10 Evolution of the flower

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Introduction

The origin and evolution of the flower have been intensively studied not only because of the great importance of flowers (and especially the fruits they produce) in providing human food, but also because of their crucial role in angiosperm sexual reproduction and many plant–animal interactions. The past centuries of morphologically and taxonomically based studies of flowers generated much information, but left some of the most critical questions of flower origin and evolution unresolved. Recent progress in understanding angiosperm (and seed plant) phylogeny provides a solid framework for evaluating evolutionary innovation, and identifies the taxa that provide the best insights into key innovations. The recent growth of developmental genetics provides exciting new data for understanding flower evolution; the interplay of developmental genetics with focused studies of morphology, development and phylogeny has generated a new field of study: the evolution of development (evo-devo). Evo-devo offers the best hope for rapid advance in the understanding of flower evolution. To appreciate this potential one must be cognizant of recent advances in all of these fields – phylogeny, morphology and developmental genetics – that are merging to create evo-devo.

Here we describe recent progress in the study of floral evolution, beginning with advances in phylogeny and the reconstruction of trends in floral evolution. We include a brief comparative review of some of the genes known to regulate flower development, with an emphasis on recent studies relevant to the classic ABC model of flower development. We conclude with a perspective on future research on floral biology at the genomic level. Throughout our discussion we describe how experimental genetic and phylogenetic analyses are together improving our understanding of the evolution of floral architecture and the molecules regulating floral development.

 Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants (ed. R.J. Henry)

Trends in Floral Evolution Inferred from Phylogeny

Background

A clear understanding of angiosperm phylogeny has recently emerged (e.g. Qiu *et al.*, 1999; Soltis *et al.*, 1999, 2000; Barkman *et al.*, 2000; Zanis *et al.*, 2002, 2003). These well-resolved and highly concordant DNA-based phylogenies have important implications for interpreting the morphology of early angiosperms and subsequent patterns of floral evolution.

Before the application of explicit phylogenetic methods, several investigators proposed that the first angiosperms had large, *Magnolia*-like flowers (Arber and Parkin, 1907; Bessey, 1915; Takhtajan, 1969; Cronquist, 1981). Stebbins (1974), in contrast, suggested that the earliest flowers were moderate in size. Endress (1987) proposed that the earliest angiosperm was bisexual, but that the transition to unisexuality was relatively easy, the perianth was undifferentiated and could be easily lost, and that the number of floral parts was labile.

Early phylogenetic studies focused attention on several herbaceous lineages (e.g. Nymphaeaceae, Piperaceae and Chloranthaceae; Fig. 10.1) as possible first-branching extant angiosperms (Donoghue and Doyle, 1989; Doyle *et al.*, 1994). Based on these results, it was suggested that early flowers were small, with a trimerous perianth, and with few stamens and carpels. However, more recent analyses (e.g. Mathews and Donoghue, 1999; Parkinson *et al.*, 1999; Qiu *et al.*, 1999; Soltis *et al.*, 1999, 2000; Barkman *et al.*, 2000; Doyle and Endress, 2000; Graham and Olmstead, 2000; Zanis *et al.*, 2002, 2003; Borsch *et al.*, 2003; Hilu *et al.*, 2003) place *Amborella*, Nymphaeaceae (including Cabombaceae; see APG II, 2003) and Austrobaileyales as basal to other extant angiosperms (Fig. 10.2). This topology suggests instead that the earliest flowers were small to moderate in size, with an undifferentiated perianth, stamens lacking a well-differentiated filament, and a gynoecium composed of one or more distinct carpels.

Fossils are critical for inferring the origin and early diversification of angiosperms, but fossil flowers of the earliest angiosperms are scarce. None the less, early Cretaceous angiosperm fossils are consistent with the hypothesis that the first flowers were small to moderate in size, with an undifferentiated perianth (Crane, 1985; Friis *et al.*, 1994, 2000; Crane *et al.*, 1995), although *Magnolia*-like forms also occurred during the same geological time (e.g. *Archaeanthus*; Dilcher and Crane, 1984). In addition, some early angiosperms lacked a perianth (e.g. *Archaefructus*; Sun *et al.*, 2002), but these may not be basal within angiosperms (Friis *et al.*, 2003). There are no known fossils representing unequivocal stem-group angiosperms (i.e. angiosperms that attach below the basal node leading to *Amborella*, Nymphaeaceae and all other living angiosperms).

One way to infer ancestral states is to employ character-state reconstruction with phylogenetic trees and programs such as MacClade (Maddison and Maddison, 1992). Using this approach, the evolution of specific floral characters in basal angiosperms has been reconstructed (e.g. Albert *et al.*, 1998; Doyle and Endress, 2000; Ronse De Craene *et al.*, 2003; Zanis *et al.*, 2003; Soltis *et al.*, 2004). We review some of the findings of these character-state reconstructions below using the most conservative optimization method (all most parsimonious states; Maddison and Maddison, 1992). Other reconstructions, using other optimization methods and tree topologies, are provided in the references noted above. Most of the same general conclusions are supported regardless of optimization.

Perianth differentiation

A differentiated or bipartite perianth has an outer whorl of sepals clearly differentiated from the inner whorl(s) of petals. In contrast, an undifferentiated perianth lacks clear differentiation between the outer and inner whorls, or the perianth may consist of undifferentiated spirally arranged parts. These undifferentiated perianth organs

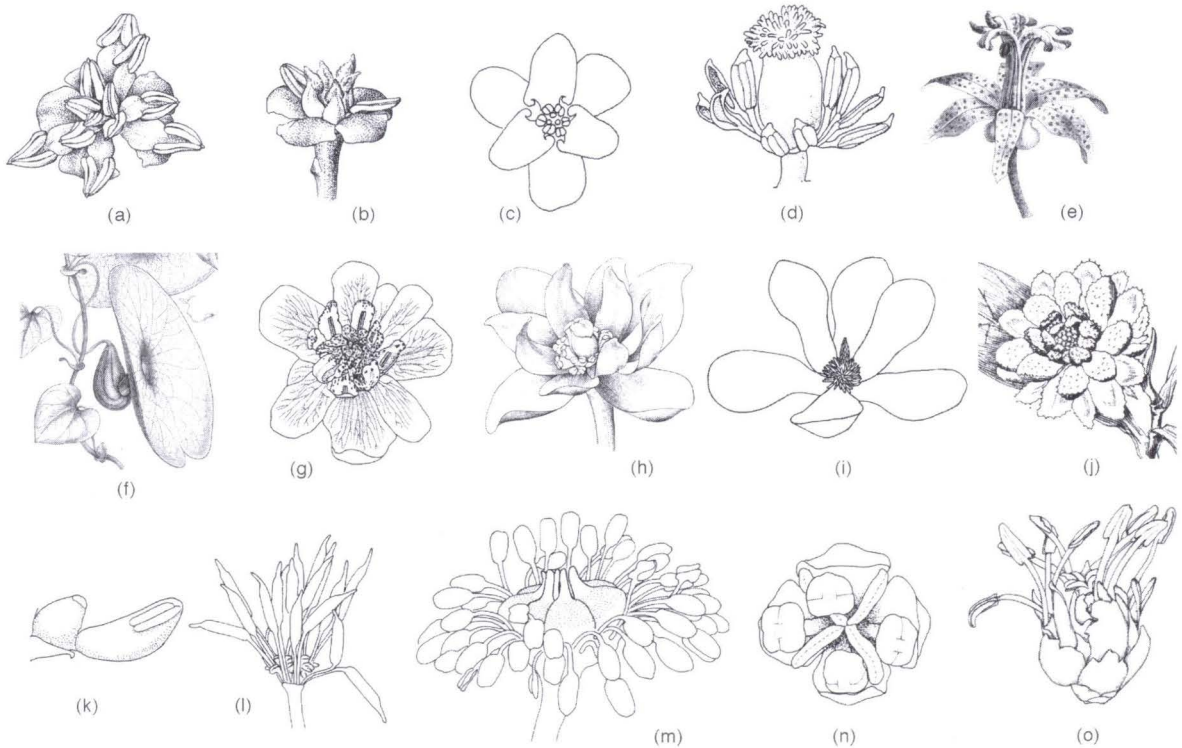

Fig. 10.1. Floral diversity in basal angiosperms (a–i) and early-diverging eudicots (j–m). (a) *Amborella trichopoda* (Amborellaceae), staminate flower (from Endress and Igersheim, 2000). (b) *A. trichopoda* (Amborellaceae), pistillate flower (from Endress and Igersheim, 2000). (c) *Cabomba aquatica* (Nymphaeaceae) (from Endress, 1994b). (d) *Trimenia papuana* (Trimeniaceae) (from Endress and Sampson, 1983). (e) *Tricyrtis pilosa* (Liliaceae), flower (from Engler in Engler and Prantl, 1887–1915). (f) *Aristolochia* (Aristolochiaceae) flower (from Solereder in Engler and Prantl, 1887–1915). (g) *Austrobaileya scandens* (Austrobaileyaceae) (from Endress, 1980). (h) *Takhtajania perrieri* (Winteraceae; Canellales) (from Endress *et al.*, 2000). (i) *Magnolia* × *soulangiana* (Magnoliaceae; Magnoliales) (from Endress, 1987). (j) *Eupomatia* (Eupomatiaceae) flowering shoot (from Uphof in Engler and Prantl, 1959). (k) *Sarcandra chloranthoides* (Chloranthaceae) (from Endress, 1987). (l) *Euptelea polyandra* (Eupteleaceae) (from Endress, 1986). (m) *Trochodendron* (Trochodendraceae) (from Endress, 1986). (n) *Tetracentron* (Trochodendraceae) (from Endress, 1986). (o) *Buxus balearica* (Buxaceae), inflorescence with lateral staminate flowers and terminal carpellate flower (from Von Balthazar and Endress, 2002).

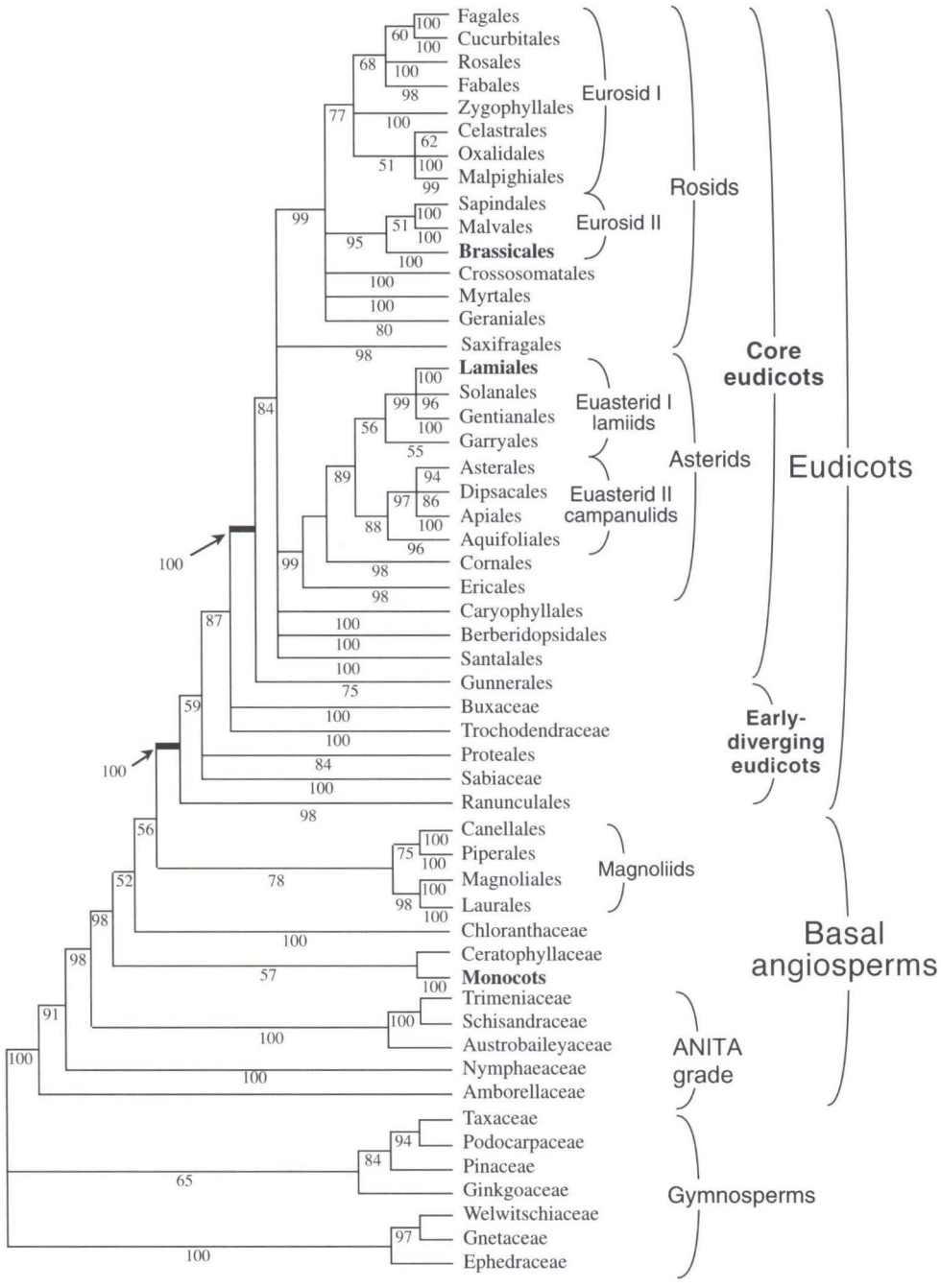

Fig. 10.2. Summary topology for angiosperms showing general positions of model organisms (in bold). Modified from Soltis *et al.* (2003).

have traditionally been referred to as tepals. The term tepal was coined by De Candolle (1827) to describe perianth organs (sepals and petals) that are not clearly differentiated morphologically; thus, the entire perianth may be petaloid. Takhtajan (1969), in con-

trast, used the term 'tepal' in a phylogenetic sense such that all monocots have tepals. Takhtajan's definition limits the application of tepal to specific groups of angiosperms and requires different terms for an undifferentiated perianth in other groups. Following other recent investigators, we will use the term tepal as defined by De Candolle.

Distinguishing sepals from petals is not always straightforward (Endress, 1994a; Albert *et al.*, 1998). Whereas sepals and petals are readily distinguished in most eudicots (~ 75% of all angiosperms), this is often not the case in basal angiosperms (Fig. 10.1), many of which have numerous undifferentiated perianth parts arranged in spirals, rather than in distinct whorls, a condition long considered ancestral (e.g. Bessey, 1915; Cronquist, 1968; Takhtajan, 1969).

