalized later in angiosperm evolution.

Timing is everything

One of the key choices a plant has to make is when to reproduce. Not surprisingly, subtle and complex mechanisms have evolved, triggered by a combination of environmental and endogenous cues, which permit plants to flower at the optimum time to maximize their reproductive potential. Shrestha *et al.* (2014) compare mechanisms for the regulation of flowering time in response to day-length in two model species, *Arabidopsis thaliana* and rice. Interestingly, they review data on the biogeography of cultivated rice varieties that carry different allelic forms of flowering regulators, which perfectly illustrates the ecological and economic significance of studying the biodiversity of flowering mechanisms. Matías-Hernández *et al.* (2014) then focus on the role of the *RAV* gene family in the control of flowering in *Arabidopsis* and also review the role of these genes in biological processes outside the flower, illustrating the way in which homologous genes and pathways are frequently recruited to a range of different functions in the course of evolution.

Flower arranging

The decision to flower might, depending on the species, result in the production of any one of a number of distinct floral architectures, including racemes, cymes, panicles, umbels, capitula and single terminal flowers. Interestingly, subtle differences in homologous gene networks appear to be responsible for generating such different inflorescence structures, which are variously adapted to different types of breeding systems, pollination vectors, fruit structures and seed dissemination mechanisms. Fernández-Nohales *et al.* (2014) identify a novel mutation that reveals one of the points at which small RNA metabolism is involved in the control of the racemous inflorescence architecture of *Arabidopsis* by repressing the key regulator *TERMINAL FLOWER1*. In a completely different type of study, Castaño *et al.* (2014) provide the first detailed description of an unusual inflorescence structure in the monoecious palm *Gaussia attenuata*, which contains small unisexual flower clusters termed acervuli. Their data should help reveal the various transitions in plant breeding systems that have occurred in the tribe Chamaedoreae to which *Gaussia* belongs.

Patterning the flower

Each flower in an inflorescence typically contains several different types of organ that arise in a precise phyllotaxy from specific domains of the floral meristem. The blueprint of the mature flower is thus laid down by early patterning processes that occur within the floral meristem. The floral organs of basally-diverging angiosperms typically arise in a spiral pattern. Such species often contain rather variable numbers of floral organs, and

intermediate organ types are frequently present in their flowers at boundary zones. By contrast, in most later-emerging angiosperm groups, whorled arrangements predominate in which organs of invariable number and unequivocal identity arise from the floral meristem in concentric rings and grow to occupy specific positions in the mature flower. Such a whorled arrangement is typically a prerequisite for the evolution of bilateral or other complex forms of floral symmetry. Such symmetries frequently lead to the formation of different organ morphologies within a single whorl, such as dorsal and ventral petals of distinct shapes and sizes. In addition, the congenital fusion of floral organs to produce, for example, corolla tubes or syncarpous gynoecia, is established by patterning events in the floral meristem. Engelhorn *et al.* (2014) focus on floral patterning in arabidopsis by analysing genetic interactions involving *ULTRAPETALAI* and *LEAFY*, and conclude that these regulators operate in separate pathways that converge to induce *AGAMOUS* expression in the centre of the floral meristem. Mantegazza *et al.* (2014) provide an extensive initial analysis of the *REM* gene family, for which few functional data were previously available. They postulate that these genes may play redundant roles in both floral patterning and early flower development.

Inside the floral organs

In contrast to certain other topics in flower development, comparatively fragmentary information is as yet available on the mechanisms that control development within each of the distinct organ types that make up the flower. Floral reproductive organs (carpels and stamens) are structurally and developmentally complex, while sterile perianth organs can also, in some cases, form quite complex structures such as nectar spurs or corolla tubes. Perianth organs may additionally contain regions of pigmentation, secretory tissues and surface micro-structures that function in interactions with animal pollinators, and which also add to their complexity. In addition, the basic forces and mechanisms that control growth in all plant organs, including those of the flower, are far from completely understood. The study of floral organ development thus represents a huge area of plant biology whose elucidation will require multidisciplinary approaches, particularly to unite high-level molecular-genetic mechanisms to the physical forces that ultimately control plant growth at the cellular level. Despite these difficulties, numerous groups are using a range of methods to make increasingly good progress in understanding floral organ development. Beauzamy *et al.* (2014) look into one very important physical force that contributes directly to growth, namely turgor pressure. They review the importance of turgor pressure in plant development and present three case studies relating to the flower. These authors particularly emphasize progress in, and further needs for, technical advances for the measurement of differential pressure within living tissues. At the higher level of transcriptional control mechanisms, Fourquin *et al.* (2014) analyse the orthologs from two legume species, *Pisum sativum* and *Medicago truncatula*, of the CRABS CLAW (CRC) transcription factor, which regulates multiple aspects of carpel and nectary development in arabidopsis. They demonstrate the conservation of key elements of CRC function between arabidopsis and legume species, but also reveal novel roles of legume CRC orthologs in vascular tissues that were not apparent from arabidopsis-based

