



The Origin of Angiosperms

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Abstract

The origin of the angiosperms, or flowering plants, is a major question of evolutionary biology, famously described by Charles Darwin as an *abominable mystery*. This group arose from a yet-to-be-identified ancestral lineage and diversified to form over 350,000 species alive today. Recent advances in molecular phylogeny and genetics have combined to provide much information on the origin of the angiosperms and their synapomorphic features, such as the carpel, outer ovule integument, bisexual reproductive axis, and double fertilization. This chapter covers the likely character states of the first angiosperms, their date and place of origin, and possible contenders for close relatives of the angiosperm stem lineage. Later sections show how molecular analyses of living groups are

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providing clues on the origin of angiosperm-specific developmental programs and suggest how this information might be integrated with paleobotanical data to refine hypotheses on the origin of the angiosperms.

Keywords

Angiosperms · Flowering plants · Flower · Carpel · Outer integument · Evo-devo

Introduction: What Are Angiosperms?

The angiosperms are a monophyletic group of seed plants in which the ovules form inside a specialized female reproductive organ termed the carpel. Indeed, the term *angiosperm* derives from the Greek for seeds within a vessel and contrasts with *gymnosperm*, the name given to the remaining seed plants (conifers, etc.), which refers to naked seeds. The angiosperm carpel functions to protect the ovules within it, receive pollens grains, and guide pollen-tube growth towards the ovules. The carpel can also act as a selective barrier to fertilization, thus preventing close inbreeding and/or interspecific crosses. After fertilization, the ovary tissues of the carpel become the fruit, which protects the developing seeds and may finally contribute to their dissemination through a wide variety of mechanisms. The carpel, fruit, and other specific features of the angiosperms are thought to have contributed to the great evolutionary success of this group, which arose abruptly in the early Cretaceous and rapidly expanded to dominate most terrestrial habitats, today numbering over 350,000 species. In parallel, the gymnosperms have declined to only around 1000 living species.

Carpels almost invariably arise at the center of the angiosperm reproductive axis, or flower. While the flower is also considered unique to angiosperms, it is difficult to provide a simple and rigorous definition of this structure, other than the presence with it of the carpel (and even that definition fails to cover unisexual male flowers). Rather, it is easier to list the typical features of flowers, noting both exceptions to these and cases of evolutionary convergence with the angiosperms' sister group, the living gymnosperms.

Flowers, like gymnosperm cones, generally form as compact reproductive axes from which lateral organs arise in close juxtaposition. Flowers in some angiosperms display, like gymnosperm cones, a spiral phyllotaxy leading to somewhat variable number of organs, though the majority of angiosperms show a whorled arrangement, resulting in relatively fixed numbers and positions of floral organs. A typical flower contains, from inside to outside: a gynoecium of carpels, an androecium of pollen-producing stamens, and a perianth of sterile, bract-like organs. The perianth is most frequently divided into an outer whorl of sepals, which typically function to protect the floral bud, and an inner whorl of petals, which may facilitate pollination by interacting with animal pollinators. However, the perianth may alternatively be undifferentiated, in which case its organs can be termed *tepals*. The perianth may also contain specialized organs such as the lodicules of Poaceae (grasses) or the

nectar spurs sporadically present in several families including Orchidaceae, Ranunculaceae, and Asteraceae.

Angiosperm ovules are generally surrounded by two integuments, except in specific groups that show a secondary reduction to one integument, such as the asterids or certain *Prunus* spp. Gymnosperm ovules, by contrast, possess a single integument, though are surrounded by additional tissue layers in Gnetales. Arils, which take on a fruit-like appearance, also surround the ovules and seeds in some gymnosperm groups including *Juniperus*, Taxaceae, and Podocarpaceae. However, true fruits, derived from the ovary tissues of the carpel, are specific to angiosperms. Though gymnosperms contain pollen-producing microsporangia, which may be considered as homologous to the anthers of angiosperm stamens, the latter have a unique 4-loculate structure, not seen in gymnosperms. Angiosperms possess double fertilization (though a distinct form of this is also present in Gnetales), leading to the production of both an embryo and a biparental reserve tissue termed the endosperm.

Angiosperms also show a number of synapomorphies in their vegetative anatomy and ecophysiological traits (Feild and Arens 2005). These include the presence of xylem vessels (though vessels are absent in some basally diverging angiosperms), and net-veined leaves. Interesting, xylem vessels, though with bordered pits resembling those of conifer tracheids, are also present in Gnetales, while net-veined leaves are present in the Gnetales genus *Gnetum*. Novel ecophysiological adaptations to cope particularly with falling atmospheric carbon dioxide concentrations, may have facilitated the rapid expansion of the angiosperms in the Cretaceous (see the chapter “► [The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis Over the Past 450 Ma](#)”). Another factor which almost certainly contributed to the success of the angiosperms was extensive coevolution with insect pollinators, while the relatively short life cycle and rapid growth of early angiosperms may also have helped to improve their fitness by reducing herbivory by the low-browsing herbivorous dinosaurs which came to prominence at that time, as discussed by Willis and McElwain (2013).

When, Where and from What Did the Angiosperms Arise?

Most molecular phylogenetic analyses indicate that the living gymnosperms and angiosperms form two sister clades whose lineages separated some 300 million years ago (MYA). However, the radiation of the extant angiosperms dates from much more recently: most molecular clock estimates place this divergence between 180 and 140 MYA (Bell et al. 2005), while the earliest unequivocal fossilized angiosperm pollen, found in Israel, Morocco and southern England, dates from around 135 MYA (Willis and McElwain 2013). Molecular and fossil data therefore combine to suggest a paleotropical origin for the flowering plants in the late Jurassic/early Cretaceous. Accordingly, we may conclude the living angiosperms to derive from a stem lineage of perhaps some 140 MY in length, from which no other living groups are available for study.

