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 andlemmas 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. Syncarp 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.
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 flowers 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 racemose 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 monocoeous 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 Chamaedoreeae 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 ULTRAPETALAL1 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 or turgor with 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-Ureña 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 annum (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
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 2007).

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., 2005). (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).
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 widespread application are chosen for their representativity, 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 to 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 Airoldi 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 crocodilians possessed infra-red-shifted retinal photoreceptors and that, as a consequence, it probably enjoyed excellent night vision (Chang et al., 2002)!
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


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