studies. Again focusing of the development of floral organs, as well as on earlier events in flower development and inflorescence architecture, Costanzo *et al.* (2014) review the roles of the *WOX* gene family. These authors particularly emphasize the importance of obtaining functional data from a range of model species to uncover all the biological functions of a given gene or family.

MORE MODELS REQUIRED

Until recently, the vast majority of molecular-genetic studies of flower development, which have succeeded in associating floral phenotypes with underlying regulatory genes and proteins, were performed in only a handful of species. Of these, arabidopsis and *Antirrhinum majus* (Fig. 1) were by far the most productive experimental systems in the early years, thanks initially to efficient forward genetic screening, coupled with map-based cloning and/or insertional mutagenesis. *Zea mays* (maize) also formed an important early molecular-genetic model, and genes involved in sex determination and other important reproductive traits were first identified in that species (e.g. Calderon-Urrea and Dellaporta, 1999). Over the last decade or so, a larger number of angiosperm species has been developed as molecular-genetic models including, for example, *Oryza sativa* (rice), *Triticum aestivum* (wheat) and *Brachypodium distachyon* from the monocots, and numerous eudicots including *Petunia × hybrida* (Fig. 1), *Solanum lycopersicum* (tomato), *Capsicum annuum* (sweet pepper), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Medicago truncatula*, *Pisum sativum* (pea), *Lotus japonicus*, *Arabidopsis lyrata*, *Brassica oleracea* and *Cardamine hirsuta*. All these species possess traits that lend themselves to the generation and screening of large mutant collections: reasonably short generation time, manageable physical size, ease of cultivation and good seed-related characteristics (adequate yield, ease of storage, high longevity and uncomplicated germination requirements). Such mutant collections have been formed through chemical, radiation-based or insertional mutagenesis, and screened using a range of forward and reverse genetic techniques. In particular, recent developments of the TILLING procedure (Wang *et al.*, 2012) allow the reverse-genetic identification of chemically generated point mutations in an increasing range of model species.

Despite the growing number of molecular-genetic angiosperm models, the taxonomic coverage of these is far from even. Indeed, the model species for which large-scale mutant collections have been established are almost exclusively from the rosid and asterid clades of the eudicots or from Poaceae (grasses) in the monocots. No molecular-genetic models have been established in the early-diverging ANA-grade (Amborellales, Nymphaeales and Austrobaileyales) angiosperms or in gymnosperms: two groups of particular relevance to studies of the origin of the flower (Viallette-Guiraud *et al.*, 2011). Furthermore, no molecular-genetic models have yet been developed in the moderately large magnoliid clade, whose stem lineage appears to have diverged prior to the split between eudicots and monocots (Bremer *et al.*, 2009). It should also be noted that, of the existing molecular genetic models, arabidopsis is by far the best developed, with unprecedented resources for the isolation of mutants and the analysis of gene expression, epigenetics, transcriptional and post-transcriptional regulation, etc. Indeed, the extreme concentration of research effort, infrastructure and funding on arabidopsis is