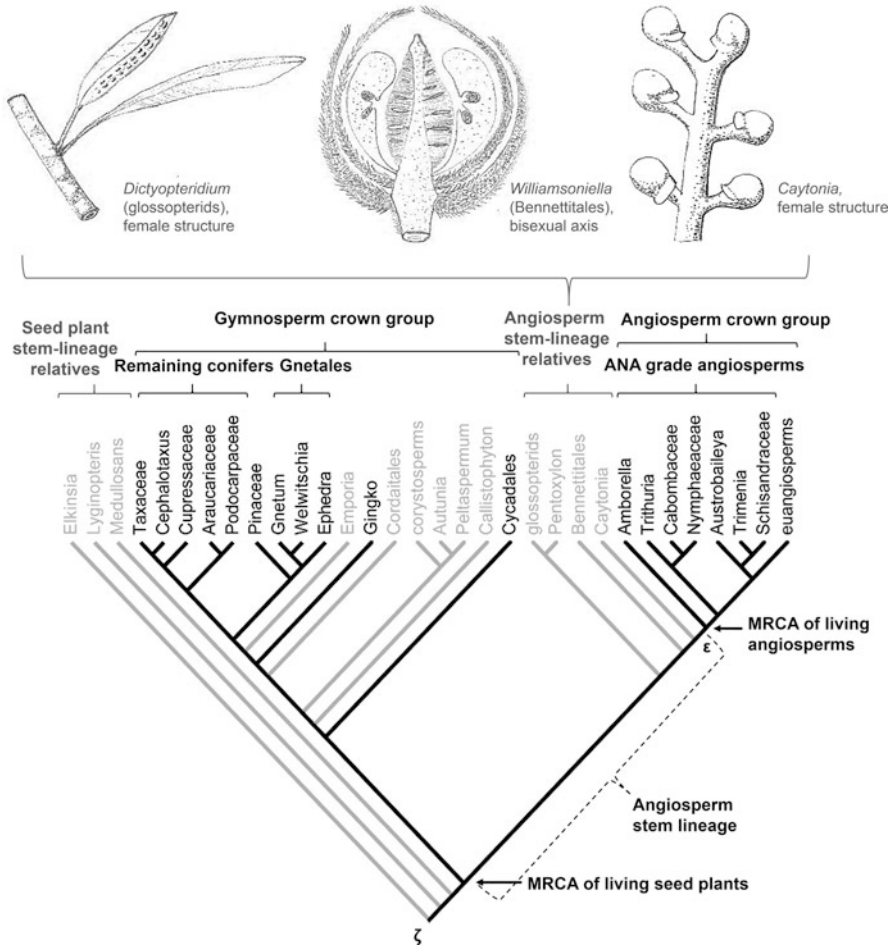


Fig. 1 A phylogeny of extant and extinct seed plants, redrawn from Doyle (2012), focusing on ANA-grade angiosperms and potential angiosperm stem-lineage relatives. Fossil taxa are shown in gray. The approximate positions of the zeta (ζ) and epsilon (ϵ) whole genome duplications, of probable key importance for the origin of seed plants and angiosperms, respectively, are indicated. Reconstructions of three angiosperm stem-lineage relatives are illustrated (of which *Williamsoniella* is shown in median longitudinal section), redrawn from published sources

Molecular phylogenetic studies (Fig. 1) indicate the earliest bifurcations in angiosperm phylogeny to separate the three orders, Amborellales, Nymphaeales, and Austrobaileales, collectively termed the ANA-grade, from all other extant angiosperms, termed the euangiosperms or mesangiosperms. Amborellales are represented by the single living species *Amborella trichopoda*, which is a scrambling shrub, endemic to the understory of the tropical rainforests of New Caledonia. Most molecular phylogenetic analyses suggest Amborellales (and hence *A. trichopoda*) to be sister to all other angiosperms, though a few studies support a slightly different topology in which Amborellales are sister to Nymphaeales (reviewed by Fogliani

et al. 2017). Nymphaeales include three families of aquatic or semiaquatic angiosperms, while Austrobaileyales comprise three families of woody plants, including shrubs, trees, and lianas. By contrast to the situation in angiosperms, the internal phylogeny of living gymnosperms is less clear (Doyle 2012), though in the topology which most frequently emerges, shown in Fig. 1, cycads occupy the most basal position, while Gnetales are nested within conifers as sister to Pinaceae.

Morphological phylogenetic analyses have permitted the tentative placement of extinct gymnosperm groups on a molecular phylogenetic backbone of living seed plants (Doyle 2008, 2012; Fig. 1). Two of the most likely relatives of the angiosperm stem lineage are Caytoniales and Bennettitales. The first member of Caytoniales to be discovered was the female reproductive structure *Caytonia* (Fig. 1), which is probably of the same species as the pollen-bearing *Caytonanthus* and leaf-bearing *Sagenopteris* fossils. These plants, like many gymnosperms, possessed saccate pollen grains and a probable droplet-based pollination mechanism but had angiosperm-like net-veined leaves. *Caytonia* ovules developed within laminate cupules, whose potential homology to reproductive structures in angiosperms is discussed below. Interestingly, several further extinct gymnosperms also possessed multiovulate cupules. These include glossopterids (Fig. 1), corytosperms, and peltasperms, of which the former group is considered as a possible stem-lineage relatives of the angiosperms, while the latter two may be more closely related to extant gymnosperms (Fig. 1).

Bennettitales, which are considered as particularly strong candidates for a close relationship to angiosperms, lacked multiovulate cupules but showed the angiosperm-like features of net-veined leaves, nonsaccate pollen, and, in some species, a bisexual reproductive axis (Fig. 1). Interestingly, Bennettitales and angiosperms also share the capacity to synthesize oleananes, which are highly resistant terpenoid compounds that persist even in fossils. Potential strategies to use data from living groups and reconstructed ancestors to help choose between fossil gymnosperm candidates for the ancestor of angiosperms are discussed in the final section of this chapter.