The origin of a differentiated perianth of sepals and petals has long been of interest (e.g. Eames, 1931; Hiepko, 1965; Kosuge, 1994; Albert *et al.*, 1998; Kramer and Irish, 1999, 2000). It has been proposed that petals evolved first and that sepals evolved later (e.g. Albert *et al.*, 1998) and that petals have evolved multiple times from different floral organs in different groups (e.g. Eames, 1961; Takhtajan, 1969; Kosuge, 1994; Albert *et al.*, 1998; Zanis *et al.*, 2003).

Takhtajan (1969, 1997) suggested two origins of petals, one from stamens and one from bracts. Support for multiple, independent origins of petals has come from morphological studies showing that 'petals' of various angiosperms exhibit major differences and can be grouped into two basic classes (e.g. Endress, 1994a; Kramer and Irish, 2000). In one group are petals that resemble stamens. The petals are developmentally delayed and are similar in appearance to stamen primordia at inception (Endress, 1994a). These petals have sometimes been termed andropetals. The second type of petaloid organ (conventionally termed tepals; Cronquist, 1981) is found in undifferentiated perianths and is more leaf-like in general characteristics. These petals initiate and mature much earlier than do the stamens and are generally more leaf-like in appearance than are other petals (Smith, 1928; Tucker, 1960; Takhtajan, 1969, 1997).

Following Albert *et al.* (1998), two or more whorls of perianth parts must be present for an unambiguous interpretation of sepals and petals. If only a single perianth whorl is present, it may be difficult to interpret as 'sepals' or 'petals' (see also Endress, 1994a,b). Is the single whorl an undifferentiated perianth, composed of neither sepals nor petals, or is the single whorl composed of either sepals or petals with the other perianth whorl absent? A single-whorled perianth has traditionally been referred to as being composed of 'sepals' as a matter of convention (e.g. Cronquist, 1968). Families of basal angiosperms that contain taxa with a single-whorled perianth include nearly all Aristolochiaceae (except *Saruma*), all Myristicaceae and Chloranthaceae (*Hedyosmum*). In some cases, however, the nature of a single-whorled perianth can be determined through comparison with the perianths of closely related taxa. In Aristolochiaceae, most taxa have a single-whorled perianth that is considered a calyx (Cronquist, 1968, 1981; Tucker and Douglas, 1996; Takhtajan, 1997). In contrast, *Saruma* has two perianth whorls that are differentiated into sepals and petals. Furthermore, in some species of *Asarum*, petals apparently begin to develop, but the only traces are small, thread-like structures (Leins and Erbar, 1985).

In recent reconstructions (Ronse De Craene *et al.*, 2003; Zanis *et al.*, 2003; Soltis *et al.*, 2004) (Fig. 10.3), the ancestral state for the angiosperms is an undifferentiated perianth. *Amborella* and Austrobaileyales have an undifferentiated perianth. In contrast, the ancestral state for Nymphaeaceae is reconstructed as equivocal because some Nymphaeaceae (e.g. *Cabomba*, *Brasenia*, *Nuphar*) have a differentiated perianth whereas more derived waterlilies (*Victoria*, *Nymphaea*) have an undifferentiated perianth. Above the basal angiosperm grade, the undifferentiated perianth continues to be ancestral for the remaining angiosperms (Fig. 10.3). Importantly, all reconstructions indicate that a differentiated perianth evolved multiple times (see Albert *et al.*, 1998). Separate origins include some Nymphaeaceae, monocots, some Magnoliaceae, Annonaceae, Canellaceae, some

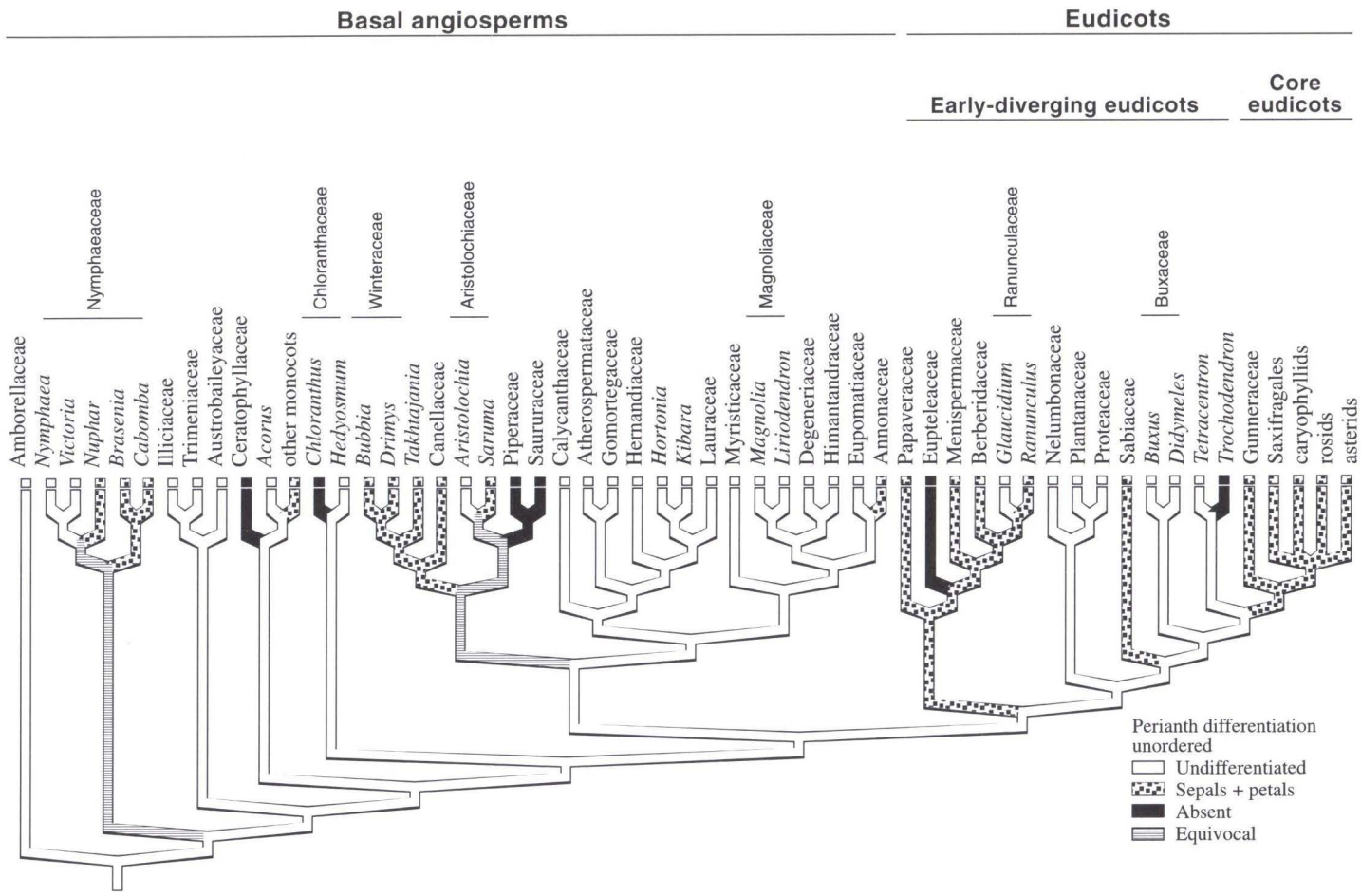

Fig. 10.3. MACCLADE reconstruction using all most parsimonious states optimization (Maddison and Maddison, 1992) of the evolution of perianth differentiation in angiosperms, with an emphasis on basal angiosperms and early-diverging eudicots. Topology is based on Zanis *et al.* (2002, 2003) and Soltis *et al.* (2000, 2003). Data are from Zanis *et al.* (2003) and Ronse De Craene *et al.* (2003). Modified from Soltis *et al.* (2004). For impact of other optimization methods (ACCTRAN, DELTRAN) see Zanis *et al.* (2003), Ronse De Craene *et al.* (2003) and Soltis *et al.* (2004).

Aristolochiaceae and Siparunaceae with additional origins in early-diverging eudicots (e.g. Papaveraceae, Menispermaceae, *Ranunculus*, Sabiaceae) and core eudicots. Comparative developmental studies are required to test whether multiple origins of perianth differentiation were driven by similar changes in gene function and regulation.

Phyllotaxis

Amborella has spiral phyllotaxis (Fig. 10.1), as do members of Austrobaileyales. In some basal families, phyllotaxis is complex. For example, in some Nymphaeaceae, phyllotaxis has been considered spiral, but it now appears to be primarily whorled, or in some cases irregular (Endress, 2001). In some Winteraceae (*Drimys* and *Pseudowintera*), phyllotaxis is primarily whorled, but occasionally spiral (Doust, 2000). In *Drimys winteri*, flowers within one tree vary between spiral and whorled (Doust, 2001).

The distinction between spiral and whorled is not always clear. In *Amborella*, recent developmental studies indicate that some floral organs (e.g. carpels) are initiated in a nearly whorl-like manner, although they are commonly described as spirally arranged (Buzgo *et al.*, 2004b). Studies of other basal angiosperms reveal that in some cases floral organs that appear to be whorled in mature flowers actually result from spiral initiation of primordia and a bimodal distribution of long and short time intervals between the initiation of consecutive organ primordia (Tucker, 1960; Leins and Erbar, 1985; Endress, 1994a). Thus, both spiral and whorled phyllotaxis of mature flowers result from the organs developing in a spiral sequence (Endress, 1987). For example, *Illicium* has spiral phyllotaxis in developing buds, but in mature flowers the carpels have an apparently whorled arrangement. Furthermore, even in some eudicots the sepals initiate in a spiral sequence, with the later-arising sepals positioned slightly inside the earliest to originate, as reflected in their imbricate arrangement at maturity. The inner organs arise in precise whorls, and even the sepals have traditionally been considered whorled, because of their close apposition at maturity.

Although spiral phyllotaxis is present in Amborellaceae and Austrobaileyales, the presence of whorled (and irregular) phyllotaxis in Nymphaeaceae makes the ancestral reconstruction for perianth phyllotaxis for the angiosperms dependent on the coding of the outgroup. However, outgroup coding is problematic because the immediate sister group of the angiosperms is unknown. Furthermore, no fossil group is known to have possessed flowers. If the outgroup is coded as lacking a perianth, then either a spiral or whorled phyllotaxis is reconstructed as equally parsimonious for the base of the angiosperms. If the outgroup is coded as having a spirally arranged perianth, then a spiral perianth is reconstructed as ancestral for the angiosperms. If the outgroup is coded as having a whorled perianth, then a whorled perianth is ancestral for the angiosperms with a spiral perianth evolving several times.

Above the Amborellaceae, Nymphaeaceae, Austrobaileyales grade, whorled perianth phyllotaxis is reconstructed as ancestral for all remaining angiosperms with multiple shifts to a spiral perianth occurring in basal lineages, including Calycanthaceae, Atherospermataceae, Gomortegaceae, some Monimiaceae, Degeneriaceae and some Magnoliaceae (Fig. 10.4). A possible transformation from whorled to spiral phyllotaxis may have occurred in *Drimys* and *Pseudowintera* (Winteraceae), which have a complex phyllotaxis involving spirals and multiple whorls (Doust, 2000, 2001; Endress *et al.*, 2000). Still additional reversals to a spiral perianth are found in the early-diverging eudicots *Nelumbo* (Proteales) and *Xanthorhiza*, *Caltha* and *Ranunculus* (Ranunculaceae). Thus, perianth phyllotaxis is highly labile in basal angiosperms and in basal eudicots (Endress, 1994b; Albert *et al.*, 1998; Ronse De Craene *et al.*, 2003; Zanis *et al.*, 2003; Soltis *et al.*, 2004). Again, comparative developmental studies are necessary to determine whether unrelated taxa with convergent phyllotaxis share common regulatory networks for organ initiation.

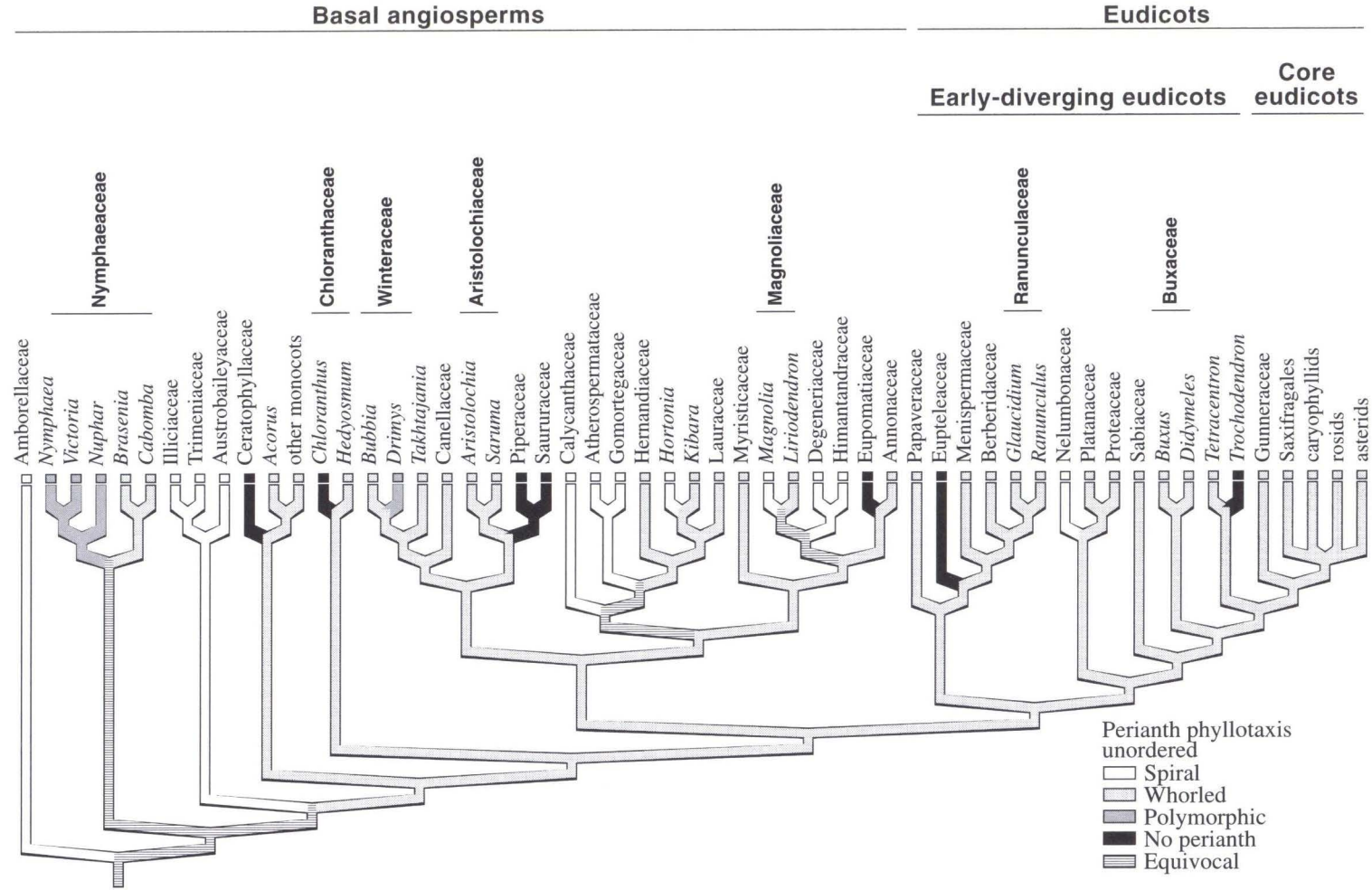

Fig. 10.4. MACCLADE reconstruction using all most parsimonious states optimization (Maddison and Maddison, 1992) of the evolution of perianth phyllotaxis in angiosperms, with an emphasis on basal angiosperms and early-diverging eudicots. Topology is based on Zanis *et al.* (2002, 2003) and Soltis *et al.* (2000, 2003). Data are from Zanis *et al.* (2003) and Ronse De Craene *et al.* (2003). Modified from Soltis *et al.* (2004). For impact of other optimization methods (ACCRAN, DELTRAN) see Zanis *et al.* (2003), Ronse De Craene *et al.* (2003) and Soltis *et al.* (2004).