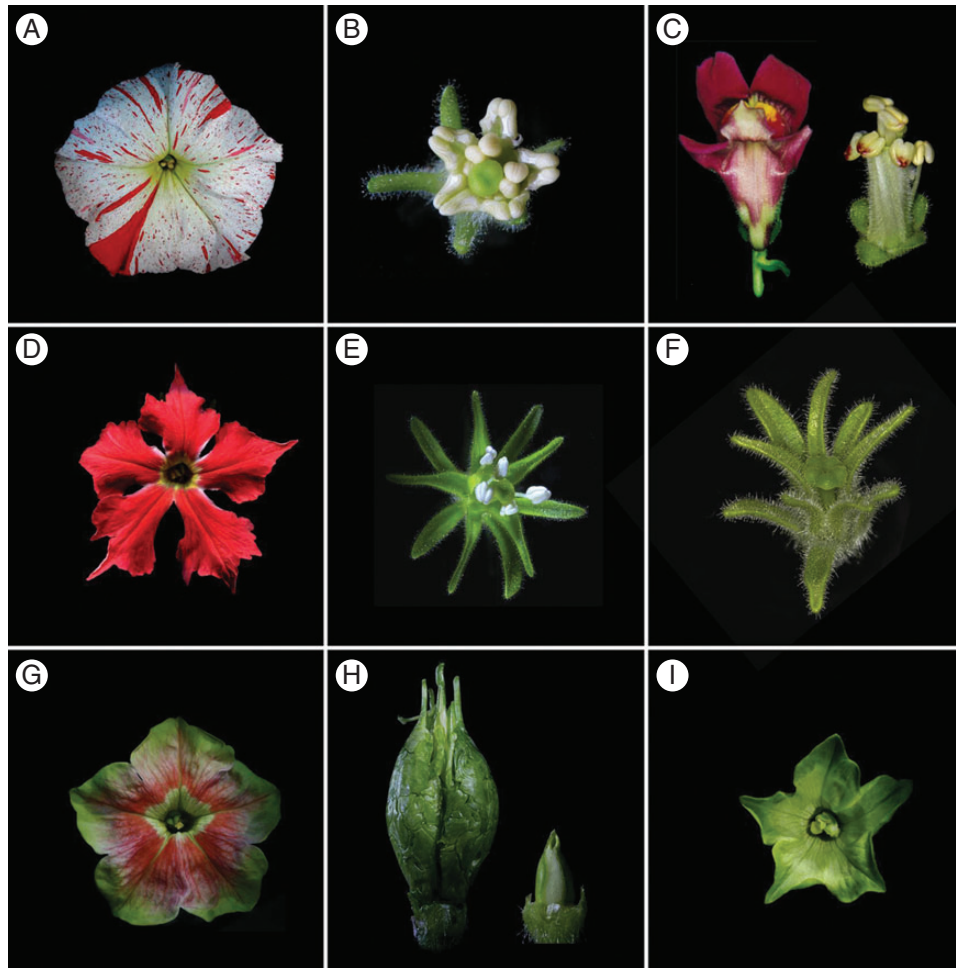


FIG. 1. Floral mutant phenotypes in *Petunia × hybrida* and *Antirrhinum majus*, two well-developed eudicot models for studies of flower development. (A) *Petunia* W138 high-copy transposon line for insertion mutagenesis (Vandenbussche *et al.*, 2008). (B) *Petunia blind* (*bl*) mutants, and (C) *fistulata* (*fis*) mutants (*fis* right; wildtype, WT, left) in snapdragon display an A-class mutant phenotype (homeotic conversion of petals to stamens due to expansion of the C-function). Surprisingly, *FIS* and *BL* encode an orthologous microRNA of the MIR169 family, demonstrating molecular divergence of the A-function compared to arabidopsis (Cartolano *et al.*, 2007). (D) Flower of the *maw* mutant showing defects in petal fusion (Vandenbussche *et al.*, 2009). (E) *phdef* mutants display a single whorl B-class phenotype due to rescue of stamen identity by PhTM6, an ancestral B-class protein present in most flowering species, but lost in arabidopsis. (F) Full B-class phenotype in *phdef tm6* double mutants (Vandenbussche *et al.*, 2004; Rijpkema *et al.*, 2006). (G) *fbp2* (SEP3 ortholog) mutants display clear defects as a single mutant. (H) Complete ovule-to-sepal conversion in *fbp2 fbp5* ovaries (left) compared to WT (right) provides direct evidence for the requirement of SEP proteins in ovule development (Vandenbussche *et al.*, 2003). (I) The phenotype of *fbp2 agl6* flowers (compare with G) reveals functional overlap between AGL6 and SEP MADS-box transcription factors (Rijpkema *et al.*, 2009).

probably one of the main reasons why the plant evo-devo field, which is principally based on inter-species comparisons, remains underdeveloped compared to its animal counterpart.

The advantages of broadening the range

A better understanding of flower development throughout the angiosperms will only be achieved by broadening the range of molecular-genetic model plants available for study and, in parallel, deepening the resources available in some of the existing model systems to rival those currently available in arabidopsis. There are numerous reasons why the study of additional models should provide unique insights into flower development and other developmental processes. Firstly, plant genomes are the result of a complex history of whole-genome or large-scale duplication events, often followed by further, local duplications.