ANA-Grade Angiosperms Provide Clues on the Morphological, Ecological, and Molecular Characteristics of the First Flowering Plants

By mapping the character-states of ANA-grade and other angiosperm species onto molecular phylogenies, it has been possible to reconstruct numerous aspects of the first angiosperms (Fig. 2). Earlier work generally used parsimony-based methods to reconstruct angiosperm features, but some recent studies have incorporated more sophisticated maximum likelihood and/or Bayesian model-based reconstructions (e.g., Willis et al. 2014; Sauquet et al. 2017). A synthesis of reconstruction studies indicates that early angiosperms were probably rapidly growing shrubs, perhaps with some liana-like tendencies, that grew in shaded and disturbed environments such as the borders of rapidly flowing streams running through dense forest (Feild and Arens

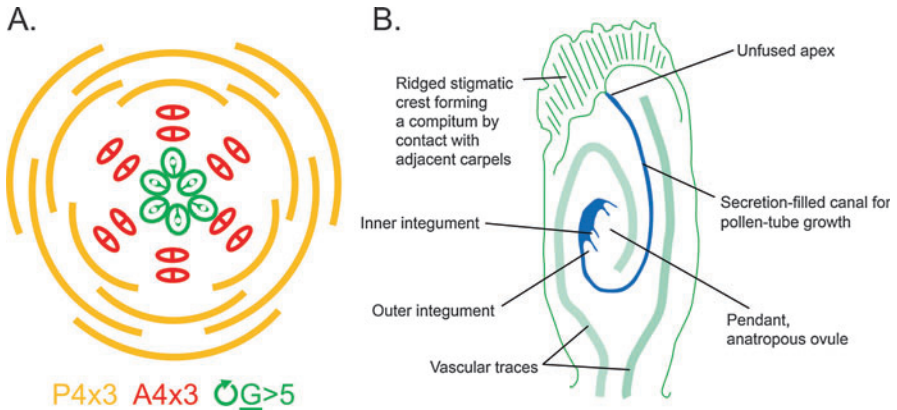


Fig. 2 (a) A floral diagram and floral formula representing a reconstruction of the MRCA of living angiosperms, based on Sauquet et al. (2017) and other references in the text. (b) Diagram of carpel anatomy in *Trimenia* (ANA grade, Austrobaileyales), redrawn from Doyle (2012), which conserves, with the possible exception of ovule-number (see Sauquet et al. 2017), the major inferred features of the carpel in the reconstructed MRCA of living angiosperms

2005). Their flowers were probably small (Endress 2001), bisexual (Sauquet et al. 2017), protogynous, actinomorphic and contained an undifferentiated perianth of tepals (Endress and Doyle 2015). Both the perianth and androecium may have contained several subwhorls, each composed of three organs (Sauquet et al. 2017).

The first angiosperms were probably pollinated by generalist insect pollinators and provided pollen rather than nectar as a reward (Endress 2001; Thien et al. 2009). The gynoecium in the MRCA of living angiosperms was almost certainly superior and probably contained more than five carpels (Sauquet et al. 2017). Sauquet et al. (2017) conclude these carpels to have probably been spirally arranged, in contrast to the likely whorled arrangement proposed by these same authors for the perianth and androecium in the MRCA of living angiosperms. However, Sokoloff et al. (2018) have pointed out that flowers may be constrained to be either whorled or spiral throughout, rather than composed of a mixture of spiral and whorled territories, questioning therefore the interpretation of Sauquet et al. (2017). Whatever their phyllotaxy, the carpels of the MRCA of living angiosperms were most likely separate, ascidiate (bottle-shaped), and incompletely fused at the apex, being closed instead by the secretion of substances into an aperture or canal that permitted the penetration of pollen tubes (Endress 2001). By contrast, the carpels of many of the more recently evolved angiosperm groups are plicate (folded), completely closed at the apex, and fused together into a syncarpic gynoecium.

The stigmatic surfaces in the MRCA of living angiosperms were probably covered with multicellular striations, rather than the unicellular papillae more prevalent in later diverging angiosperms. These surfaces may have been in physical contact between adjacent carpels and thus contributed to a compitum that permitted the growth of pollen tubes between carpels and thereby improved the efficiency of fertilization (Endress and Doyle 2015). The ovary of each carpel probably

contained one or a few pendant, anatropous ovules. Each ovule almost certainly possessed two integuments and a large nucellus (or maternal nutritive tissue), and probably contained a four-celled embryo sac (Friedman and Ryerson 2009). Double fertilization would have occurred to generate a diploid zygote and a diploid endosperm. Interestingly, in a few ANA-grade angiosperms, including *Trimena* (Austrobaileyales), several embryo sacs persist in the mature ovule and grow through the nucellus towards the pollen tubes to bring about fertilization. Thus, the growth of female gametophytes, as well as that of pollen tubes, may have contributed to a filter for fitness in early flowering plants (Bachelier and Friedman 2011). Seeds of the ancestral angiosperm probably possessed a form of morphophysiological dormancy, in which the embryo remained relatively small in the mature seed (Willis et al. 2014; Fogliani et al. 2017).

A comparative approach in living species can also be used to determine the likely molecular mechanisms underpinning the phenotypic characters of early angiosperms. Such studies mostly begin from functional genetic data in model angiosperms. Comparative studies in ANA-grade angiosperms can then be used to determine which of the molecular mechanisms under consideration have likely been conserved since the MRCA of extant angiosperms, and which were added later. For the moment, there are no viable functional genetic models among the ANA-grade angiosperms. The methods currently used to ascertain gene functions in these species are therefore largely indirect and are often based on the conservation of expression patterns (Fig. 3) and/or of protein functions in vitro or in heterologous in vivo systems (e.g., in transgenic model angiosperms).

Using a range of comparative molecular approaches, it has been possible to conclude that many of the mechanisms controlling flower development in eudicot models such as *Arabidopsis* have probably been conserved since the MRCA of extant angiosperms. Notably, the ABC model of flower development, initially constructed in *Arabidopsis* and *Antirrhinum*, shows considerable conservation in ANA-grade angiosperms. In this model, which is extensively described in the chapter “► Evolution of Floral Organ Identity”, the overlapping expression domains in the floral meristem of several classes of transcription factors, almost all of which belong to the MADS-box gene family, control the identity of sepals, petals, stamens, and carpels in the first to fourth floral whorls, respectively. The ABC model was extended from a genetic to a biochemical model by the addition of the MADS-box E-function, which is expressed in all floral whorls. E-function MADS-box proteins are capable of forming heterotetramers of different combinations with other classes of floral MADS-box proteins, and each type of complex formed is hypothesized to interact with pairs of so-called CArG motifs (of consensus sequence CC[A/T]₆GG) in the promoters of distinct sets of target genes, thus bringing about the development of the various different floral organ types. The expression domains of floral MADS-box genes appear generally conserved in ANA-grade angiosperms (Kim et al. 2005; Fig. 3), suggesting the ABCE model to have already functioned in early angiosperms. One of the main differences in floral MADS-box gene expression between ANA-grade angiosperms such as *Amborella* and eudicot models such as *Arabidopsis* is that expression domains appear less clearly delimited in the ANA-grade (Fig. 3b),