Merosity

Among basal angiosperms, many lineages have numerous parts, some clades are trimerous, and others defy simple coding of merosity. In Winteraceae, the outermost floral organs are in dimerous whorls, followed by a switch to tetramerous whorls, and finally (in *Takhtajania*) a change to pentamerous whorls (Endress *et al.*, 2000). Similarly, in Magnoliaceae, the perianth of some species of *Magnolia* is an indeterminate spiral, whereas that of *Liriodendron* and other species of *Magnolia* is in three trimerous whorls and may represent a transition from spiral to whorled phyllotaxis (Tucker, 1960; Erbar and Leins, 1981, 1983).

Amborella and Austrobaileyales have an indeterminate spiral (Fig. 10.1). However, within Nymphaeaceae, *Cabomba*, *Brasenia* and *Nuphar* they are trimerous; other genera (e.g. *Victoria*, *Nymphaea*) are trimerous or tetramerous (Endress, 2001). As found for phyllotaxis (above), reconstruction of the ancestral merosity of extant angiosperms is dependent on the coding of merosity for the outgroup. If the outgroup of the angiosperms is coded as having an indeterminate number of perianth parts, then an indeterminate number is also ancestral for the angiosperms. Alternatively, if the ancestor of the angiosperms is considered to lack a perianth, then it is equally parsimonious for the base of the angiosperms to be either trimerous or indeterminate in perianth merosity (see Zanis *et al.*, 2003; Soltis *et al.*, 2004).

However, regardless of outgroup coding, above the basal grade of *Amborella*, Nymphaeaceae and Austrobaileyales, the ancestral character state for all remaining angiosperms is a trimerous perianth (Fig. 10.5) (e.g. Ronse De Craene *et al.*, 2003; Zanis *et al.*, 2003; Soltis *et al.*, 2004). Thus, although the trimerous condition is typically associated with monocots, these results indicate that trimery played a major role in the early evolution and diversification of the flower (Kubitzki, 1987).

Following the origin of a trimerous perianth, there was a return to an indeterminate spiral perianth in several basal lineages, including Calycanthaceae (e.g. *Calycanthus*), the clade of Atherospermataceae and Gomortegaceae, Himantandraceae, some Monimiaceae (e.g. *Hortonia*) and some Magnoliaceae (*Magnolia*). A perianth has also been lost several times (e.g. Eupomatiaceae (see below), Piperaceae, most Chloranthaceae, and Ceratophyllaceae) (Fig. 10.5).

These reconstructions indicate that perianth merosity is labile in basal angiosperms (see also Endress, 1987, 1994b; Albert *et al.*, 1998; Zanis *et al.*, 2003), a condition that continues through the early-diverging eudicots (Fig. 10.5). Dimery is often seen in early-diverging eudicots. However, trimery is also prevalent (Ranunculales), and pentamery is seen in some taxa. In contrast, in core eudicots, pentamery predominates. Interestingly, dimery is found in *Gunnera*, sister group to all other core eudicots. Thus, reconstructions not only indicate that perianth merosity is labile in basal angiosperms and early-diverging eudicots, but also suggest that a dimerous perianth could be the immediate precursor to the pentamery characteristic of eudicots (Soltis *et al.*, 2003). Once more, comparative developmental studies are required to elucidate the molecular basis of changes in merosity throughout angiosperm history.

Genes Controlling Early Floral Development

The models

Developmental genetic analyses have provided unprecedented insights into the molecular mechanisms that determine identities of the principal floral organs, at least in the eudicot model organisms used for these studies. *Arabidopsis thaliana* and *Antirrhinum majus*, two derived eudicots, were the first models studied, and are still the best understood. Investigations of these models have resulted in the identification and understanding of over 80 genes critical for normal floral development, including genes involved in flower initiation; however, the true number is bound to be much larger (Zhao *et al.*, 2001a; Ni *et al.*, 2004) (Fig. 10.6). Careful morphological developmental

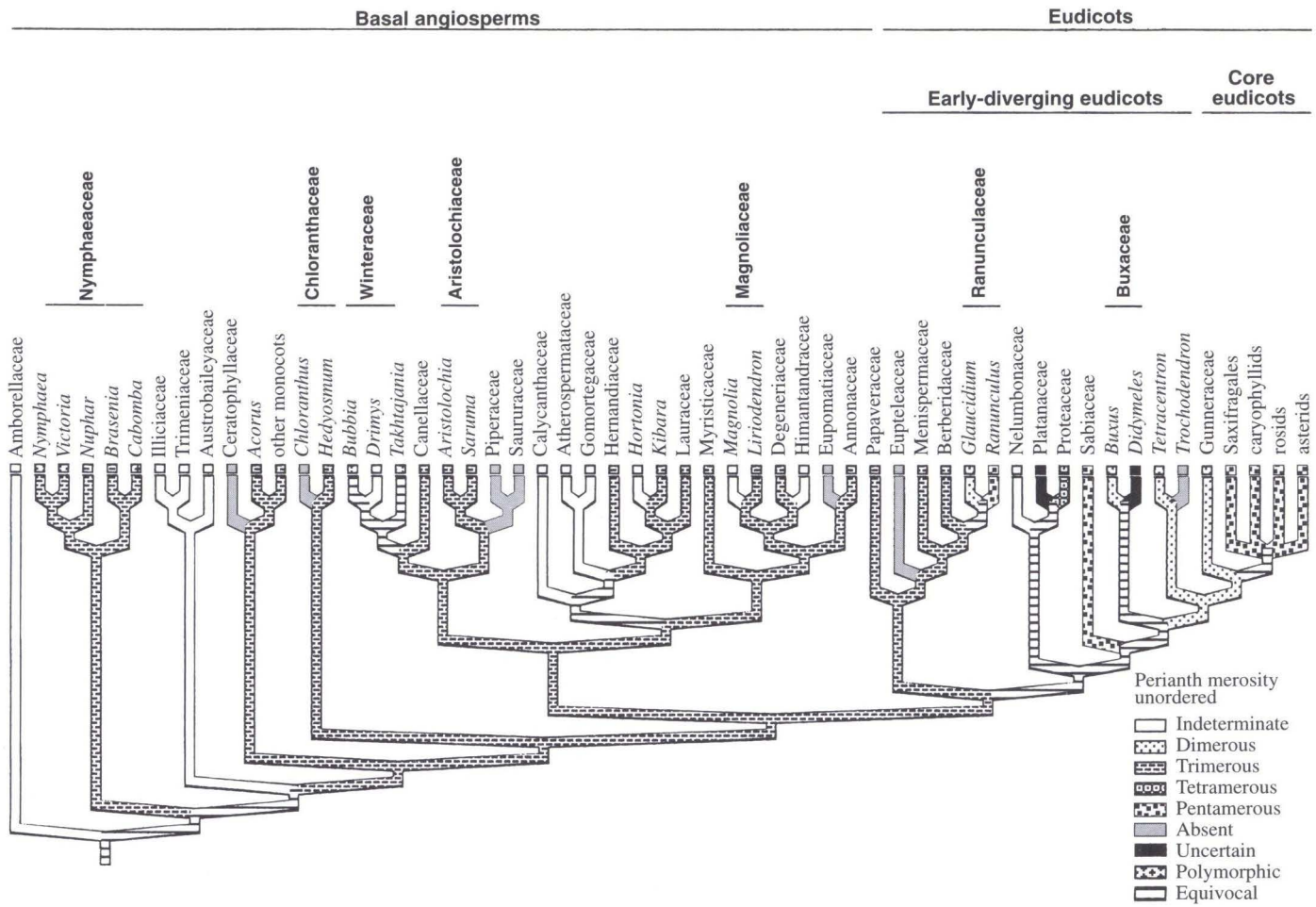

Fig. 10.5. MACCLADE reconstruction using all most parsimonious states optimization (Maddison and Maddison, 1992) of the evolution of perianth merosity (merism) in angiosperms, with an emphasis on basal angiosperms and early-diverging eudicots. Topology is based on Zanis *et al.* (2002, 2003) and Soltis *et al.* (2000, 2003). Data are from Zanis *et al.* (2003) and Ronse De Craene *et al.* (2003). Modified from Soltis *et al.* (2004). For impact of other optimization methods (ACCTRAN, DELTRAN) see Zanis *et al.* (2003), Ronse De Craene *et al.* (2003) and Soltis *et al.* (2004).

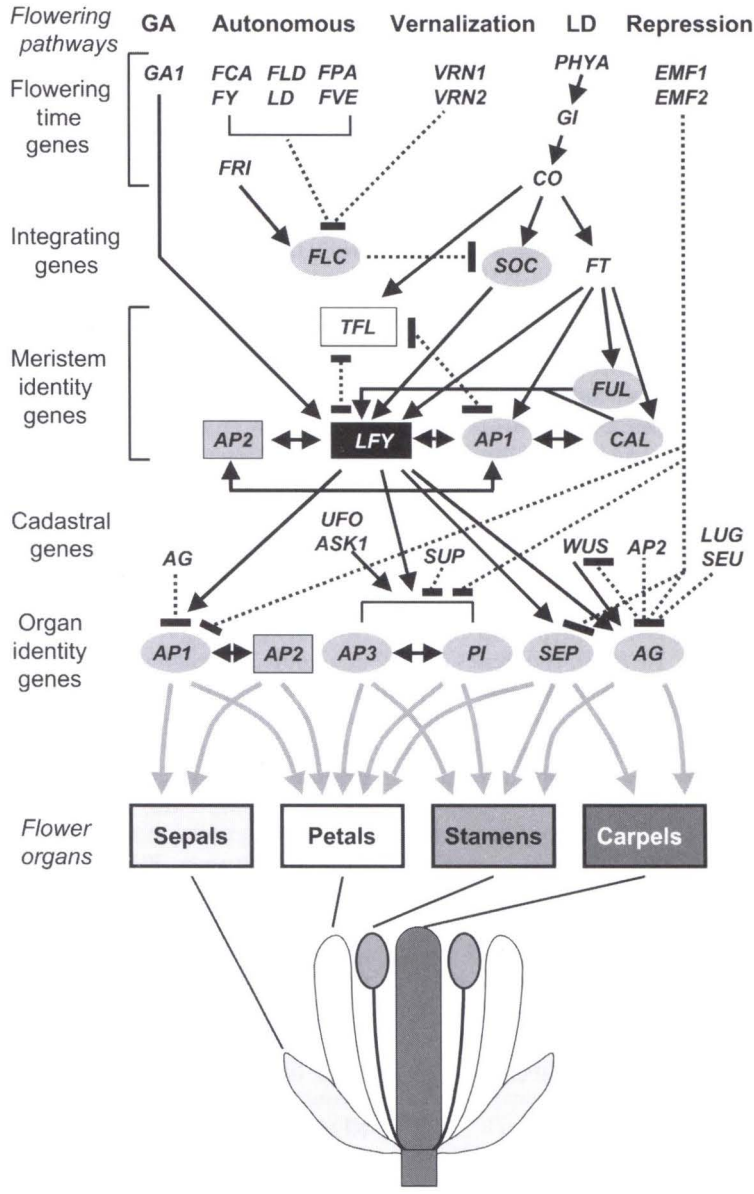

Fig. 10.6. Genes that have been demonstrated genetically to regulate flowering time, floral meristem and organ identities in *Arabidopsis*. MADS-box genes are shown in ovals. For genes that encode other types of proteins, only those that play critical roles in floral meristem and organ identities are shown in boxes. The black lines and arrows indicate positive genetic interaction; the dotted lines with a short bar at the end represent negative genetic interactions. The arrows indicate that the specific organ identity gene(s) is (are) required for the identity of the corresponding organ. See Fig. 10.7 for an illustration of the ABC model. Although few genes have been identified that function downstream of the organ identity genes (Sablowski and Meyerowitz, 1998), a number of putative downstream genes for *LFY* and *AP3/PI* have been reported recently from microarray analysis (Schmid *et al.*, 2003; Zik and Irish, 2003). Modified from a figure in Soltis *et al.* (2002), with recent information on the regulation of floral meristem identity genes by *CO* and *FT* (Schmid *et al.*, 2003) and regulation of floral organ identity genes by *EMF1*, *EMF2*, *LUG* and *SEU* (Franks *et al.*, 2002; Moon *et al.*, 2003; Schmid *et al.*, 2003).

studies (Smyth *et al.*, 1990) provided a foundation for evaluating the effects of mutations and defining gene functions. This integration of morphological and developmental genetic investigations has characterized the work on several other model systems as well, including the derived monocots *Zea mays* and *Oryza sativa* (Poaceae), and to a lesser extent *Petunia hybrida* and *Lycopersicon esculentum* (= *Solanum lycopersicum*), both of Solanaceae (Coen and Meyerowitz, 1991; Meyerowitz *et al.*, 1991; Ma, 1994, 1998; Weigel and Meyerowitz, 1994; Weigel, 1995; Yanofsky, 1995; Ma and dePamphilis, 2000; Zhao *et al.*, 2001a; Irish, 2003).

The best-known genes controlling floral organ identity are the A, B and C function genes (Coen and Meyerowitz, 1991; Meyerowitz *et al.*, 1991). According to the ABC model, three overlapping gene functions, A, B and C, act alone or in combination to specify the four types of floral organs (Fig. 10.7). In 1990, the genes representing *deficiens* (B class) and *agamous* (C class) mutants were cloned from *Antirrhinum* and *Arabidopsis*, respectively.