Gene and genome duplications frequently lead to genetic redundancy, with the result that many gene functions cannot easily be revealed by classical genetic screening. Indeed, such redundancy is probably the main reason why the majority of arabidopsis genes remain to be functionally characterized. However, gene and genome duplication events arise in an essentially random manner. Consequently, genetic functions that are masked by redundancy in one species may be uncovered in a sufficiently distant species that will probably contain very different sets of redundant and unique gene functions.

A second reason for broadening the range of models used in plant molecular-genetics concerns genes that have been lost in existing models. Gene losses follow distinct patterns in different plant lineages, as do duplications. Accordingly, arabidopsis appears to have lost a number of genes that play important developmental roles in other species (e.g. Rijpkema *et al.* 2006; Lloyd

and Davies, 2013) and may indeed do so in the majority of the extant angiosperms. Clearly, studies in a broad range of molecular-genetic models are required to gain insight into the roles of such genes, which happen to have been lost in some of the most-studied current models.

A third and rather obvious reason for studying numerous model angiosperms is the presence of characters of interest in some plants that are absent in others. In general, models of wide-spread application are chosen for their representivity, which often implies the absence of specific or unusual features. Thus, typical model species may lack interesting floral features such as extra perianth whorls, particular forms of symmetry, petal microstructures that cause structural colour, monoecy, dioecy, post-genital carpel fusion and floral resupination, etc. The study of such specific floral characteristics clearly requires the use of specific molecular-genetic models, chosen for the presence of characters of interest. In addition, many such specific characteristics have clearly been acquired independently in different plant clades. For example, such development traits as dioecy may be regulated by different sets of genes, even in closely related genera. Occasionally, traits of particular interest may be limited to species that are poorly adapted as models, and the particular problems of studying these ‘awkward plants’ are described in the following section.

Awkward plants

Although model plants that are well suited to molecular-genetic analyses are invaluable for the study of flower development, plant scientists frequently need to work on species that are poorly adapted to such methods. Such non-model species may, for example, have a very large physical size, or a long life cycle, or require growth conditions that are difficult to provide. They may also be refractory to genetic transformation, obligately outbreeding, or produce seed in very small quantities or with poor storage characteristics. The need to study such non-model species may result from their key phylogenetic positions, their high economic or strategic importance, or because they possess traits of particular interest that are absent from more tractable species (Fig. 2). To study flower development in such interesting but awkward plants, a number of options are available.

Next-generation sequencing (NGS)-based methods for gene identification in non-models. In species whose life cycle or physical size is not adapted to mutant screening, or in which map-based cloning and insertional mutagenesis cannot be performed, mutant-based methods of gene-identification are not available. However, NGS methods are beginning provide solutions to the problems of gene identification in such non-models. For example, Muyle *et al.* (2012) have developed methods based on RNA-seq data from a population of plants that can be used to identify genes present at or closely linked to sex-determination loci, which have consequently undergone an arrest of genetic recombination. These techniques can be used to identify the regulators of sex, and to analyse the evolution of sex chromosomes, in any dioecious species, with no prior knowledge of the genes involved.

In many cases, it may be useful to determine whether a particular developmental mechanism is conserved between model and non-model species. As relevant mutant or knock-down plants are unlikely to be available in non-model species of interest,

other approaches are needed. In such cases, the availability of the complete genome sequence of non-model species, facilitated by the advent of NGS technology, can be invaluable in identifying from these the close relatives of genes of interest from model species. Once such candidate genes have been identified, detailed studies of spatial and temporal gene expression can be undertaken to determine which of these might play conserved roles with their model plant homologues, bearing in mind that switches in function between closely related, although non-orthologous, genes can occur as a result of shifting patterns of redundancy in multi-gene families (reviewed by Airoidi and Davies, 2012).

Biophysical and other methods to provide functional data in non-models. Phylogenetic and expression data, as mentioned above, may provide support for a particular hypothesis, but arguments based on such data alone fall considerably short of proof of the conservation of gene function. One possible way forward in these cases is to analyse, in both model and non-model species, the biochemical properties of regulatory molecules of interest. For example, the DNA-binding properties of transcription factors can be investigated by such *in vitro* methods as systematic evolution of ligands by exponential enrichment (SELEX; Wang *et al.*, 2011) and protein-binding microarrays (Godoy *et al.*, 2011; Franco-Zorrilla *et al.*, 2014) to produce position–weight matrices (PWMs) that represent the DNA-binding preferences of the factor of interest. PWMs from model and non-model species can be compared to determine likely conservation of function, and these can also be used to scan, in a procedure known as SELEX-seq, the genomic sequences of their native species to determine conservation and differences in likely transcription factor–target gene interactions. Protein–protein interactions involving transcription factors and other classes of regulatory molecules involved in flower development can be assayed by a whole range of *in vitro* and *in vivo* methods including yeast-2-hybrid, bimolecular fluorescence complementation (BiFC), gel-shift and surface plasmon resonance analyses.