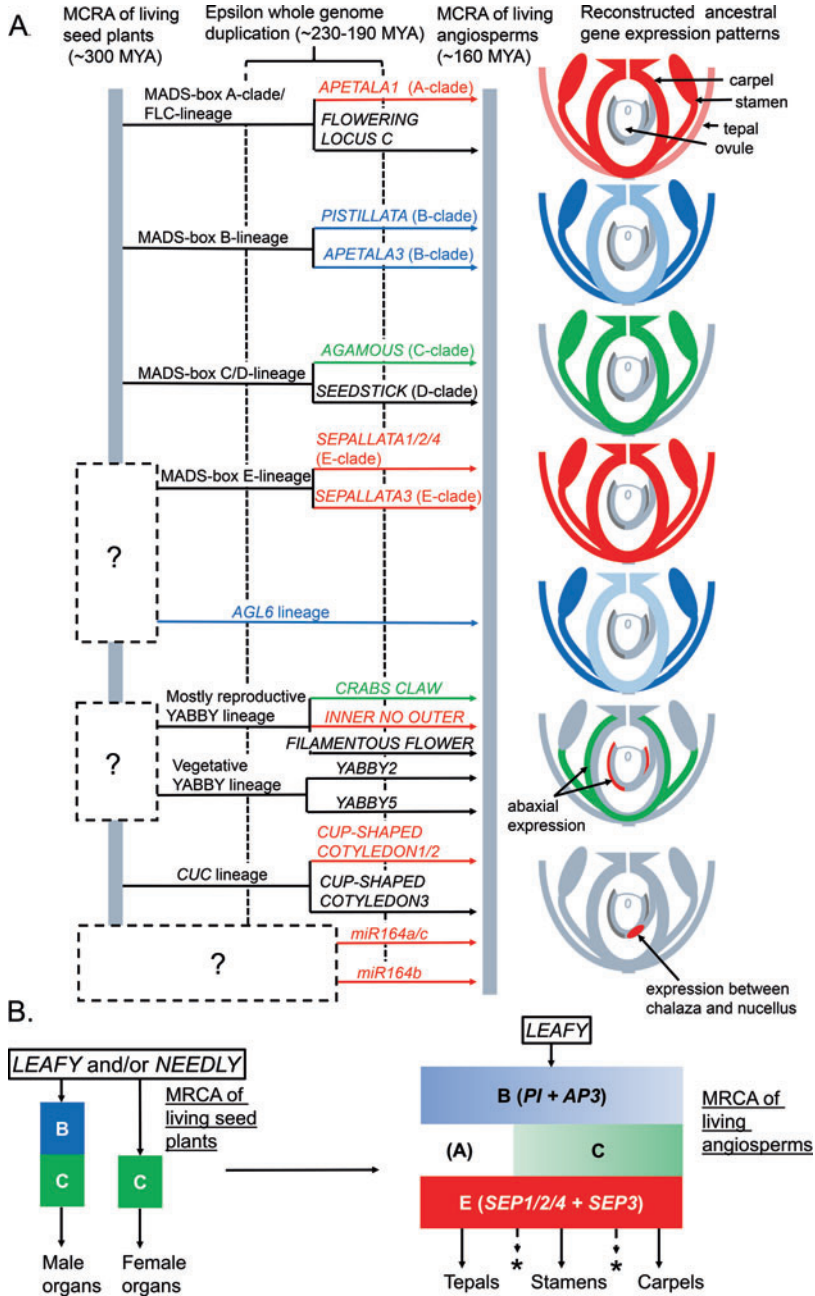


Fig. 3 (a) Evolution of flower-development regulators along the angiosperm stem lineage and their reconstructed expression patterns in the MRCA of living angiosperms. Gene lineages are named following their first-published descendent(s) in *Arabidopsis*. Uncertainties in the order or timing of gene duplications are indicated by dotted boxes or polytomies. Lighter shades indicate weaker regions of gene expression. Data were collated from Fourquin et al. (2005), Kim et al. (2005),

which may explain the typically gradual transformations observed between floral organ types in the ANA grade, particularly in species such as *Amborella* in which floral organs are arranged spirally rather than in whorls.

Besides MADS-box genes, numerous other classes of regulators are known to control floral development in model angiosperms, and some of these have also been investigated in ANA-grade angiosperms to evaluate the potential conservation of their functions since the MRCA of extant angiosperms. These regulators include the YABBY transcription factors INNER NO OUTER (INO) and CRABS CLAW (CRC), which have been concluded to control abaxial-adaxial polarity and organ expansion in the outer integument and carpel, respectively, since the base of living angiosperms (Yamada et al. 2003; Fourquin et al. 2005). The developmental module involving CUP-SHAPED COTYLEDON (CUC)/NO APICAL MERISTEM (NAM) transcription factors and their microRNA regulator *miRNA164* plays a role in defining the boundary between the nucellus and chalaza that also appears to have been conserved since the MRCA of extant angiosperms (Jasinski et al. 2010; Vialette-Guiraud et al. 2011). It furthermore seems likely that expression of *miR164* may downregulate *CUC* genes to facilitate the fusion of carpel margins in ANA-grade angiosperms as it does in eudicots (Vialette-Guiraud et al. 2016b).