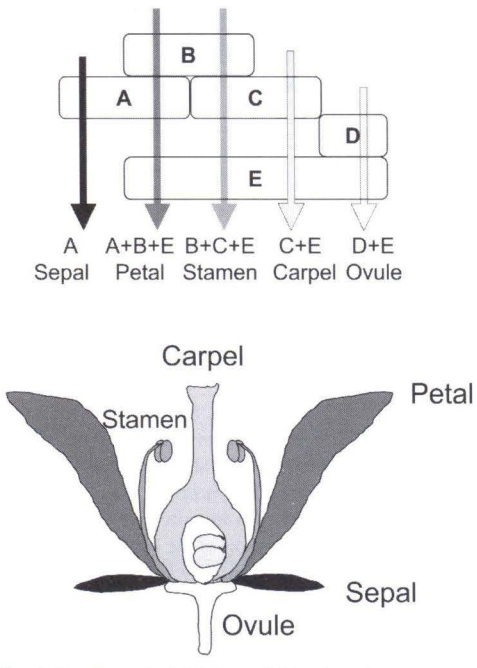

Fig. 10.7. Extended ABC model for floral organ specification (modified from Theißen, 2001).

Homologous genes from these two models sometimes have different names, creating some confusion for newcomers to the field; we therefore often provide both names in our overview. The protein products of *DEFICIENS* (*DEF* = *APETALA3* (*AP3*) in *Arabidopsis*) and *AGAMOUS* (*AG* = *PLENA* (*PLE*) in *Antirrhinum*) were found to be from the same family of transcription factors, which are regulators of the expression of other genes (Schwarz-Sommer *et al.*, 1990). This family was named MADS-box genes after a DNA-binding amino acid domain present in *MCM1* (mini-chromosome maintenance-1; from yeast), *AG*, *DEF* and *SRF* (serum response factor; from humans). MADS-box genes encode a conserved domain that constitutes most of the DNA-binding domain.

It had been hypothesized from mutant phenotypes that the *DEF* (= *AP3*) and *AG* (= *PLE*) genes control floral organ identity in a combinatorial, whorl-specific fashion: A function directs sepal identity; B function together with A specifies petals; B plus C function designates stamens; and C alone promotes carpel development (Meyerowitz *et al.*, 1991; Ma, 1994; Weigel and Meyerowitz, 1994; see below; Fig. 10.7). The *DEF* and *AG* gene products were assigned to the B and C functions, respectively.

As noted, in *Arabidopsis*, the A function genes are *AP1* and *AP2* (Fig. 10.7), the B function genes are *AP3* (= *DEF*) and *PISTILLATA* (*PI* = *GLO* in *Antirrhinum*), genes that resulted from an ancient duplication event (discussed below), and the C function is specified by *AG* (= *PLE*) (reviewed in Ma, 1994; Ma and dePamphilis, 2000). Genetic studies were crucial for the identification of these gene functions, with mutations in each of these genes affecting two adjacent whorls. For example, *ap3* mutants produce sepals and carpels instead of petals and stamens, respectively. Double- and triple-mutant analyses in *Arabidopsis* have further clarified the genetic interactions among A, B, C class genes. Expression studies have also been important in confirming aspects of the ABC model. All of the ABC MADS-box genes are expressed in the regions of the floral meristem that they help specify. The model is sup-

ported by over-expression studies of the ABC genes in *Arabidopsis*, which can place any of the four flower organs in any of the four whorls.

Recently, in *Arabidopsis*, the role of the class E genes, *SEPALLATA1*, *SEPALLATA2* and *SEPALLATA3*, has been demonstrated: they act redundantly to specify petals, stamens and carpels (Pelaz *et al.*, 2000, 2001; Theißen, 2001). These genes were identified through their sequence similarity to *AG*, rather than through individual mutant phenotypes. Triple-null mutants of *SEP1-3* produce 'flowers' consisting only of sepal-like organs, suggesting that these related genes have redundant functions in controlling the identity of petals and reproductive organs (Pelaz *et al.*, 2000, 2001). Floral MADS-domain proteins can form homodimers, heterodimers and tetramers, providing a mechanism for the interaction of genes within and between the A, B, C and E functions (Theißen, 2001) (see Fig. 10.10).

In addition to A, B, C and E function genes, numerous other genes are also regulators of normal floral development. Furthermore, not all floral regulators are MADS-box genes. In *Arabidopsis*, the non-MADS *APETALA2* (*AP2*) confers A function along with the MADS gene *APETALA1* (*AP1*= *SQUAMOSA* (*SQUA*) in *Antirrhinum*). *LEAFY* (*LFY*), which controls the entire floral developmental programme, codes for a previously unknown type of transcriptional regulator (Weigel *et al.*, 1992). Space does not permit review of all of the numerous genes involved in floral development here. Readers are encouraged to consult recent reviews (e.g. Ma, 1998; Zhao *et al.*, 2001a; Ni *et al.*, 2004; Fig. 10.6). Because most genes with known functions in flower development have been detected through their single-gene mutant phenotypes, genes such as the *sepallata* genes with redundant function (Pelaz *et al.*, 2000; Theißen, 2001), or genes that are lethal when disrupted, are not usually discovered except through detailed follow-up analysis. As a result, even this rapidly growing collection of genes of known function must be considered an underestimate of the genes with critical roles in flower development.

Genes are also known that specify the floral character of the apical meristem that forms the flower. The genes *FLORICAULA* (*FLO*) and *LEAFY* (*LFY*) of *Antirrhinum* and *Arabidopsis*, respectively, are transcription factors of a family unique to land plants. *FLO/LFY* is single copy in diploid angiosperms. *FLO/LFY* is expressed in a graded manner and acts synergistically with the MADS-box gene *SQUAMOSA/AP1* to specify the floral character of the apex. These genes integrate signals from multiple pathways involved in the transition to flowering. Some of the additional genes involved in floral specification are shown in Fig. 10.6 (e.g. Coen *et al.*, 1990; Weigel *et al.*, 1992; Weigel, 1995; Riechmann and Meyerowitz, 1997; Ma, 1998; Theißen *et al.*, 2000; Theißen, 2001; Zhao *et al.*, 2001a).

New model plants

Exhaustive studies of a few key model plants, chiefly *Arabidopsis* and *Antirrhinum*, have provided enormous insights into the genetic control of flower development. However, a key question is, are the models of the genetic control of floral development in these derived eudicots applicable to all angiosperms? Interestingly, the conservation of A function is unclear in angiosperms other than Brassicaceae. Another floral developmental model emphasizing the B and C functions alone (called at that time A and B) was developed even before the ABC model, and this focus might be more broadly applicable (Schwarz-Sommer *et al.*, 1990) (Fig. 10.8). The genetic architecture of floral development in angiosperms other than the well-known models should also be investigated (e.g. Albert *et al.*, 1998; Kramer and Irish, 2000; Soltis *et al.*, 2002). To obtain maximal benefit from the enormous resources afforded by well-developed models for floral developmental genetics, it is imperative that researchers expand their emphasis to include additional species representing a wider phylogenetic coverage of angiosperms.

The rapid increase in interest in the evolutionary developmental biology ('evo-devo') of the flower has stimulated the investiga-

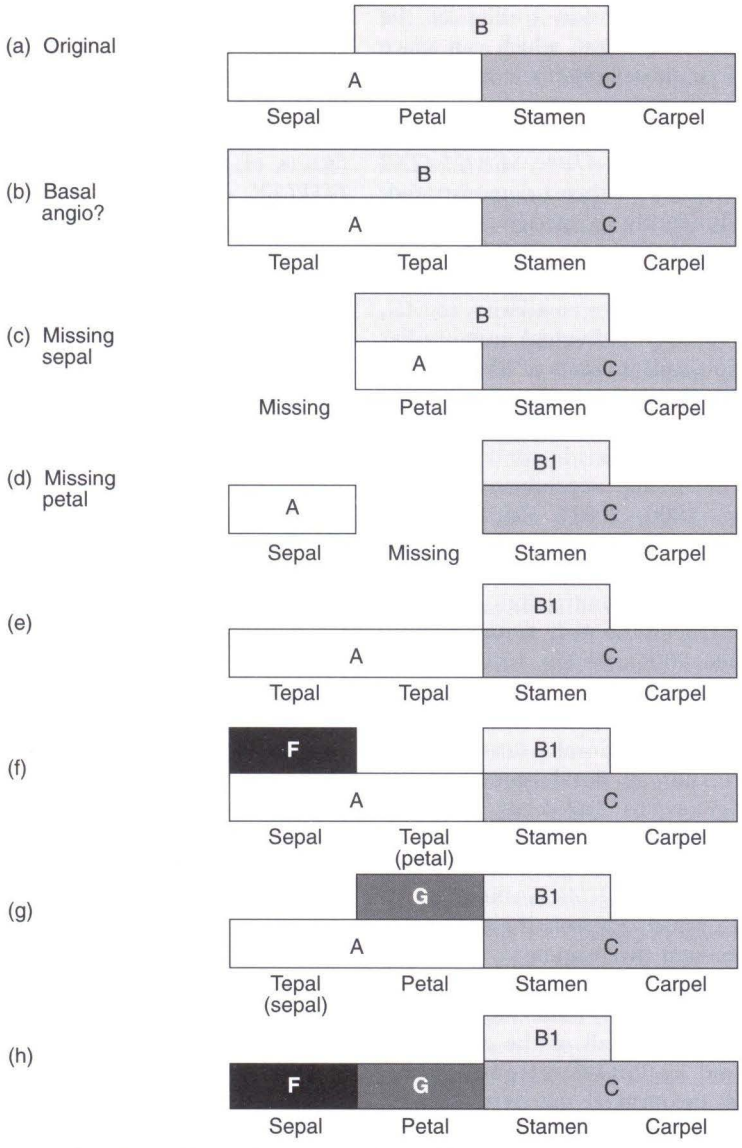

Fig. 10.8. The original ABC model (a) with variations that could explain morphological changes. Versions (b–d) simply allow the change of the domains of A and B functions to account for the diversity in the perianth. Version (e) makes the control of the tepal identity similar to that of the sepal identity in derived eudicots, although tepals are often morphologically similar to petals. Versions (f–h) propose 'F and G functions' different from the ABC functions in distribution and in consequence to control perianth identities. B1 is used instead of B when the function is only used to control the stamen identity.

tion of a number of new 'model' plants, and many of these are under investigation as part of genomics initiatives (Soltis *et al.*, 2002; De Bodt *et al.*, 2003). New models have typically been chosen based on their significant phylogenetic positions (Fig. 10.2). *Amborella* (Amborellaceae) and waterlilies (Nymphaeaceae) were chosen because they represent the sister groups to all other angiosperms. Other basal angiosperms (e.g.

Lauraceae and Magnoliaceae) are also the focus of study, as is *Acorus* (Acoraceae), the sister to all other monocots. Poppies (*Papaver* and *Eschscholzia*) are important choices because they represent an early-diverging eudicot lineage (Fig. 10.2) and provide a critical link between derived eudicot models (e.g. *Arabidopsis* and *Antirrhinum*) and basal angiosperms.

The growing list of new models not only expands the phylogenetic diversity under study, but also the diversity of floral form that is currently under molecular and genetic investigation (Soltis *et al.*, 2002; De Bodt *et al.*, 2003). In addition to the standard whorled arrangements of parts, new models such as *Amborella* exhibit a spiral perianth that is undifferentiated. *Gerbera*, a derived asterid in the sunflower family, is also a useful model because of its divergent inflorescence format: multiple flowers of different phenotypes borne together in a dense head (Yu *et al.*, 1999; Kotilainen *et al.*, 2000).

The new models also have important limitations. For most, genetic studies are not yet possible. Although developmental morphological and molecular studies can lead to the formulation of useful hypotheses regarding the evolution of gene functions, these await testing using genetic studies. For basal angiosperms that are woody (e.g. *Amborella*, *Persea*) and not readily analysed genetically, definitive conclusions about gene functions will be difficult to achieve. Therefore, herbaceous basal angiosperms (e.g. the waterlily *Cabomba*) and herbaceous basal eudicots (e.g. *Papaver* or *Eschscholzia*) may have the greatest potential as new models because of their short life cycles and the transformability of *Papaver* (Baum *et al.*, 2002).

New technologies might provide effective methods for reverse genetic analysis of new genetic models. Methods that use viruses to generate small, interfering RNA and to post-transcriptionally silence a gene of interest (Lu *et al.*, 2003) might be applicable in mature plants, even in long-lived perennials. Such new methods, if perfected, that allow easy elucidation of gene function in diverse plants by mutation or by gene silencing, could become as important for evo-devo studies as PCR has been for molecular phylogenetics.

Limits on the generality of floral developmental genetics

Through molecular evolutionary and gene exchange studies, it was determined that *AP3* represents the *Arabidopsis* homologue of *DEF* from *Antirrhinum*. Similarly, sequence and functional homologies were found between *AG* and *LFY* and their *Antirrhinum* counterparts (*PLE* and *FLO*). However, the situation with A function genes is more complex. Mutations in the *Antirrhinum SQUAMOSA* gene, a likely orthologue of *AP1* from *Arabidopsis*, cause floral meristem defects similar to *ap1* mutants. Flowers of *squamosa* mutants also exhibit defects in petal development, although the role of *SQUAMOSA* in controlling petal identity is thought to be less important than that of *AP1* in *Arabidopsis*. Recently, two *Antirrhinum* homologues, *LIPLESS1* and *LIPLESS2* (*LIP1* and *LIP2*), of the *Arabidopsis AP2* gene, have been shown to have redundant functions in controlling sepal and petal development (Keck *et al.*, 2003) in a manner similar to that of *AP2*. However, unlike *AP2*, *LIP1/2* do not seem to be involved in the negative regulation of the C-function gene *PLENA* (*PLE* = *AG*). Therefore, results from *Antirrhinum* support a critical role for A function in determining perianth identities, but the interactions between known genes involved in A and C functions seem to be different between *Arabidopsis* and *Antirrhinum*. *AP1*- and *AP2*-like genes have been identified from a diverse array of angiosperms (Litt and Irish, 2003); however, whether they play a role in A function is not known.

Therefore, the existence of a conserved A function in angiosperm flower development is still uncertain, although there should be gene functions that specify sepal identity in species that produce a differentiated perianth. It is possible that different genes serve this function in different flowering plant lineages, or that the determination of sepal and petal identity is more complex than depicted in the ABC model. Based on the presence of B- and C-function MADS-box genes in gymnosperms (which lack flowers), it has been hypothesized that determination of flower organ identity has evolved from a

more ancient role of these genes in sex determination (Hahn and Somerville, 1988; Münster *et al.*, 1997; Winter *et al.*, 1999). It has also been hypothesized that 'true' sepals of the kind expressed by *Arabidopsis* and *Antirrhinum* are a relatively recent evolutionary innovation, because basal eudicots and monocots characteristically lack discrete sepals and petals and bear tepals instead.