The types of biophysical methods mentioned above are of particular use in studies of the evolution of developmental processes, which frequently involve non-model species that occupy important phylogenetic positions. Interestingly, comparison of the biochemical properties of proteins in an evo-devo context is not limited to present-day regulators. It is possible, in a procedure known as protein resurrection, to use specialized phylogenetic methods to reconstruct ancestral protein sequences from given nodes on a molecular phylogeny (Thornton and Bridgham, 2007). Sequences encoding these ancestral proteins can then be reconstructed from long, double-stranded oligonucleotides, and inserted into expression or transformation vectors for protein production or plant transformation, respectively. The resulting resurrected ancestral proteins can be assayed using the same range of *in vitro* and *in vivo* methods as their descendant molecules from present-day species. Protein resurrection has not yet been extensively used to investigate the evolution of flower development, although its use in other domains indicates the potential of this method, which has, for example, been used to conclude that a common ancestor of dinosaurs, pterosaurs, birds and crocodylians possessed infra-red-shifted retinal photoreceptors and that, as a consequence, it probably enjoyed excellent night vision (Chang *et al.*, 2002)!

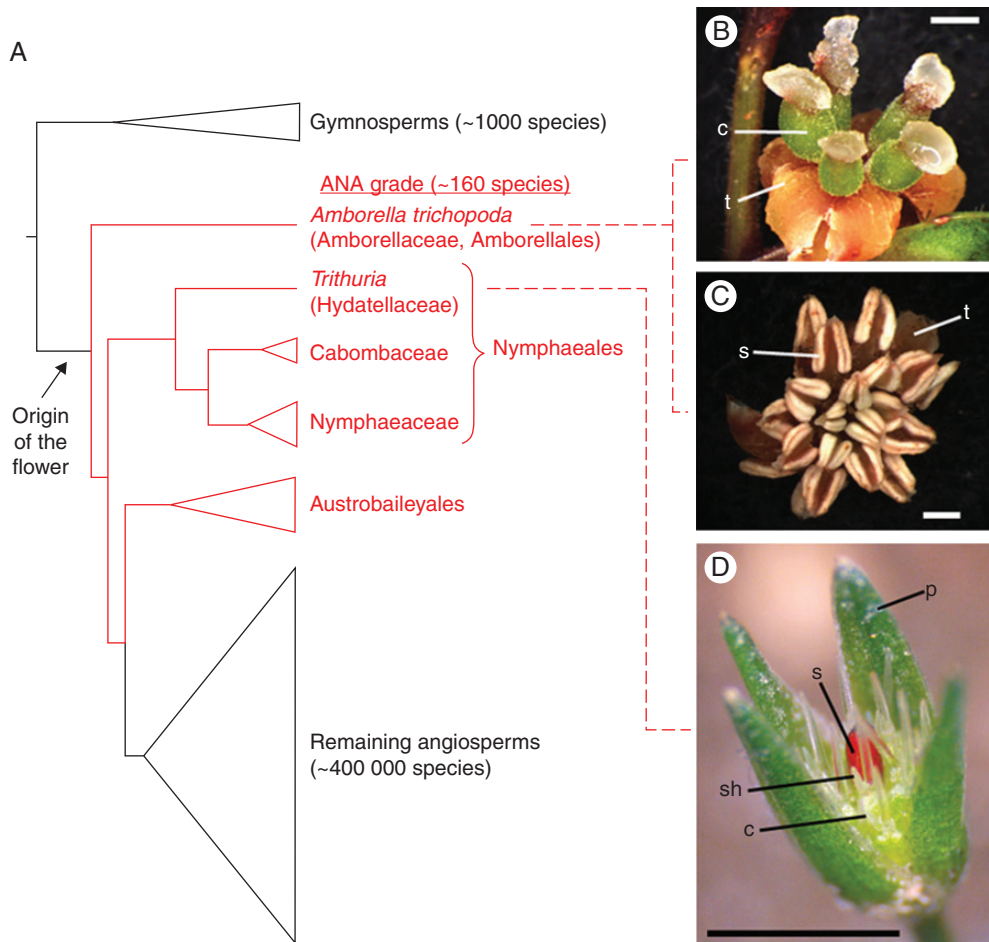


FIG. 2. Two non-model angiosperm species, *Amborella trichopoda* and *Trithuria submersa*, studied both for their early-diverging phylogenetic positions and the presence of unusual floral features: dioecy and inside-out reproductive axes, respectively. (A) A schematic phylogeny of the living seed plants (angiosperms + gymnosperms), showing (in red) the ANA grade (Amborellales, Nymphaeales and Austrobaileyales), representing the three most basally-diverging angiosperm lineages and revealing the key phylogenetic positions of *Amborella trichopoda* and *Trithuria*. (B) Female and (C) male flowers of *Amborella trichopoda*, the probable sister to all other living angiosperms, respectively containing around five carpels (c) and 12–20 stamens (s), surrounded in both cases by a perianth, typically containing 7–8 tepals (t) in females and 9–11 tepals in males (Endress, 2001). (D) A bisexual reproductive unit (RU) of *Trithuria submersa*, one of approximately 12 species of *Trithuria*, the only known genus of Hydatellaceae, which is sister to remaining Nymphaeales (Nymphaeaceae + Cabombaceae). RUs of *T. submersa* typically contain 1–2 stamens (s) at the centre, surrounded by numerous carpels (c) and four bract-like phyllomes (p). Each carpel bears several long stigmatic hairs (sh) at maturity. *Trithuria* RUs have been interpreted as either inverted flowers or condensed inflorescences of extremely reduced flowers (Rudall *et al.*, 2009). Bisexual species of *Trithuria* and the monocot *Lacandonia schismatica* (Rudall, 2008) are the only known examples of such inside-out reproductive axes among the ~400 000 known species of extant flowering plants. Scale bars = 1 mm.