Many further classes of regulators have been analyzed using phylogenomic techniques to attempt to correlate changes in the structure of gene families (duplications, losses, etc.) with morphological evolutionary history (Pfannebecker et al. 2017a, b). The addition of expression studies to these phylogenomic data should provide stronger evidence for potential roles of the genes analyzed in early flowering plants. In the longer term, direct methods for functional genetic analysis in the ANA grade will be essential to a rigorous demonstration of the developmental roles of regulatory genes in these species (Scutt and Vandenbussche 2014). Such direct functional data in ANA-grade angiosperms should help to refine morphological reconstructions of the ancestral flower. For example, recent reconstruction work (Sauquet et al. 2017) has indicated with high confidence the presence of a whorled, trimerous perianth and androecium in the MRCA of euangiosperms and suggested, but with lower confidence, that these features were already present in the MRCA of all living angiosperms. It is clear that this latter conclusion must depend strongly on the presence of a whorled, trimerous arrangement in some taxa of Nymphaeales, in addition to euangiosperms. If the whorled, trimerous arrangements in Nymphaeales and euangiosperms prove to involve similar molecular mechanisms, brought about



Fig. 3 (continued) Jasinski et al. (2010), Jiao et al. (2011), Yamada et al. (2011), Gramzow et al. (2014), Vialette-Guiraud et al. (2016a), and Moyroud et al. (2017). **(b)** Molecular models of reproductive development in the MRCAs of living seed plants and angiosperms, based on the (A) BC model of flower development proposed of Causier et al. (2010), modified to fit reconstructed ancestral gene expression patterns. MADS-box gene expression patterns in early angiosperms, as in some living ANA-grade taxa, may have had *fuzzy* boundaries, as indicated by color gradients, leading to the production of intermediate floral organ types, as indicated by asterisks

by orthologous sets of regulators, such a result would provide very strong support for the conclusion that a trimerous, whorled arrangement had evolved prior to the split between Nymphaeales and the common ancestral lineage of Austrobaileyales and euangiosperms.

Evo-Devo Hypotheses for the Origin of Angiosperm-Specific Reproductive Features

The Outer Integument and Carpel

The inner integument of angiosperms appears homologous to the single integument of gymnosperms and therefore can be concluded to have arisen over 300 MYA in a common ancestor of living seed plants. The outer integument and carpel, by contrast, are unique to angiosperms and therefore must have arisen along the angiosperm stem lineage, before the MRCA of living angiosperms that probably lived some 160 MYA. Distinct sets of genes control organ polarity in the inner and outer integument, at least in *Arabidopsis* (Kelley et al. 2009), reflecting the separate temporal origins of these organs.

Doyle (2008) summarizes several evolutionary hypotheses that could account for the approximately simultaneous evolution of the carpel and outer integument. One of these mechanisms postulates the cupule-bearing megasporophyll of a *Caytonia*-like gymnosperm as a progenitor of the female reproductive arrangement in angiosperms (Scenario 1 in Fig. 4). *Caytonia* cupules appear to be pinnately distributed along a radially symmetrical rachis, and each cupule contains 8–30 unitegmic ovules that form on its adaxial surface. The outer integument may have evolved from the cupule wall by a reduction in ovule-number to one-per-cupule, while the carpel may then have been formed by the expansion and conduplicate folding of the rachis around the resulting bitegmic ovules. This potential evolutionary mechanism leads elegantly to the anisotropous ovule, thought to have been present in early angiosperms (Endress and Doyle 2015).

Another cupule-bearing structure proposed as a potential progenitor of the carpel is the glossopterid-type sporophyll (Scenario 2 in Fig. 4). In this case, the carpel could be interpreted as a compound structure that includes both the cupulate sporophyll and its subtending bract. However, fossil evidence of glossopterids considerably predates the likely origin of the angiosperms, and these plants have also been suggested as possible ancestors of Caytoniales and therefore as possible indirect ancestors of the angiosperms. A further possibility is that the angiosperms evolved from Bennettitales (Scenario 3 in Fig. 4), in which case, the progenitor organs to the carpel and outer integument of angiosperms would remain unclear.

The origin of the outer integument and carpel probably took place at around the time of the epsilon whole genome duplication event (Jiao et al. 2011; Fig. 1). Numerous gene families involved in outer integument and/or carpel development contain paralogs that were apparently retained in this duplication (Fig. 3a) or in localized duplications that occurred at a similar evolutionary stage. Carpel