Conservation of control over floral specification of the flower apex by *FLO/LFY* has been shown for several eudicots, but additional functions are also known. For example, the *LFY* homologue of *Pisum* (Fabaceae) controls compound leaf development in addition to the transition to flowering. In grasses, *LFY* homologues probably direct the development of inflorescence meristems rather than only floral meristems, as in *Arabidopsis*.

As noted above, the *SEPALLATA* (*SEP*) genes of *Arabidopsis* provide redundant function required for floral organ identity. However, research on other organisms such as *Gerbera* has shown that *SEP*-class homologues play divergent roles in development of the condensed, head inflorescence as well as in the different floral forms that are borne by it. Specifically, one *SEP*-like gene confers the C function only in the staminal whorl of flowers borne at the periphery of the inflorescence, whereas another *SEP* homologue (the probable duplication partner of the first) appears to confer the C function only in carpels (Teeri *et al.*, 2002). This partitioning of genetic function has probably had morphological evolutionary consequences because the outer flowers of *Gerbera* inflorescences are male-sterile and highly asymmetrical, with fused, elongate petals, whereas the inner flowers are bisexual and very close to symmetrical, with non-elongate petals. The *Gerbera* inflorescence looks very much like a single flower, and probably attracts pollinating insects in the same capacity.

The ABC Model: New Data, New Views

ABCs of basal angiosperms

Recent investigations of basal angiosperms have provided an important assessment of the applicability of the ABC model to all angiosperms. Certainly much of the ABC framework is conserved in a number of eudicots and grasses, but there are important variations on the ABC theme in some flowering plants (Fig. 10.8). For example, in contrast to the well-differentiated sepal and petal whorls of eudicots such as *Arabidopsis* and *Antirrhinum*, the two outer floral whorls in many members of the monocot family Liliaceae (lily family) are petaloid and almost identical in morphology (Fig. 10.1). Importantly, in *Tulipa* (tulip), the B-class genes are expressed in both petaloid whorls, as well as in stamens (Kanno *et al.*, 2003). This situation supports the idea that petals and petal-like organs require B function, regardless of the position of these organs within the flower.

Similarly, in some Nymphaeaceae (waterlilies) such as *Nuphar*, the outer whorl of the flower, sometimes referred to as sepals, exhibits B class gene expression, as do the petals, stamens and staminodes (Kim *et al.*, 2004) (Fig. 10.8). In *Amborella*, which has a spirally arranged perianth with parts that are not differentiated into sepals and petals (Fig. 10.1), a similar pattern is observed, with B class genes expressed throughout the perianth, as well as in the stamens (Fig. 10.8). Similar expression data have been forthcoming for basal angiosperms in the magnoliid clade. In *Magnolia* (Magnoliaceae), B class gene expression has been documented throughout the perianth whorls, as well as in stamens and staminodes (Kramer and Irish, 2000; Kim *et al.*, unpublished). A similar pattern of B-class gene expression has been observed in basal eudicots such as *Papaver* (Papaveraceae) and various members of Ranunculaceae (Kramer and Irish, 2000; Kramer *et al.*, 2003). The expression of B-function genes in sepal-like organs suggests that these B-function genes are not sufficient to specify petal identity.

The expression of C-class genes has also been examined in several basal angiosperms, and the results for this gene match the predictions of the ABC model. For example, homologues of *AGAMOUS* have been isolated from *Amborella*, and these are expressed in carpels, stamens and sta-

minodes (Kim *et al.*, unpublished). Data for the expression of A-class genes from the basal-most angiosperms remain fragmentary.

Thus, recent data suggest a modified ABC model for basal angiosperms, with B-class genes expressed and presumably functioning throughout the perianth and stamens (Fig. 10.7) (see Van Tunen *et al.*, 1993; Albert *et al.*, 1998) following the original 'BC model' idea put forth by Schwarz-Sommer *et al.* (1990). From a phylogenetic standpoint, the ABC model may reflect a more recent programme that is important in *Arabidopsis* and possibly other eudicots. The specification of sepals, which may have evolved more than once (Albert *et al.*, 1998), may well be encoded by different genes in different angiosperm lineages. The pattern of B-class gene expression observed in basal angiosperms and basal eudicots probably represents the ancestral condition, with the model originally proposed for *Arabidopsis* and *Antirrhinum* a derived modification (Fig. 10.8).

An important evolutionary question now becomes: at which node in the angiosperm tree did the switch from the more general BC model occur? Functional studies in phylogenetically critical taxa are required before this question can be answered, but the switch probably coincided with the evolution of the core eudicots (Fig. 10.2). Other important changes in floral genes similarly appear to coincide with the origin of core eudicots, including duplication of *AP3* yielding the eu*AP3* gene lineage, as well as the origin of *AP1* (Kramer *et al.*, 1998; Litt and Irish, 2003).

Molecular phylogenetic analyses of the gene families involved in floral development are elucidating the important role that gene duplication has played in the evolution of flower development. The gene duplications and losses evident in gene family phylogenies can confuse discussions of functional evolution when the genes with equivalent function in different species are not orthologous. At the same time, orthology does not always coincide with strict functional equivalence (e.g. the discussion of *LIP* genes in Keck *et al.*, 2003). Given the lack of perfect correspondence between gene function and phylogeny, a clear distinction should be made between functional and phylogenetically based classifications of gene relationships (Becker and Theißen, 2003).

AP3/PI-like genes: an ancient duplication

The evolution of MADS-box genes has involved a series of gene duplications and subsequent diversification, as well as losses. Several investigators have conducted phylogenetic analyses of the floral MADS-box genes (e.g. Purugganan, 1997; Theißen *et al.*, 2000; Johansen *et al.*, 2002; Nam *et al.*, 2003; Becker and Theißen, 2003). For example, a duplication yielding the A and E + *AGL6* class genes occurred approximately 413 million years ago (mya) (Nam *et al.*, 2003), and the ages of several other prominent MADS-box gene duplications have also been estimated (e.g. Purugganan *et al.*, 1995; Purugganan, 1997; Nam *et al.*, 2003).

Whereas angiosperms possess two B-class paralogues (*AP3* = *DEF*, and *PI* = *GLO*), only one certain B-class homologue has been found in gymnosperms, suggesting that an ancient duplication led to the presence of the *AP3* and *PI* homologues. However, the accelerated rate of evolution of *AP3* and *PI* relative to other MADS-box genes precluded estimation of the age of the *AP3/PI* duplication by molecular clock-based substitution rate methods (Purugganan *et al.*, 1995; Purugganan, 1997; Kramer *et al.*, 1998; Nam *et al.*, 2003). Tree-based methods using a data set of over 20 new *AP3* and *PI* gene sequences for basal angiosperms estimated that the *AP3/PI* duplication occurred approximately 260 mya (range of 230–290 mya) (Kim *et al.*, 2004). This date places the duplication shortly after the split between extant gymnosperms and angiosperms and on the 'stem' lineage of extant flowering plants. This indicates that the *AP3/PI* duplication occurred perhaps 100 million years before the oldest fossil flowers (generally placed at 125–131.8 mya; Hughes, 1994). Thus, this suggests that the joint expression of *AP3* and *PI* did not immediately result in the formation of petals, structures for which they control development in extant angiosperms, because no such structures are present in the

fossil record at that time. This raises the question: what was the early (pre-angiosperm) role of the *AP3* and *PI* homologues? The co-expression of *AP3* and *PI* homologues could reflect an evolutionary innovation of animal-attractive, petal-like organs well before the appearance of angiosperms in the fossil record. Indeed, some fossil, non-angiosperm seed plants from the appropriate timeframe, such as the glossopterids, had sterile spathe-like organs attached to male or female reproductive structures (e.g. Crane, 1985).

Transcription-factor complexes: early flexibility?

A striking result of Kim *et al.* (2004) is the strong similarity between *Amborella* AP3 and PI C-domain amino acid sequences (Fig. 10.9). The C domains, as well as K- and MADS-domains, signal the assembly of multimers for several MADS proteins in core eudicots (Egea-Cortines *et al.*, 1999; Ferrario *et al.*, 2003). Indeed, higher-order multimers are probably the active state of B-function MADS-box proteins (Egea-Cortines *et al.*, 1999; Honma and Goto, 2000; Theißen, 2001; Ferrario *et al.*, 2003).

Heterodimerization of AP3 and PI proteins is required for DNA binding in the core eudicots that have been studied. However, PI/PI homodimers are possible in some monocots, at least *in vivo*, although it is not clear whether these can bind DNA (Fig. 10.10). (Even the *Arabidopsis* PI proteins can form homodimers, but these cannot bind DNA (Riechmann *et al.*, 1996).) Selective fixation of heterodimerization has been hypothesized for the morphologically more stereotyped core eudicots (Winter *et al.*, 2002). However, the phylogenetic point at which heterodimerization became enforced is not yet clear. Hints from sequence comparison suggest that *Amborella*, and perhaps some other basal lineages (e.g. Nymphaeaceae), may have retained some capacity for B-class homodimerization.

Because *Amborella* proteins may have K-domain heterodimerization signals that differ from those in *Arabidopsis* and other well-studied angiosperms, the data suggest that *Amborella* B-function proteins may have different dimerization dynamics from monocots and core eudicots. Two different *AP3* genes are present in *Amborella* (*Amborella AP3-1* and *AP3-2*). *Amborella* may be capable of forming PI/PI, AP3-1/AP3-1 and AP3-2/AP3-2 homodimers and perhaps AP3-1/AP3-2 heterodimers. Furthermore, if the amino acid residues in the K1 subdomain of *Amborella* AP3 and PI are not sufficient to prevent heterodimerization, but only weaken it, perhaps *Amborella* can also form AP3-1/PI and AP3-2/PI heterodimers (Fig. 10.10). Recent studies using transgenic *Arabidopsis* plants indicate that the C terminus of AP3 is sufficient to confer AP3 functionality on the paralogous PI protein (Lamb and Irish, 2003). This finding, when considered in the light of *Amborella* and its indistinct AP3 and PI C domains, also supports the possibility of AP3-1/AP3-2 heterodimerization.

A simple extension of the *Arabidopsis* 'quartet model' for MADS protein function (Fig. 10.10; Theißen, 2001) can accommodate both

(a)

		Exon 1	Exon 2	Exon 3	Exon 4	**Exon 5**	Exon 6	Exon 7
PI family	*Am.tr.PI* (*Amborella*) *Nu.ad.PI* (*Nuphar*)	113<	67	62	100	**42**	45	132
	GLO (*Antirrhinum*)	188	67	62	100	**30**	45	132
	PI (*Arabidopsis*)	255		62	100	**30**	45	132
AP3 family	*DEF* (*Antirrhinum*)	188	67	62	100	**42**	45	132
	AP3 (*Arabidopsis*)	188	67	62	100	**42**	45	132
	SILKY1 (*Zea*)	188	67	62	100	**42**	45	132

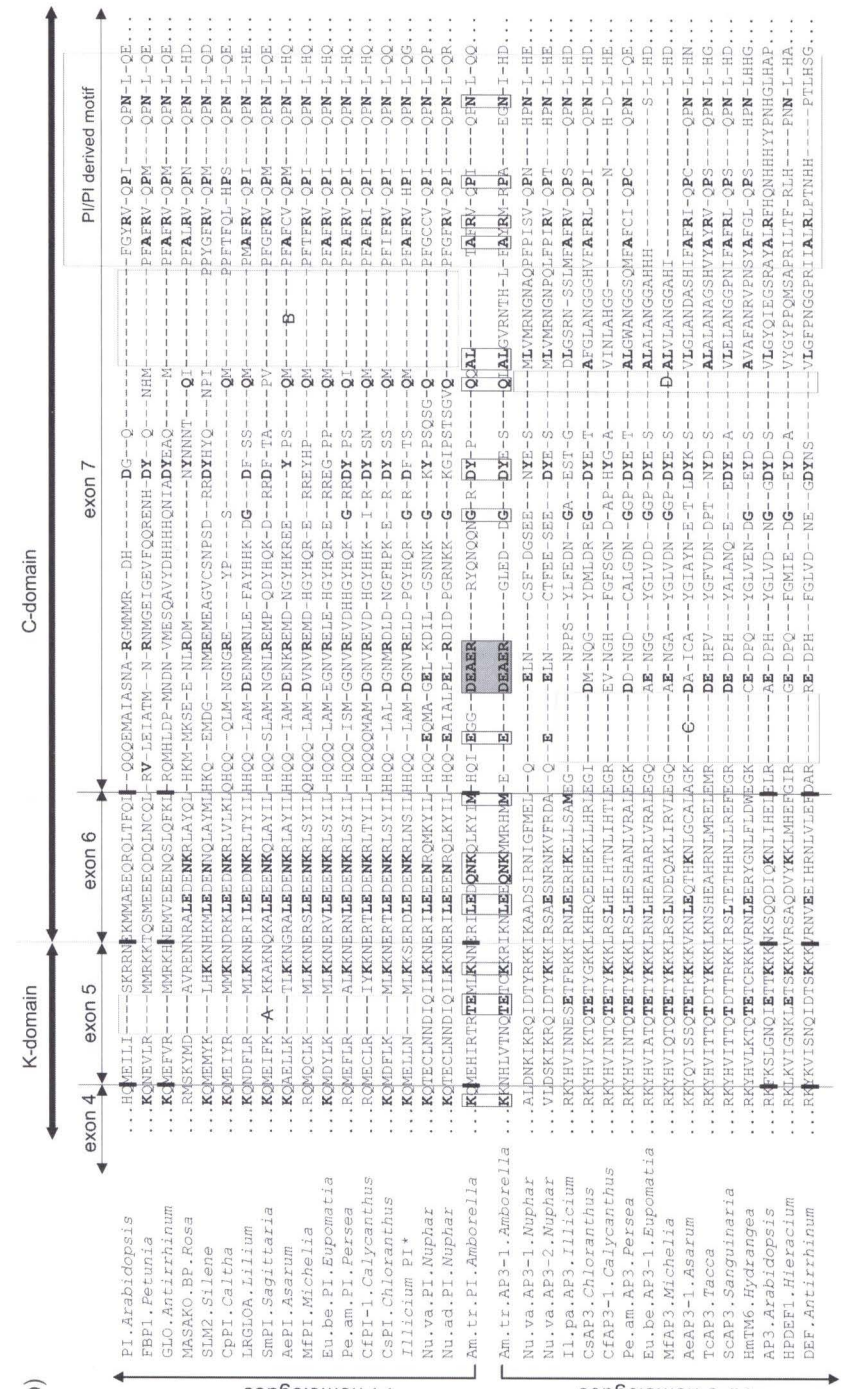

Fig. 10.9. *AP3/PI* gene structure in basal angiosperms. (a) (Opposite.) The size of exon 5 in *Amborella* and *Nuphar* compared with that observed in *Arabidopsis* and other eudicots. (b) Comparison of *AP3/PI* domain similarities in *Amborella* and other basal angiosperms (from Kim *et al.*, 2004).