Semi-models

Despite the possibilities afforded by next-generation sequencing and *in vitro* functional studies, it remains desirable, in most studies of plant development, to link genes to phenotypes through mutants, gene knock-downs and mis-expression analyses, etc. Although not all plant species may be amenable to forward or reverse genetic screens, which typically require the generation of large mutant collections and the growth of many thousands of individuals, a number of technologies are available to provide functional-genetic data in species that are not particularly well adapted as models, making of these what has been termed 'semi-models' (Frohlich, 2006). Plant genetic transformation makes many possibilities available, from gene knock-down through RNAi and artificial micro-RNAs to mis-expression analyses using recombinant promoter-coding sequence fusions.

Although generation time is an issue in some species, particularly where flower phenotypes are of primary interest, plant transformation may be still worth attempting to gain insight into flower development. If a species can be transformed, positive regulators of flowering might be co-transformed with genes of interest to circumvent an otherwise long juvenile phase (Weigel and Nilsson, 1995). Interestingly, even some gymnosperms are amenable to genetic transformation using *Agrobacterium*-based protocols (Tang *et al.*, 2007), and the absence of published transformation protocols in a given case may reflect a lack of previous studies, rather than a species that is refractory to transformation. If transformation protocols are available or can be established for a species of interest, methods for homologous recombination involving the use of transcription activator-like effector nucleases (TALENs; Chen and Gao, 2013) now enable precise gene replacements to be

performed. This method can, therefore, be used to generate null mutations, or any other precise mutations that may be required, in species in which mutant screening is technically impossible.

A further possibility to obtain gene functional data is made available through the use of virus-induced gene silencing (VIGS). A number of VIGS vectors with various different spectra of host plant species have been developed (Senthil-Kumar and Mysore, 2011; Burch-Smith *et al.*, 2004). An increasing number of studies of flower development make use of VIGS technology, which has notably been used to provide much-needed functional data in basal eudicots, thus providing an external reference for the evolutionary processes within the core eudicots – a group containing > 300 000 species (Hileman *et al.*, 2005; Gould and Kramer, 2007; Wege *et al.*, 2007) and representing the majority of all extant flowering plants.