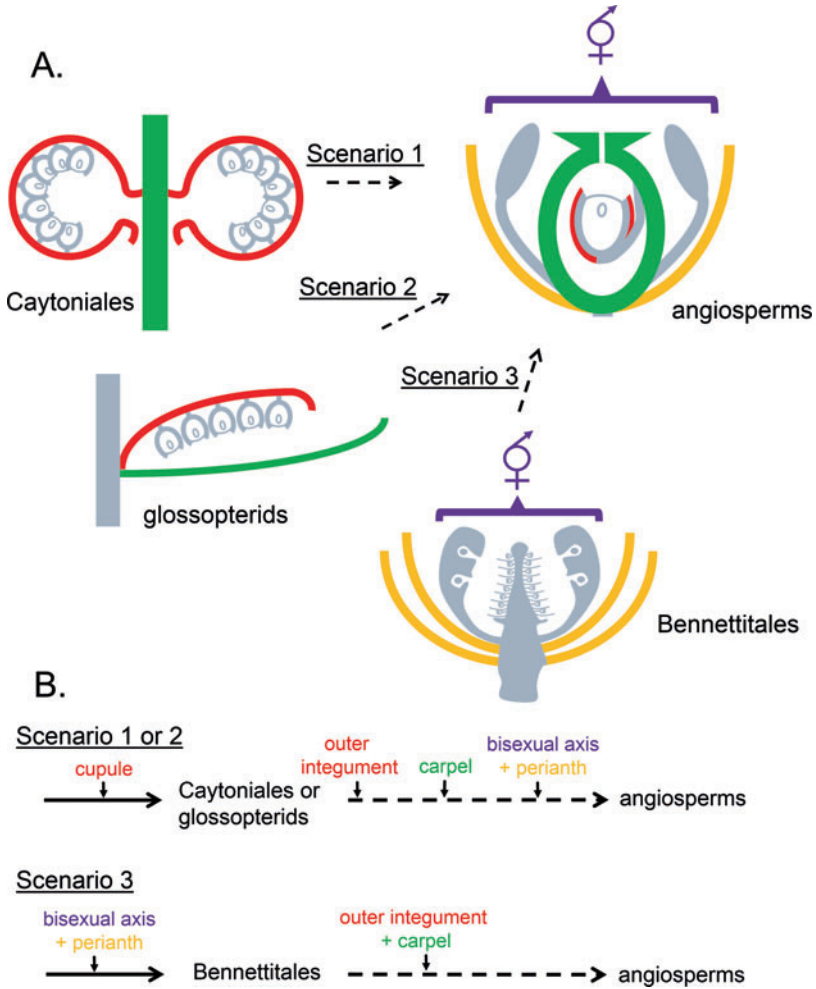


Fig. 4 (a) Three alternative evolutionary scenarios for the origin of major specific features of the flower (the outer integument, carpel, perianth, and bisexual axis) from ancestors resembling Caytoniales, glossopterids, and Bennettitales (also illustrated in Fig. 1), based in part on Doyle (2008, 2012). Potentially homologous features of stem-lineage relatives and angiosperms are indicated using colors. (b) Two alternative timelines for the acquisition of angiosperm-specific features, depending on the evolutionary scenario (from a) considered

development in model eudicots is under the control of C- and E-function MADS-box transcription factors, which are thought to act as heterotetramers, each composed of two C- and two E-function proteins (see the chapter “► Evolution of Floral Organ Identity”). The angiosperm C-lineage was derived from a duplication that took place along the angiosperm stem lineage and separated this from the angiosperm D-lineage, whose genes play various roles in carpel and ovule development. The gymnosperm pro-orthologs of angiosperm C/D genes are also expressed in female

tissues. Hence, neofunctionalization processes that followed the separation of the C and D MADS-box lineages may have been key events in the origin of the carpel, by adapting part of the molecular toolkit that previously specified megasporophyll and ovule development in a gymnospermous progenitor of the angiosperms.

The early evolution of the E-clade of MADS box genes, also involved in carpel development, is enigmatic. These genes are present only in angiosperms, but there were apparently already two separate E-class lineages in the MRCA of living angiosperms (Fig. 3a). The nearest relatives to E-function genes in gymnosperms are genes of the *AGAMOUS LIKE6* (*AGL6*) lineage, which is also present in angiosperms. It is not currently clear whether an ancient E-clade was lost in gymnosperms or whether the angiosperm E-clade was derived by a duplication in the angiosperm *AGL6* lineage, followed by unequal rates of evolution in the resulting paralogous lineages (Fig. 3a). It is also not currently clear whether the gymnosperm orthologs of angiosperm MADS-box floral homeotic proteins form tetramers *in planta*, and if so, of what composition. If MADS-box protein tetramer formation proves to be unique to the angiosperms, then clearly the origin of this molecular feature may have played a key role in the origin of the carpel and other floral organs.

Bisexuality

Bisexuality is predominant in the ANA-grade, as in the angiosperms as a whole, and ancestral reconstruction suggests this character was present in the MRCA of living angiosperms (Sauquet et al. 2017; Fig. 2a). Bisexuality is not, however, completely specific to angiosperms, as flower-like bisexual reproductive axes were present in the extinct gymnosperm order Bennettitales (Figs. 1 and 4), while morphologically bisexual, though functionally unisexual, reproductive axes are present in Gnetales. In addition, mutations causing bisexuality have been reported in gymnosperms including *Picea*, *Pinus*, *Pseudotsuga*, *Juniperus*, *Sequoia*, *Abies*, and *Agathis* (Lanner 1966), suggesting that the transition from unisexual to bisexual reproductive axes may not require very numerous or complex genetic changes.

Despite the apparent genetic simplicity of transitions to bisexuality, a number of hypotheses for the origin of the angiosperms have focused particularly on the acquisition of this character along the angiosperm stem lineage. Most of these hypotheses, reviewed in detail in the chapter “► [Evolution of Floral Organ Identity](#)”, emphasize the role of the transcription factor *LEAFY* (*LFY*), a master controller of floral patterning in angiosperms. In *Arabidopsis*, *LFY* is a unique gene that is expressed specifically in the floral meristem and interacts with cofactors including *UNUSUAL FLORAL ORGANS* (*UFO*, F-box ubiquitinase family) and *WUSCHEL* (*WUS*; homeobox transcription factor family) to set up the correct expression of MADS floral homeotic genes in overlapping zones of the floral meristem. This includes the expression of B-, C- and E-function MADS genes in the zones that will give rise to the androecium and gynoecium. An ortholog of *LFY* is present in gymnosperms, and most gymnosperms also possess a second *LFY*-like gene termed *NEEDLY* (*NLY*), whose angiosperm ortholog was apparently lost before the

radiation of extant angiosperms. Recent biochemical work has shown that *LFY* in *Welwitschia* (Gnetales) is specifically able to bind to B-function MADS-box gene promoters, indicating the conservation of early steps in the patterning of the reproductive axis between angiosperms and gymnosperms (Moyroud et al. 2017, Fig. 3b).

According to the mostly male theory (MMT; Frohlich 2003), bisexuality in a common ancestor of living angiosperms first arose through the ectopic production of ovules on microsporophylls situated towards the apex of its cone-like axes. The basal microsporophylls of these cones would have remained unisexual, while the subsequent loss of the microsporangia from the now bisexual sporophylls at the apex would have led to a bisexual axis composed of apical megasporophylls and basal microsporophylls. These transitions were, it is hypothesized, accompanied by the loss of *NLY* and all downstream pathways uniquely regulated by this factor. The MMT thus proposes that all angiosperm reproductive tissues, other than those of the ovule itself, are derived from previously male developmental programs. However, *LFY* and *NLY* have not proven to show strictly respective male- and female-specific expression patterns in all gymnosperms (Vazquez-Lobo et al. 2007), as might be predicted from the MMT, while studies of the sex-specific expression patterns of orthologous genes in angiosperms and gymnosperms have also failed to support this hypothesis (Tavares et al. 2010).