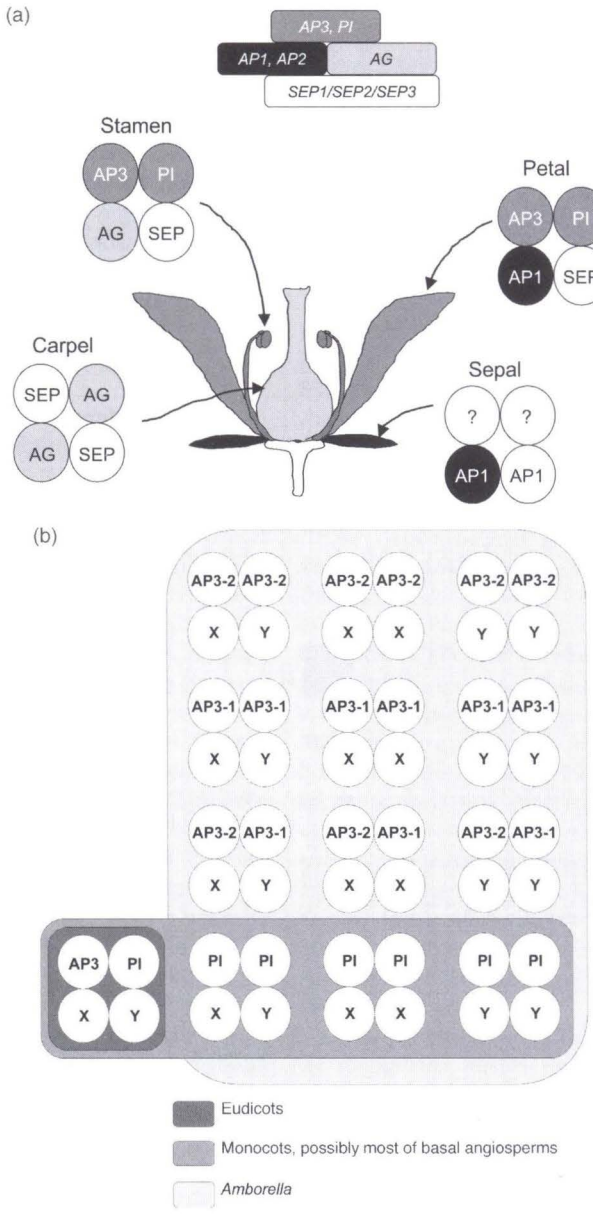

Fig. 10.10. Transcription-factor complexes. (a) Quartet model of floral organ specification in *Arabidopsis* (Theißen, 2001). (b) Extension of the quartet model for determination of floral organ identity to include *Amborella*. MADS protein tetramers are shown schematically (as in (a); see Theißen, 2001). One possible model is presented with the following assumptions: (i) AP3/PI obligate heterodimerization occurs in core eudicots; (ii) additional MADS proteins X and Y are available in cells; (iii) PI/PI dimers can tetramerize with all three configurations of X and Y in monocots (Winter *et al.*, 2002) and possibly other basal angiosperms, whereas only the XY configuration is possible in core eudicots; (iv) *Amborella* AP3/AP3 and PI/PI dimers possess similar capacities for C-domain tetramerization specification; and (v) *Amborella* AP3-1 and AP3-2 proteins are able heterodimerize. Tetramer potential would be 12:4:1 for *Amborella*, monocots (and perhaps other basal angiosperms) and core eudicots, respectively. If *Amborella* AP3 and PI can also heterodimerize to some extent, the ratio of possible quartets in *Amborella*:monocots:core eudicots becomes 18:4:1.

the monocot and *Amborella* cases. In this hypothetical example, the MADS protein tetramer AP3/PI/X/Y specifies a particular organ identity in *Arabidopsis*. Assuming that homodimerization is the ancestral state for B-function proteins (Winter *et al.*, 2002), we can invoke a model whereby PI/PI homodimers, as known from and argued to have functional significance in monocots (Münster *et al.*, 2001; Winter *et al.*, 2002), are more flexible in their protein partnerships. This scenario could call for the possibility of PI/PI/X/Y, PI/PI/X/X and PI/PI/Y/Y tetramers in monocots and most other basal angiosperms (Fig. 10.10).

Although this hypothesis must be tested using gel shift and yeast 2-, 3- and 4-hybrid assays (Winter *et al.*, 2002; Ferrario *et al.*, 2003), the implications of this model (Fig. 10.10; Kim *et al.*, 2004) are that *Amborella* would have 12 times more tetramer possibilities than a core eudicot and three times greater tetramer potential than a monocot or other basal angiosperm with limited homodimerization potential. Given that *Amborella* may have the capacity to form more different protein quartets for a given number of genes, it should possess more distinct controls (and therefore flexibility) over organ identity and development than any other flowering plant. The waterlily *Nuphar* also has considerable C-domain similarity for the AP3 and PI proteins, and this may be sufficient to provide the Nymphaeaceae with at least some extra tetramerization possibilities. By contrast, *Illicium*, which represents the next most basal clade of angiosperms after the Nymphaeaceae (Austrobaileyales; Fig. 10.2), has lost most of the C-domain AP3/PI similarity. Furthermore, a deletion in the K domain of *PI* (Fig. 10.9) first appears in *Illicium* (Austrobaileyales) and is fixed in all other angiosperms. Although most flowering plants are canalized in their possibilities for heterodimerization and multimer formation (Theißen, 2001), several eudicots (e.g. Ranunculales, Kramer *et al.*, 2003; *Petunia*, Ferrario *et al.*, 2003) and monocots (Münster *et al.*, 2001) may have regained some potential for developmental flexibility by a different mechanism involving later duplications of *AP3* homologues, *PI* homologues, or both.

The data suggest that the evolution of the control of B-function MADS-box genes in the development of the earliest flowers was dynamic, with different 'experiments' tried. *Amborella*, which may be the most flexible living angiosperm in its developmental genetics, is the sole surviving representative of its clade. Some of this same biochemical flexibility may also be present in waterlilies. These are testable hypotheses, to be pursued with more rigorous molecular investigations. None the less, *Amborella* B-function proteins would have represented a considerable increase in complexity over the demonstrated B-protein homodimerization known for conifers (Sundström *et al.*, 1999) and *Gnetales* (Winter *et al.*, 2002). However, the amino acid structural evidence suggests that this flexibility was rapidly lost before the bulk of the angiosperm radiation occurred. The unique phylogenetic position of *Amborella* and waterlilies, coupled with their apparently ancestral and flexible mode of B-gene function, make them model organisms that should be studied more intensively.

The Early Floral Genome

Rice and *Arabidopsis*: similarity in gene copy number

Early angiosperms clearly had the basic framework of B- and C-function genes in place. However, these genes are only a few of those involved in floral organ development and identity (Fig. 10.6) (Zhao *et al.*, 2001a). Complete sequencing of the rice and *Arabidopsis* genomes has made it possible to conduct comparisons of floral gene homologues shared by a derived monocot and a derived eudicot. These comparisons reveal a striking similarity in the number of homologues of genes involved in floral identity in the two species (Fig. 10.11). The similarity in gene family sizes is surprising given that the genome of rice is four times larger than that of *Arabidopsis* and the predicted number of protein-coding genes is just over twice as large in rice (Goff *et al.*, 2002; Yu *et al.*, 2002). Similarities in gene family size may be due to conservation of orthologous sets of rice and *Arabidopsis* genes or conservation of

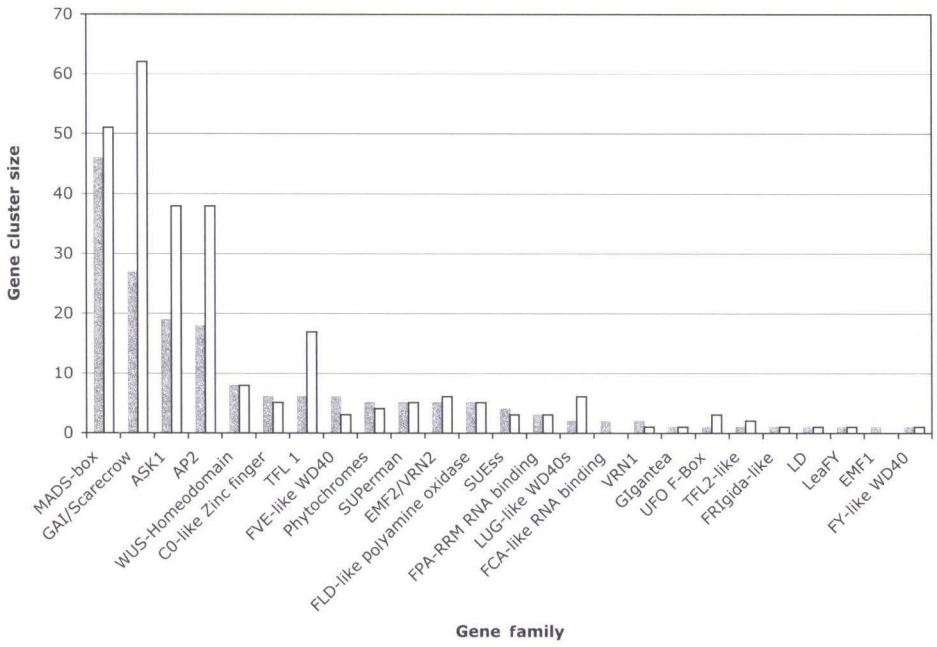

Fig. 10.11. Similarity of size of gene families that contain key floral regulators (Fig. 10.6) in two distantly related flowering plant species, *Arabidopsis* (shaded bars) and rice (open bars). The proteomes of *Arabidopsis* (26,993 proteins) and rice (62,657 proteins) were gathered into 20,934 'tribes' or putative gene families (Wall *et al.*, unpublished) using the Markov-clustering method of Enright *et al.* (2002).

gene number following independent gene duplications and losses after the split of the monocot and eudicot lineages at least 125 mya. Phylogenetic analyses of gene families allow us to test these hypotheses and investigate the evolution of gene function.

Basal angiosperms: a diverse tool kit of floral genes

As more data have emerged from major EST (expressed sequence tag) projects on angiosperms, it has become possible to make broader comparisons of some of the numerous genes and gene families that are involved in normal floral development. Particularly useful have been ESTs obtained for several basal angiosperms (www.floralgenome.org). Many genes identified in rice and *Arabidopsis* have clear homologues in basal angiosperms. Given that extant basal angiosperms represent old lineages (the waterlily lineage, for example, is among the oldest in the fossil record of angiosperms; Friis *et al.*, 2001), the data suggest that early angiosperms possessed a diverse tool kit of floral genes.

As more genes are examined phylogenetically, it is also clear that there are different types of floral gene histories. In some cases, the gene phylogenies roughly track organismal phylogeny. This is the case for the B-class genes, *PI* and *AP3*. The single-copy gene *Gigantea* also appears to track organismal phylogeny (Chanderbali *et al.*, unpublished). However, several gene families present in rice and *Arabidopsis* exhibit an array of different evolutionary patterns (see below).

AP3/PI-*like genes*

Phylogenetic analyses of *AP3* and *PI* homologues (Kim *et al.*, 2004) resulted in gene trees that generally track the organismal phylogeny (Fig. 10.12) inferred from analyses of large data sets of plastid, mitochondrial and nuclear rDNA sequences. *Amborella* and *Nuphar* (Nymphaeaceae) appear as sisters to all other angiosperms, in complete

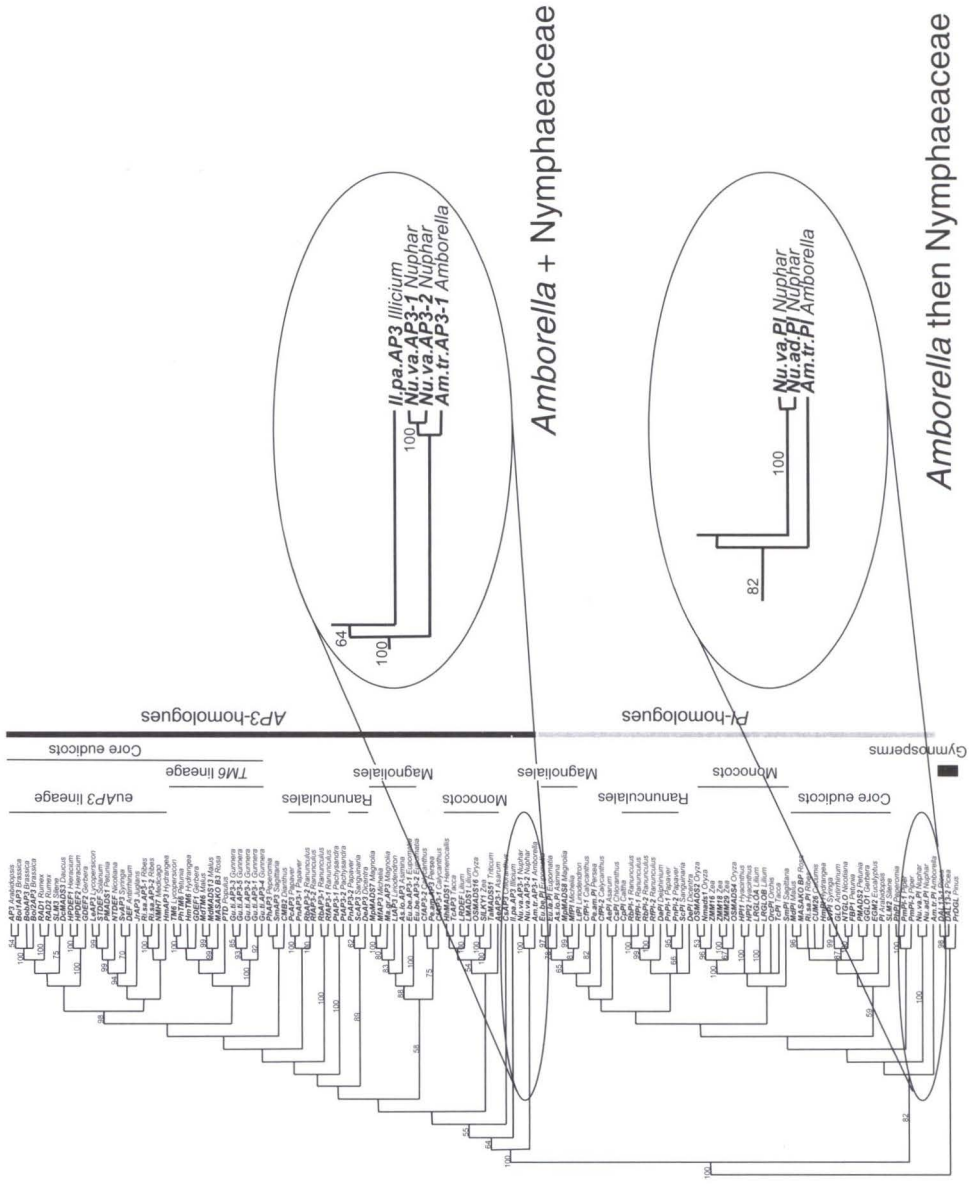

Fig. 10.12. *AP3* and *PI* gene trees. Strict consensus of 72 equally most parsimonious trees (shown as a phylogram) using M-, I-, K- and C-domain regions of amino acid sequences. Numbers above branches are bootstrap values; only values above 50% are indicated (from Kim *et al.*, 2004).

agreement with the organismal phylogeny (see references in 'Background'). Several clades of *AP3* and *PI* homologues that correspond to well-supported organismal clades were consistently recognized by Kim *et al.* (2004), including Magnoliales and monocots. The eu*AP3* gene clade, which was previously described (Kramer *et al.*, 1998), was recovered in most analyses.