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LITERATURE CITED

- Airoldi CA, Davies B. 2012. Gene duplication and the evolution of plant MADS-box transcription factors. *Journal of Genetics and Genomics* **39**: 157–165.
- Armbruster WS, Debevec EM, Willson MF. 2002. Evolution of syncarpy in angiosperms: theoretical and phylogenetic analyses of the effects of carpel fusion on offspring quantity and quality. *Journal of Evolutionary Biology* **15**: 657–672.
- Beauzamy L, Nakayama N, Boudaoud A. 2014. Flowers under pressure: ins and outs of turgor regulation in development. *Annals of Botany* **114**: 1517–1533.
- Bremer B, Bremer K, Chase MW, *et al.* 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.
- Burch-Smith TM, Anderson JC, Martin GB, Dinesh-Kumar SP. 2004. Applications and advantages of virus-induced gene silencing for gene function studies in plants. *Plant Journal* **39**: 734–746.
- Calderon-Urrea A, Dellaporta SL. 1999. Cell death and cell protection genes determine the fate of pistils in maize. *Development* **126**: 435–441.
- Cartolano M, Castillo R, Efreanova N, *et al.* 2007. A conserved microRNA module exerts homeotic control over *Petunia hybrida* and *Antirrhinum majus* floral organ identity. *Nature Genetics* **39**: 901–905.
- Castaño F, Stauffer F, Marquinez X, Crèvecoeur M, Collin M, Pintaud J-C, Tregear J. 2014. Floral structure and development in the monoecious palm *Gaussia attenuata* (Arecaceae; Arecoideae). *Annals of Botany* **114**: 1483–1495.
- Chang BSW, Jonsson K, Kazmi MA, Donoghue MJ, Sakmar TP. 2002. Recreating a functional ancestral archosaur visual pigment. *Molecular Biology and Evolution* **19**: 1483–1489.
- Chen K, Gao C. 2013. TALENs: customizable molecular DNA scissors for genome engineering of plants. *Journal of Genetics and Genomics* **40**: 271–279.
- Costanzo E, Trehin C, Vandenbussche M. 2014. The role of *WOX* genes in flower development. *Annals of Botany* **114**: 1545–1553.
- Endress PK. 2001. The flowers in extant basal angiosperms and inferences on ancestral flowers. *International Journal of Plant Sciences* **162**: 1111–1140.
- Endress PK, Igersheim A. 2000. Gynoecium structure and evolution in basal angiosperms. *International Journal of Plant Sciences* **161**: S211–S223.
- Engelhorn J, Moreau F, Fletcher JC, Carles CC. 2014. ULTRAPETALA1 and LEAFY pathways function independently in specifying identity and determinacy at the Arabidopsis floral meristem. *Annals of Botany* **114**: 1497–1505.
- Fernández-Nohales P, Domenech MJ, Martínez de Alba AE, Micol JL, Ponce MR, Madueño F. 2014. AGO1 controls arabidopsis inflorescence architecture possibly by regulating *TFL1* expression. *Annals of Botany* **114**: 1471–1481.
- Fourquin C, Primo A, Martínez-Fernández I, Huet-Trujillo E, Ferrándiz C. 2014. The *CRC* orthologue from *Pisum sativum* shows conserved functions in carpel morphogenesis and vascular development. *Annals of Botany* **114**: 1535–1544.
- Franco-Zorrilla JM, Lopez-Vidriero I, Carrasco JL, Godoy M, Vera P, Solano R. 2014. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proceedings of the National Academy of Sciences of the USA* **111**: 2367–2372.
- Friedman WE. 2009. The meaning of Darwin's 'abominable mystery'. *American Journal of Botany* **96**: 5–21.
- Frohlich MW. 2006. Recommendations and goals for evo-devo research: scenarios, genetic constraint and developmental homeostasis. *Aliso* **22**: 172–187.
- Gramzow L, Weilandt L, Theißen G. 2014. MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. *Annals of Botany* **114**: 1407–1429.
- Godoy M, Franco-Zorrilla JM, Perez-Perez J, Oliveros JC, Lorenzo O, Solano R. 2011. Improved protein-binding microarrays for the identification of DNA-binding specificities of transcription factors. *Plant Journal* **66**: 700–711.
- Gould B, Kramer EM. 2007. Virus-induced gene silencing as a tool for functional analyses in the emerging model plant *Aquilegia* (columbine, Ranunculaceae). *Plant Methods* **3**: 6.
- Hileman LC, Drea S, de Martino G, Litt A, Irish VF. 2005. Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot species *Papaver somniferum* (opium poppy). *Plant Journal* **44**: 334–341.
- Lloyd JPB, Davies B. 2013. SMG1 is an ancient nonsense-mediated mRNA decay effector. *Plant Journal* **76**: 800–810.
- Mantegazza O, Gregis V, Mendes MA, Morandini P, Alves-Ferreira M, Patreze CM, Nardeli SM, Kater MM, Colombo L. 2014. Analysis of the arabidopsis *REM* gene family predicts functions during flower development. *Annals of Botany* **114**: 1507–1515.
- Matías-Hernández L, Aguilar-Jaramillo AE, Marín-González E, Suárez-López P, Pelaz S. 2014. *RAV* genes: regulation of floral induction and beyond. *Annals of Botany* **114**: 1459–1470.
- Melzer R, Härter A, Rümpler F, Kim S, Soltis PS, Soltis DE, Theißen G. 2014. DEF- and GLO-like proteins may have lost most of their interaction partners during angiosperm evolution. *Annals of Botany* **114**: 1431–1443.
- Muyle A, Zemp N, Deschamps C, Mousset S, Widmer A, Marais GAB. 2012. Rapid *de novo* evolution of X chromosome dosage compensation in *Silene latifolia*, a plant with young sex chromosomes. *Plos Biology* **10**: e1001308.
- Rijkema AS, Royaert S, Zethof J, van der Weerden G, Gerats T, Vandenbussche M. 2006. Analysis of the *Petunia TM6* MADS box gene reveals functional divergence within the DEF/AP3 lineage. *Plant Cell* **18**: 1819–1832.
- Rijkema AS, Zethof J, Gerats T, Vandenbussche M. 2009. The *petunia AGL6* gene has a SEPALLATA-like function in floral patterning. *Plant Journal* **60**: 1–9.
- Rudall PJ. 2008. Fascicles and filamentous structures: comparative ontogeny of morphological novelties in Triuridaceae. *International Journal of Plant Sciences* **169**: 1023–1037.
- Rudall PJ, Remizowa MV, Prenner G, Prychid CJ, Tuckett RE, Sokoloff DD. 2009. Nonflowers near the base of extant angiosperms? Spatiotemporal arrangement of organs in reproductive units of Hydatellaceae and its bearing on the origin of the flower. *American Journal of Botany* **96**: 67–82.
- Senthil-Kumar M, Mysore KS. 2011. New dimensions for VIGS in plant functional genomics. *Trends in Plant Science* **16**: 656–665.
- Shrestha R, Gómez-Ariza J, Brambilla V, Fornara F. Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals. *Annals of Botany* **114**: 1445–1458.
- Tang W, Newton RJ, Weidner DA. 2007. Genetic transformation and gene silencing mediated by multiple copies of a transgene in eastern white pine. *Journal of Experimental Botany* **58**: 545–554.
- Thornton JW, Bridgham JT. 2007. Using ancestral gene resurrection to unravel the evolution of protein function. In: Liberlies DA, ed. *Ancestral sequence reconstruction*. Oxford/New York: Oxford Biosciences, pp. 183–199.
- Vandenbussche M, Zethof J, Souer E, *et al.* 2003. Toward the analysis of the *petunia* MADS box gene family by reverse and forward transposon insertion