Despite the above difficulties, the MMT remains a useful conceptual framework which has encouraged the development of further hypothesis for the origin of the bisexual flower. One such hypothesis by Baum and Hileman (2006) also proposes a central role for *LFY* but postulates that the origin of floral bisexuality was caused by a change in the relative binding affinity of this factor to B- and C-function MADS-box gene promoters. During the evolutionary origin of the flower, B-genes would, accordingly, have become less sensitive to *LFY* than C-genes, such that C-proteins would have predominated at the lower *LFY* concentrations found near the apex of the reproductive axis, giving rise to female organs. Meanwhile, B- and C-genes would have been transcribed at comparable levels under the higher *LFY* concentrations encountered lower down the axis, thus giving rise to an outer whorl of male organs.

The Perianth

Character state reconstructions indicate that a bract-derived perianth was likely present in the MRCA of flowering plants (Endress and Doyle 2015) and may have shown a whorled arrangement (Sauquet et al. 2017; Fig. 2a). However, perianth-like organs are not entirely specific to angiosperms as these were present in the flower-like reproductive axes of Bennettitales and also occur in the reproductive axes of all three extant genera of Gnetales: *Ephedra*, *Gnetum*, and *Welwitschia*. In addition, female cones of Pinaceae are made up of developmental modules in which each ovulate scale (or megasporophyll) is subtended by an outer or bract scale, which might thus be considered to occupy an equivalent position to a perianth organ in angiosperms. Given all of these examples of perianth-like organs external to the

flowering plants, and the lack of clearly assignable angiosperm ancestors in the fossil record, it is not currently clear at what stage the perianth arose, compared to other angiosperm synapomorphies such as the carpel, outer integument, and bisexual axis (Fig. 4b). Indeed, organs of potential homology to the carpel and outer integument are much clearer in *Caytonia* and glossopterids than in Bennettitales, whereas the latter group shares the other angiosperm characteristics of a bisexual reproductive axis and perianth. The accurate assignment of closest angiosperm stem-lineage relatives would therefore help enormously to elucidate the order of appearance of the major synapomorphies of the angiosperm flower.

It is important to note that inner perianth organs, which are often petaloid, may have a distinct evolutionary origin from outer perianth organs, particularly in later diverging angiosperm groups within the core eudicots. In these latter cases, petals are believed to be derived not from bracts, as in more basally diverging angiosperms, but from outer stamens that have become secondarily sterilized, as reviewed by Ronse de Craene and Brockington (2013).

The original ABC model for flower development has been modified recently to a more generally applicable (A)BC model, which emphasizes the role of A-clade MADS box genes in floral patterning, rather than in the identity of perianth organs (Causier et al. 2010; Fig. 3b). According to the (A)BC model, the specification of outer perianth organ (sepal) identity does not require MADS-box gene expression of the A, B, or C classes, while combined B- and E-function expression, in the absence of C-function expression, generates inner perianth organs, or petals, in the second floral whorl. As previously mentioned, gene expression studies in the ANA grade suggest the role of B- and E-function genes in the generation of petaloid perianth organs in eudicots has been conserved since the earliest stages of angiosperm evolution, even if a major switch from bract-derived *bracteopetals* to stamen-derived *andropetals* subsequently occurred within the core eudicots and other groups.

Embryo Sac Anatomy and Fertilization Mechanisms

Gymnosperm ovules consist of a haploid megagametophyte that develops from a functional megaspore within a diploid nucellus, which is, in turn, enclosed with a single integument (Linkies et al. 2010). Gymnosperm megagametophytes typically produce several archegonia, each containing an egg cell. Fertilization by sperm nuclei from pollen grains thus often leads to the development of several embryos within each ovule, though in most cases, only one of these will survive. In gymnosperms, therefore, fertilization essentially involves a single event between two haploid nuclei to generate a diploid zygote that will divide to form the embryo within the seed. The gymnosperm nucellus, which is a diploid maternal tissue, functions to store food reserves to support the growth of the embryo during and immediately after germination.

By contrast to gymnosperms, double fertilization, leading to the production of both an embryo and a biparental nutritive endosperm, is present in angiosperms

(Friedman and Ryerson 2009). A distinct form of double fertilization is known in *Ephedra* and *Gnetum* of the gymnosperm order Gnetales, though this gives rise to two zygotes, one of which subsequently degrades. Due to its biparental origin and key role in the fitness of offspring, the angiosperm endosperm is believed to play an important role in parental conflict (as does the placenta in mammals), which reflects the different interests of male and female parents in the supply of resources to developing offspring. In most angiosperms, the embryo sac is of the *Polygonum* type, containing seven cells of which the central cell, which will give rise to the endosperm, is binucleate. The two nuclei of the central cell combine with one sperm nucleus following fertilization to generate a triploid endosperm. However, in the ANA-grade genera *Nuphar* and *Trithuria* (both in Nymphaeales) and *Illicium* (Austrobaileyales), the embryo sac contains four cells, including a uninucleate central cell. Double fertilization in these taxa generates an embryo and endosperm, both of which are diploid. In *Amborella*, the only representative of the remaining ANA-grade order Amborellales, the embryo sac contains eight cells, including a binucleate central cell that produces a triploid endosperm after fertilization. The *Amborella* embryo sac arrangement is thus more similar to that of *Polygonum* and the majority of later-diverging angiosperms than to other members of the ANA grade.

Because of this distribution of characters, character state reconstruction by parsimony fails to formally resolve the embryo sac arrangement in the MRCA of living angiosperms between four-, seven-, and eight-celled types, or the ploidy of its endosperm, which might be either diploid or triploid. However, Friedman and Ryerson (2009) argue that the *Amborella* and *Polygonum* embryo sac arrangements represent variants on a doubled form of a basic module composed of four cells, which is still present in Nymphaeales and Austrobaileyales. The doubling of the embryo sac module, according to this view, occurred in parallel in the Amborellales and euangiosperm lineages but involved one extra cell division in Amborellales. Thus, according to Friedman and Ryerson (2009), the MRCA of living angiosperms would have contained a four-celled embryo sac and double fertilization leading to a zygote and endosperm, both of which were diploid. Interestingly, both of the hypothesized independent origins of a triploid biparental endosperm (in Amborellales and euangiosperms, respectively) may have had a role in increasing the female genetic component of the endosperm and thus biasing parental conflict in favor of female factors that promote an equal distribution of nutrients among sibling and/or half-sibling offspring.