SHAGGY-*like kinases*

The *SHAGGY/GSK3*-like kinases are non-receptor Ser-Thr kinases that play numerous roles in plants and animals (Kim and Kimmel, 2000). The rice and *Arabidopsis* proteomes include 69 and 79 *SHAGGY*-like kinases, respectively, but these genes can be subdivided into smaller gene families. For example, ten *Arabidopsis* genes were identified as forming a clade with *SHAGGY* itself (*AtSK* genes; Dornelas *et al.*, 2000). The *AtSK* family was shown to form four subclades in a phylogenetic analysis (Charrier *et al.*, 2003): (i) *AtSK41* and *AtSK42* formed a subclade sister to the remaining genes, which were weakly supported as a clade (bootstrap support < 50%); (ii) *AtSK31* and *AtSK32* formed a second subclade sister to the remaining genes, which formed a well-supported (88% bootstrap) clade (this clade was composed of the two remaining subclades, each of which received strong bootstrap support); (iii) *AtSK21*, *AtSK22* and *AtSK23* (100%); and (iv) *AtSK11*, *AtSK12*, *AtSK13* (98%). The *AtSK* loci appear to have diverse functions. Mutant-based analyses indicate that *AtSK11* and *AtSK12* have a role in floral development; expression analyses suggest that *AtSK31* is flower-specific (Charrier *et al.*, 2003). The *SHAGGY*-like kinases are also involved in plant responses to stress.

The Floral Genome Project research consortium has obtained ESTs for a number of *SHAGGY*-like kinase genes in basal angiosperms. Yoo *et al.* (2005) conducted a phylogenetic analysis of all *SHAGGY*-like kinase genes available in public databases, as well as the ESTs from basal angiosperms (Fig. 10.13). Plant *SHAGGY*-like kinase genes form a well-supported clade distinct from those of animals (see also Charrier *et al.*, 2003). Across all angiosperms, Yoo *et al.* identified four clades of *SHAGGY*-like kinase genes that mirrored the *AtSK* subgroups reported for *Arabidopsis*. Importantly, *SHAGGY*-like kinase genes from rice and from basal angiosperms were also represented in these four clades. For example, *SHAGGY*-like kinase ESTs from basal angiosperms appeared in all four of the subclades noted (Fig. 10.13). These data indicate that *SHAGGY*-like kinase genes diversified into these four well-marked clades early in angiosperm evolution.

SKP1-like proteins

Gene duplications within the angiosperms are also important in the history of the *SKP1* gene family. SKP1 (S-phase kinase-associated protein 1) is a core component of Skp1-Cullin-F-box protein (SCF) ubiquitin ligases and mediates protein degradation, thereby regulating many fundamental processes in eukaryotes such as cell-cycle progression, transcriptional regulation and signal transduction (Hershko and Ciechanover, 1998; Callis and Vierstra, 2000). Among the four components of the SCF complexes, Rbx1 and Cullin form a core catalytic complex, an F-box protein acts as a receptor for target proteins, and SKP1 links one of the variable F-box proteins with a Cullin (Zheng *et al.*, 2002). There is only one known functional SKP1 protein in human and yeast, and this unique protein is able to interact with different F-box proteins to ubiquinate different substrates (Ganoth *et al.*, 2001). In some plant and invertebrate species, however, there are multiple *SKP1* genes, which have evolved at highly heterogeneous rates (Farras *et al.*, 2001; Nayak *et al.*, 2002; Yamanaka *et al.*, 2002; Kong *et al.*, 2004). The extreme rate of heterogeneity observed among the 38 rice and 19 *Arabidopsis SKP1* homologues raised concerns that long-branch attraction may obscure true relationships in phylogenetic analyses of the entire gene family. For this reason, Kong *et al.* (2004) partitioned the original data set into subsets of genes with slow, medium and rapid rates of evolution and analysed each group separately. Most *SKP1* homologues observed in EST databases were included in the set of slowly evolving genes. In *Arabidopsis*, the slowly evolving *SKP1* genes were expressed more widely (in more tissues and more develop-

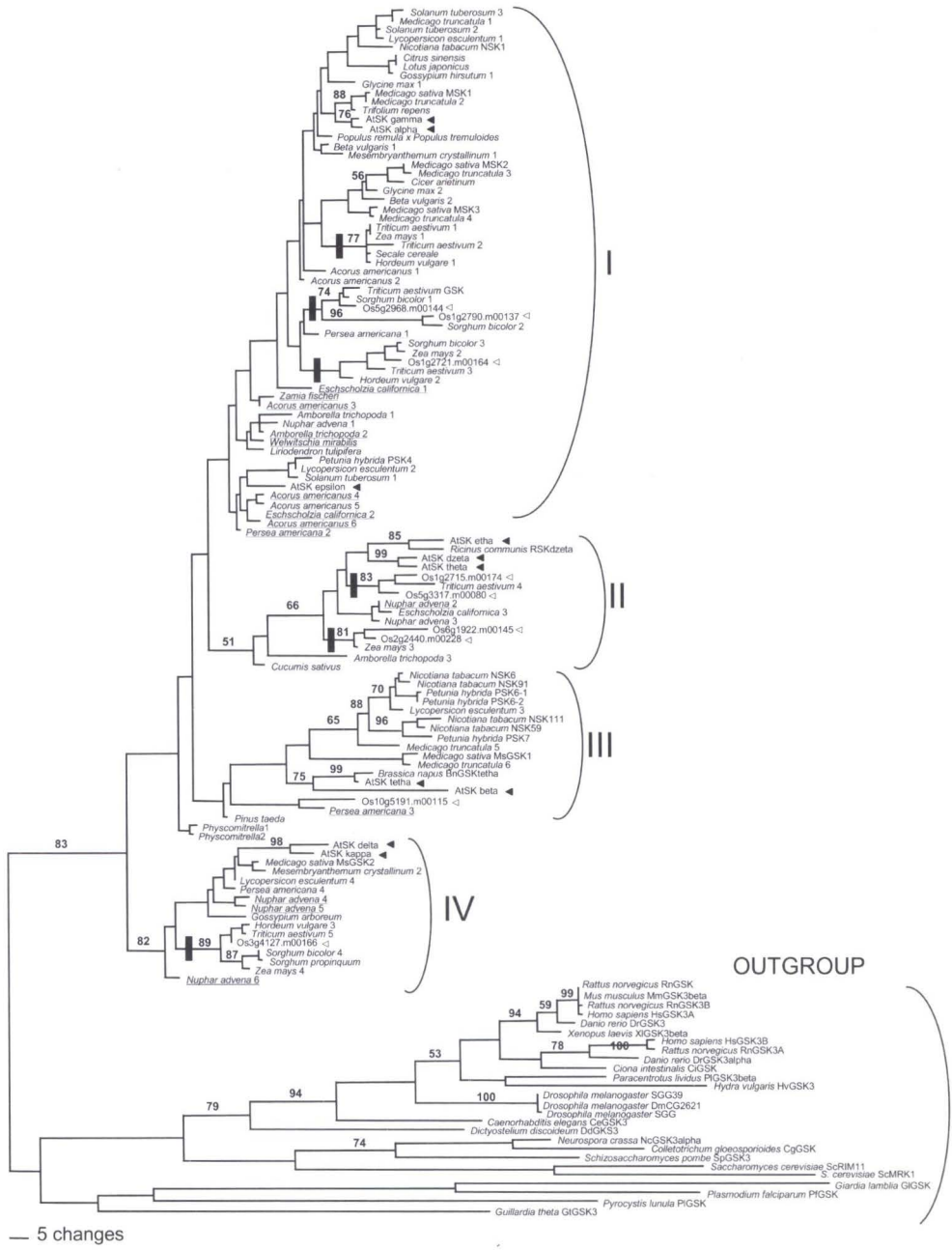

Fig. 10.13. *SHAGGY*-like kinase tree. Strict consensus of equally most parsimonious trees (shown as a phylogram) based on phylogenetic analysis of amino acid sequences. Closed triangle represents *GSK/SHAGGY*-like protein kinase from *Arabidopsis* and open triangle represents *Oryza*. Clade designations (I–IV) follow those given to *Arabidopsis* sequences (see text). ESTs provided by the Floral Genome Project (www.floralgenome.org) are underlined; monocot-specific clades are indicated by a vertical bar. Numbers above branches are bootstrap values; only values above 50% are indicated (from Yoo *et al.*, 2005).

mental stages) and at higher levels than the more rapidly evolving rice and *Arabidopsis SKP1* homologues. In addition, the strength of purifying selection was found to be significantly greater in the slowly evolving *Arabidopsis SKP1*-like genes (Kong *et al.*, 2004). Taken together, these results suggest that the slowly evolving *SKP1* homologues serve the most fundamental function(s) to interact with Cullin and F-box proteins.

The two slowly evolving *Arabidopsis SKP1*-like *1* and *2* genes, *ASK1* and *ASK2*, are important for vegetative and flower development and essential for male meiosis (Samach *et al.*, 1999; Yang *et al.*, 1999; Zhao *et al.*, 1999, 2001b, 2003). Slowly evolving *SKP1* homologues from other plant species usually have very similar sequences, suggesting that they may also serve similar fundamental functions (Kong *et al.*, 2004). Multiple slowly evolving *SKP1* homologues have been sampled in EST studies for a variety of angiosperm species, including *Liriodendron*, *Persea*, *Mesembryanthemum*, *Vitis*, *Medicago*, *Lotus*, *Rosa*, *Arabidopsis*, *Brassica*, *Gossypium*, *Helianthus* and *Solanum*. While relationships are poorly resolved across the angiosperm *SKP1* phylogeny, it is clear that gene duplication events that have occurred throughout angiosperm history have contributed to this set of conserved genes (Fig. 10.14). Recent duplication has increased *SKP1* gene diversity in *Brassica*, *Helianthus*, *Medicago* and *Triticum*. In contrast, conserved paralogues in *Liriodendron*, *Persea*, *Mesembryanthemum*, *Vitis*, *Lotus*, *Rosa*, *Arabidopsis*, *Gossypium* and Solanaceae are the products of ancient duplication events. Interestingly, the basal position of the sole *Amborella SKP1* homologue sampled from a set of 10,000 ESTs suggests that all of these duplications occurred after the origin of the angiosperms (Fig. 10.14).

Homology of Floral Organs: Extending Out from New Models

Can we use expression data to determine organ identity?

The homology of characters leading to the assessment of organ identity can be inferred from the mature phenotype, from the positions and function of organs within a flower, from developmental morphology, from phylogeny, from developmental genetics, or a combination of these approaches (Albert *et al.*, 1998; Buzgo *et al.*, 2004a). Albert *et al.* (1998) were among the first to explore the topic of using gene expression data as one means of determining floral organ identity, and this application of expression information continues to be a topic of debate. As an example, there are now divergent definitions of perianth organs and interpretations of organ identity. Sepals typically are the outermost organs of the flower, whereas petals are conspicuous organs, typically of the second perianth whorl. The two outer floral whorls in *Tulipa* may be positionally homologous to sepals and petals, respectively. However, both whorls are morphologically petaloid, and, as noted, patterns of B-class gene expression in both whorls resemble those of eudicot petals (Kanno *et al.*, 2003). If gene expression patterns are conserved across the broad phylogenetic distances from *Arabidopsis* to tulip, then these data suggest homology of both whorls to petals. Alternatively, changes in expression patterns of B-function genes may have occurred during angiosperm evolution (e.g. 'shifting borders'; Kramer *et al.*, 2003); if so, similar expression patterns may not indicate homology. Extension of expression and functional data to homology assessment of the lodicules of grasses is even more challenging. Although lodicules occur in the position of petals and exhibit B-class gene expression (e.g. Schmidt and Ambrose, 1998), as predicted for petals, their unique morphology suggests that they may not be 'petals', despite their position and gene expression patterns. Thus, morphology, developmental data and genetic data may provide conflicting evidence of homology (organ identity) and yet ultimately a more complete, and complex, view of a structure (Buzgo *et al.*, 2004a).