- mutagenesis approaches: B, C, and D floral organ identity functions require *SEPALLATA*-like MADS box genes in petunia. *Plant Cell* **15**: 2680–2693.
- Vandenbussche M, Zethof J, Royaert S, Weterings K, Gerats T. 2004.** The duplicated B-class heterodimer model: whorl-specific effects and complex genetic interactions in *Petunia hybrida* flower development. *Plant Cell* **16**: 741–754.
- Vandenbussche M, Janssen A, Zethof J, et al. 2008.** Generation of a 3D indexed *Petunia* insertion database for reverse genetics. *Plant Journal* **54**: 1105–1114.
- Vandenbussche M, Horstman A, Zethof J, Koes R, Rijpkema AS, Gerats T. 2009.** Differential recruitment of WOX transcription factors for lateral development and organ fusion in *Petunia* and *Arabidopsis*. *Plant Cell* **21**: 2269–2283.
- Vialette-Guiraud ACM, Alaux M, Legeai F, et al. 2011.** *Cabomba* as a model for studies of early angiosperm evolution. *Annals of Botany* **108**: 589–598.
- Wang J, Lu J, Gu G, Liu Y. 2011.** *In vitro* DNA-binding profile of transcription factors: methods and new insights. *Journal of Endocrinology* **210**: 15–27.
- Wang TL, Uauy C, Robson F, Till B. 2012.** TILLING in extremis. *Plant Biotechnology Journal* **10**: 761–772.
- Wege S, Scholz A, Gleissberg S, Becker A. 2007.** Highly efficient virus-induced gene silencing (VIGS) in california poppy (*Eschscholzia californica*): an evaluation of VIGS as a strategy to obtain functional data from non-model plants. *Annals of Botany* **100**: 641–649.
- Weigel D, Nilsson O. 1995.** A developmental switch sufficient for flower initiation in diverse plants. *Nature* **377**: 495–500.