Interestingly, an extensive perisperm is present in mature seeds of *Trithuria* (Nymphaeales), in addition to a diploid endosperm and a very small, underdeveloped embryo (Friedman 2008). Similarly to the endosperm, the perisperm is an embryo-nourishing tissue, though one which is derived exclusively from the maternal nucellus. The presence of a nutritive perisperm or nucellus is mainly associated with gymnosperms and the prominence of this tissue in *Trithuria* has been suggested to form a link with the gymnosperm-like ancestor of the flowering plants.

Technical Developments to Help Elucidate the Origin of Angiosperms

A number of recent developments provide much hope for efforts to understand the origin of the angiosperms. At present, complete genome sequence data, in draft form, is available only from one ANA-grade angiosperm (*Amborella trichopoda*) and two closely related gymnosperm taxa (*Pinus* spp. and *Picea abies*). However, increasing efficiency and decreasing costs of next generation sequencing (NGS) make it likely that we will soon have available a much more complete list of whole genome and transcriptome sequences. Certain recent NGS methods provide reads extending to tens of kilobases, thus simplifying the problem of genome assembly in the absence of physical linkage maps and genetic markers, etc. This will be particularly useful for the sequencing of basal angiosperms and gymnosperms, all of which, with the exceptions of Hydatellaceae and Nymphaeaceae (both in Nymphaeales), have moderately large genomes. In parallel, excellent progress has been made to develop methods, based on the combined analysis of synteny and phylogeny, to reconstruct the structure of ancestral genomes from those of their living descendants (Murat et al. 2017). Accordingly, we may soon be able to reconstruct, in considerable detail, the genome of the ancestor of living angiosperms, and even that of their more distant ancestor from before the epsilon whole genome duplication event.

To fill the current gaps in functional studies of developmental regulators in basal angiosperms and gymnosperms, a number of recent developments appear promising (Scutt and Vandenbussche 2014). First, the recently discovered *Nymphaea thermarum*, and possibly also certain *Trithuria* spp. (both from Nymphaeales), could form well-adapted model ANA-grade angiosperms, amenable to molecular-genetic studies. If transformation methods can be developed for these species, the exciting recent developments in gene editing should greatly facilitate functional studies of genes that were crucial to the origin of the angiosperms.

A second major approach that can help to elucidate the evolution of developmental regulatory mechanisms in non-model plants consists of using in vitro and heterogeneous in vivo methods to measure protein-DNA and protein-protein interactions (Viallette-Guiraud et al. 2016a). These methods can be combined with genomic-scale analyses and modeling approaches to describe the networks of posttranscriptional, transcriptional, and epigenetic interactions that control reproductive development in convenient angiosperm and gymnosperm species of importance for the study of angiosperm origin and other important evo-devo questions. Furthermore, the approach of ancestral sequence reconstruction, also known as *protein resurrection*, can be used to directly study the biophysical and biochemical properties of ancestral regulatory molecules from key stages in plant evolution.

In terms of paleobotany, hope for discoveries of further early angiosperms and stem lineage-relatives comes from mesofossils, which are fossils of up to a few millimeters in diameter, often preserved as coalified specimens that were generated by intense forest fires or similar events (Schoenenberger 2005). These coalified fossils often show anatomical details down to the cellular and even subcellular

level and can be examined thoroughly and nondestructively using recently developed tomographic methods. As the earliest angiosperms likely had small flowers and grew in the forest understory, the chances of their preservation as coalified meso-fossils appear relatively high.

Integration of Data from Neo- and Paleobotany

The task of understanding the origin of angiosperms is considerable, and it is clear that evidence from both living and extinct groups will be needed to provide the fullest possible answer to this question (see also the chapter “► [Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record](#)”). There are several ways in which data from fossils and living plants can be combined to useful effect. For example, well characterized and accurately dated fossil divergences can be used to calibrate the molecular clock and thereby provide the best possible date for key points in molecular phylogenies, such as the MRCA of living angiosperms, which are not themselves directly represented in the current meso- or macrofossil record (Bell et al. 2005).

Paleobotanical data can also be used to support or refute given phylogenetic topologies generated from molecular data. For example, molecular phylogenies performed using various different data-sets and methods suggest four distinct possible topologies for the living seed plants (Doyle 2012). However, the fossil record indicates that Gnetales and angiosperms are of more recent origin than ginkophytes and cycads, and this information is only consistent with a subset of molecular topologies in which Gnetales arose recently, within or close to conifers, such as that shown in Fig. 1. Paleobotanical data thus refute alternative topologies in which Gnetales emerge in a basal position within extant gymnosperms.

Evo-devo hypotheses based principally on data from extant species can sometimes be reinforced by the use of data from fossil plants. Such an example is provided by the mostly male theory (MMT; Frohlich 2003), whose original formulation suggested corystosperms as a potential unisexual ancestor to the bisexual angiosperms. The MMT did not depend on the identification of corystosperms in this role, or provide a test of this group as a potential angiosperm ancestor; rather, it suggested a pair of hypotheses that were mutually consistent and thus appeared stronger than either hypothesis would have on its own.

A fourth and more ambitious combination of data can be envisaged in which molecular-developmental information from living species can be used to test evolutionary hypotheses based on paleobotanical data. Though no DNA is present in the fossils of potential relevance to the origin of the angiosperms, it is possible in many cases to *resurrect*, using ancestral reconstruction, the proteins encoded by genes that were present along the angiosperm stem lineage. The structure, activity, and molecular evolution of proteins that controlled plant development along the angiosperm stem lineage may be informative on the morphology and development of the species in which they functioned, and this information might be used to choose between potential angiosperm ancestors (or close stem-lineage relatives) from the fossil record. For example, the timeline of neofunctionalization events in selected gene

lineages could be compared with hypothesized developmental evolutionary sequences to support or refute hypotheses such as the origin of the angiosperm outer integument and carpel from female structures in cupulate gymnosperms, or the origin of the angiosperm perianth from preexisting structures in the flower-like reproductive axes of Bennettitales, thus testing the relative likelihood of the alternative scenarios shown in Fig. 4.

Cross-References

- ▶ [Evolution of Floral Organ Identity](#)
- ▶ [Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record](#)
- ▶ [The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis Over the Past 450 Ma](#)

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