Eupomatia: a case study

Eupomatia (Eupomatiaceae; Fig. 10.1) is a genus of two species that possess an unusual structure (a calyptra) that encloses and presumably protects the flower in bud. The origin of the calyptra has been debated. Some

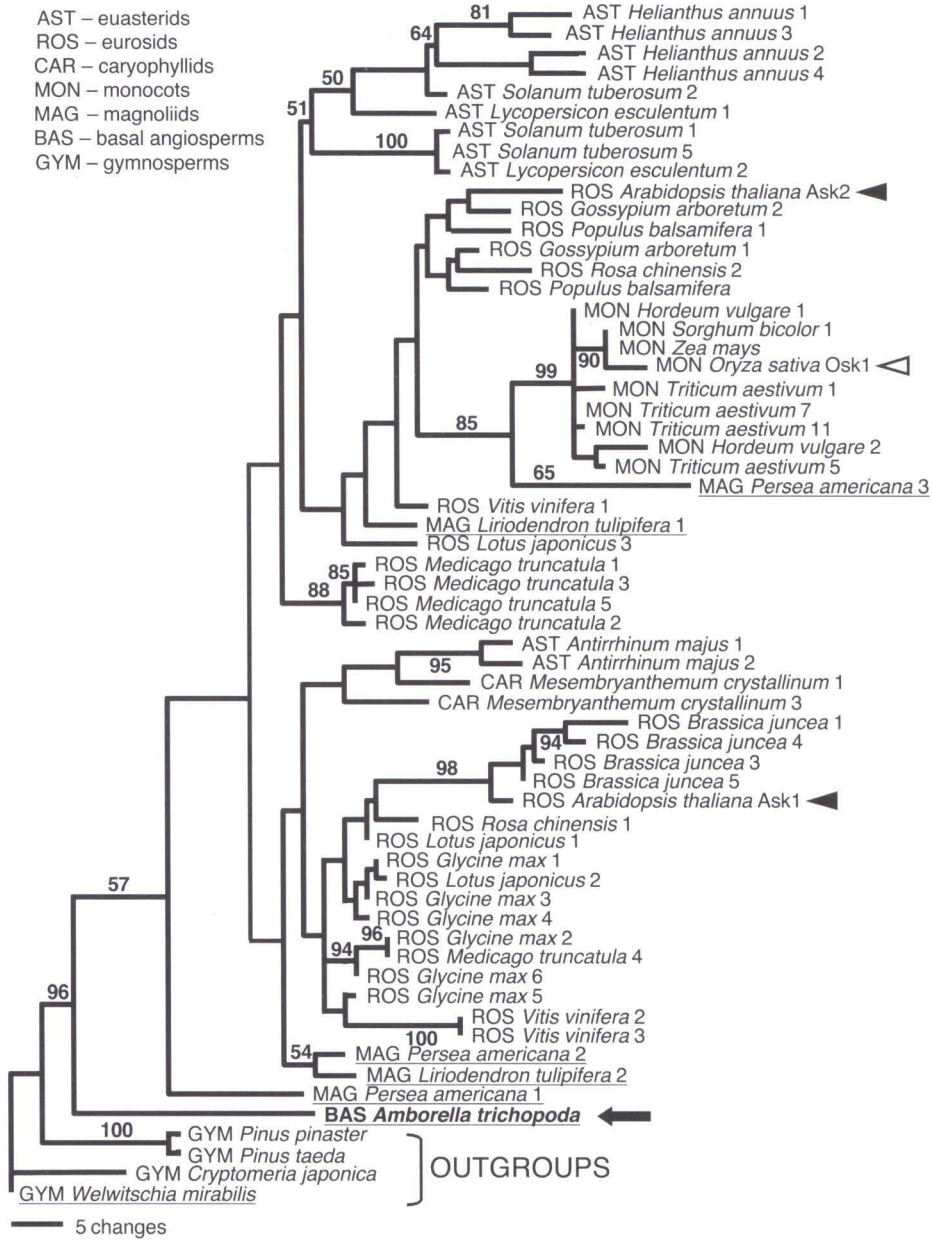

Fig. 10.14. Phylogenetic relationships of slowly evolving SKP1 proteins from select rosid (ROS, ROS1, ROS2), euasterid (AST), monocot (MON), magnoliid (MAG), basal angiosperm (BAS) and gymnosperm (GYM) species suggest that gene duplications have occurred both before and after the origin of major taxa within the angiosperms. The maximum likelihood tree shown is one of >2000 most parsimonious trees. Maximum parsimony bootstrap values higher than 50% are shown above or below the branch. Support for each branch was tested with 500 replicates of bootstrap analysis using random input order for each replicate. Note that most nodes on the tree are not well supported because the regions used for analysis are rather short (149 amino acids) and highly conserved. The taxonomic categories for these species follow Soltis *et al.* (2000). ESTs generated by the Floral Genome Project are underlined. Closed triangles indicate SKP1 homologues from *Arabidopsis* and open triangles those from rice. Modified from Kong *et al.* (2004).

have considered it to represent a modified perianth (Cronquist, 1981), but developmental data indicate that it represents a modified bract (Endress, 2003). Eupomatiaceae are closely related to the *Magnolia* family, members of which have a well-developed perianth of showy tepals, as well as bracts that enclose the flower.

Kim *et al.* (unpublished) examined the expression of A and B class genes in the calyptra. The B-function genes (*AP3* and *PI*) isolated from staminodes of *Eupomatia* species were strongly expressed in developing stamens, staminodes and carpels, but either not expressed or expressed weakly in the calyptra, at a level consistent with expression in leaves. As reviewed, recent studies suggest that in basal angiosperms and monocots an 'ancestral' ABC model (compared with *Arabidopsis*) operates with B-function genes expressed throughout the perianth. Following this model (Fig. 10.8), the pattern of expression of floral genes in the calyptra of *Eupomatia* generally matches the expectations for a non-floral organ (such as a leaf or bract) rather than predictions for perianth, consistent with Endress's (2003) interpretation based on developmental morphology.

Thus, in some situations such as a clade of related taxa, floral gene expression may be useful in addressing the origins of enigmatic structures. There are caveats, however. In the case of a basal angiosperm such as *Eupomatia*, comparisons are better made in light of the proposed 'ancestral' ABC model, rather than the classic ABC model of *Arabidopsis* and other core eudicots.

Gene Evidence and the Origin of Flowers

An understanding of the nature of the flower in basal angiosperms should help in elucidating the evolutionary origin of the flower itself. Flowers differ so greatly from the reproductive structures of living and fossil gymnosperms that the origin of the flower has long been a famous question in evolutionary biology. Numerous hypotheses based on morphological, developmental and palaeobotanical studies have been proposed (reviewed in Stebbins, 1974; Crane, 1985; Hughes, 1994), but each typically accounts for only a limited range of observations, and none is testable, unless revealing fossils happen to be discovered.

The Mostly Male Theory (Frohlich and Parker, 2000; Frohlich, 2001, 2002, 2003) arose through studies of the *LFY* gene, in particular from the observations that *LFY* is single copy in diploid angiosperms, but that there are two copies present in all extant gymnosperm groups, owing to an ancient duplication predating the divergence of angiosperms from extant gymnosperms. Angiosperms would have inherited both copies of *LFY*, but one of these has been lost. Expression of the gymnosperm *LFY*s in pine, coupled with the role of *LFY* in angiosperms, suggests that one gymnosperm *LFY* helps to specify the male reproductive unit while the other helps to specify the female unit. Angiosperms retain only the male-specifying unit, suggesting that the angiosperm flower may derive more from the male reproductive structure of the gymnosperm ancestor, rather than from the female unit. Other data from extant plants and from fossils are consistent with this view, and together suggest that bisexuality of the angiosperm reproductive structure may have arisen when the ovule antecedent became ectopic upon microsporophylls of a reproductive unit resembling that of the fossil gymnosperm group, Corystospermales. The theory is consistent with the flower antecedent originally consisting of stamen- and carpel-like structures, but without a perianth, and with insect-attractive features and insect pollination long predating the full elaboration of the flower (Frohlich, 2001), as suggested by the timing of the *AP3/PI* gene duplication described above.

Other recent gene-based hypotheses also focus on the origin of angiosperm bisexual reproductive structures from the unisexual structures typical of gymnosperms. Albert *et al.* (2002) proposed an alternative to Mostly Male. Their hypothesis stresses the possible functional replacement of one copy of *LFY* by the other, resulting in expression of both male- and female-specific genes in the reproductive unit. Theißen *et al.* (2002) suggest that changed expression patterns of B-class genes could have generated bisexual

reproductive structures from either male or female cones of gymnosperms. Both of these hypotheses, but especially that of Theißen *et al.* (2002), suggest relatively equal participation of male- and female-derived gymnosperm genes in the organization of the flower. If the distinction between gymnosperm male and female structures is fully determined by differences in B-gene expression, then changes in B-gene expression patterns should bring the full panoply of male- or female-specific genes into the (formerly) unisexual cone of the other gender.

The relative contribution of male- and female-specific gymnosperm genes to those active in the flower constitutes a direct test of these theories. The gene discovery, gene phylogeny and gene expression studies of the Floral Genome Project (see below) will provide this test.

Future Prospects

The evolution of flower morphology is being elucidated through research on the genetic mechanisms of reproductive development in diverse angiosperms. *Arabidopsis* has figured prominently in these studies. Although *Arabidopsis* was the first plant to have its nuclear genome completely sequenced (in 2000), earlier genetic studies of *Arabidopsis* (beginning in the late 1980s) paved the way for evolutionary interpretations of the molecular processes underlying floral diversity.

The evolutionary genetics of floral morphology

While the simplicity of the ABC model made it seem that diversions from 'normal' sepal/petal/stamen/carpel identity among angiosperms might be explored through comparative expression studies of A-, B- and C-function genes (as has been done analogously with homeobox genes in the segmental evolution of animals), the greater genetic complexity now recognized behind flower development indicates that this view requires revision. For example, one author of this contribution once felt that explaining the homeotic evolution of a second corolla (fused petal) whorl in the Hawaiian genus *Clermontia* (Campanulaceae) would be a simple issue of demonstrating ectopic expression of B-function genes in the first, normally sepalar, whorl. However, with the generality of the A function now in question, the mechanistic basis for the double-corolla phenotype in *Clermontia* might be other than simply out-of-place B-function gene expression. Furthermore, if altered B-gene expression is the cause, various mechanisms could generate such modified expression. The naturally occurring mutation could be within a B-function coding sequence, or perhaps in its transcriptional promoter, which might have elements that fine-tune spatio-temporal expression. It could equally well be that the double-corolla lesion is in a different gene, the product of which normally interacts with a B-function gene's promoter to regulate where it expresses. In other words, the gene that normally excludes B-function genes from the first whorl of *Clermontia*, or any gene upstream of it in its developmental regulatory cascade, could be the culprit. Analysing this problem will not be simple, because *Clermontia*, unlike weedy *Arabidopsis*, is a small tree that is much less tractable to genetic studies that require progeny analysis. Such 'forward' genetic studies, starting with a phenotypically recognizable mutation and culminating with the gene linked to it, may be difficult to accomplish outside of the model plants. Therefore, investigators are turning more and more to 'reverse' genetic approaches that start with a gene sequence that is suspected to have a particular function (e.g. through a molecular evolutionary relationship to genes of known function) and work backwards to establish this function through transgenic experiments that over- and/or underexpress the gene's protein product. In this way the *Gerbera SEP*-like genes were characterized.

The Floral Genome Project, a large-scale effort to identify genes specific to flower development and those linked to floral diversification, is under way (www.floralgenome.org; overview in Soltis *et al.*, 2002). The Floral Genome Project is sequencing genes expressed during the earliest stages of floral development in diverse

lineages of angiosperms, particularly basal angiosperms such as *Amborella*, waterlilies, tulip tree (Magnoliaceae), avocado (Lauraceae) and *Acorus* (sister to all other monocots), plus *Eschscholzia* (poppy, a basal eudicot), and will obtain expression data for genes in a subset of these taxa. Genes in common with or distinct to these species should provide valuable molecular tools for the next generation of plant evolutionary developmental research.

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References

Albert, V.A., Gustafsson, M.H.G. and Di Laurenzio, L. (1998) Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. In: Soltis, D.E., Soltis, P.S. and Doyle, J.J. (ed.) *Molecular Systematics of Plants* II. Kluwer, New York.

Albert, V.A., Oppenheimer, D. and Lindqvist, C. (2002) Pleiotropy, redundancy and the evolution of flowers. *Trends in Plant Science* 7, 297–301.

APG II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* 141, 399–436.

Arber, E.A.N. and Parkin, J. (1907) On the origin of angiosperms. *Journal of the Linnean Society, Botany* 38, 29–80.

Barkman, T.J., Chenery, G., McNeal, J.R., Lyons-Weiler, J. and DePamphilis, C.W. (2000) Independent and combined analysis of sequences from all three genomic compartments converge to the root of flowering plant phylogeny. *Proceedings of the National Academy of Sciences USA* 97, 13166–13171.

Baum, D.A., Doebley, J., Irish, V.F. and Kramer, E.M. (2002) Response: Missing links: the genetic architecture of flower and floral diversification. *Trends in Plant Science* 7, 31–34.

Becker, A. and Theißen, G. (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Molecular Phylogenetics and Evolution* 29, 464–489.

Bessey, C.E. (1915) The phylogenetic taxonomy of flowering plants. *Annals of the Missouri Botanical Garden* 2, 109–164.

Borsch, T., Hilu, K.W., Quandt, D., Wilde, V., Neinhuis, C. and Barthlott, W. (2003) Non-coding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16, 558–576.

Buzgo, M., Soltis, D.E., Soltis, P.S. and Hong, M. (2004a) Toward a comprehensive integration of morphological and genetic studies of floral development. *Trends in Plant Science* 9, 164–173.

Buzgo, M., Soltis, P.S. and Soltis, D.E. (2004b) Floral developmental morphology of *Amborella trichopoda* (Amborellaceae). *International Journal of Plant Sciences* (in press).

Callis, J. and Vierstra, R.D. (2000) Protein degradation in signaling. *Current Opinion in Plant Biology* 3, 381–386.

Charrier, B., Champion, A., Henry, Y. and Kreis, M. (2003) Expression profiling of the whole *Arabidopsis* Shaggy-like kinase multigene family by real-time reverse transcriptase-polymerase chain reaction. *Plant Physiology* 130, 577–590.

Coen, E.S. and Meyerowitz, E.M. (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353, 31–37.

Coen, E.S., Momero, J.M., Doyle, S., Elliot, R., Murphy, G. and Carpenter, R. (1990) *Floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. *Cell* 63, 1311–1322.

Crane, P.R. (1985) Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of the Missouri Botanical Garden* 72, 716–793.

Crane, P.R., Friis, E.M. and Pedersen, K.R. (1995) The origin and early diversification of angiosperms. *Nature* 374, 27–33.

Cronquist, A. (1968) *The Evolution and Classification of Flowering Plants*. Houghton Mifflin, Boston, Massachusetts.

Cronquist, A. (1981) *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.

De Bodt, S., Raes, J., Van de Peer, Y. and Theißen, G. (2003) And then there were many: MADS goes genomic. *Trends in Plant Science* 8, 475–483.

De Candolle, A.P. (1824–1873) *Prodomus systematis naturalis regni vegetabilis*. Déterville, Paris.

Dilcher, D.L. and Crane, P.R. (1984) *Archaeanthus*: an early angiosperm from the Cenomanian of the western interior of North America. *Annals of the Missouri Botanical Garden* 71, 351–383.

Donoghue, M.J. and Doyle, J.A. (1989) Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. In: Crane, P.R. and Blackmore, S. (eds) *Evolution, Systematics, and Fossil History of Hamamelidae*. Clarendon Press, Oxford, pp. 17–45.

Dornelas, M.C., Lejeune, B., Dron, M. and Kreis, M. (2000) The *Arabidopsis SHAGGY*-related protein kinase (*ASK*) gene family: structure, organization and evolution. *Gene* 212, 249–257.

Doust, A.N. (2000) Comparative floral ontogeny in Winteraceae. *Annals of the Missouri Botanical Garden* 87, 366–379.

Doust, A.N. (2001) The developmental basis of floral variation in *Drimys winteri* (Winteraceae). *International Journal of Plant Sciences* 162, 697–717.

Doyle, J.A. and Endress, P.K. (2000) Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *International Journal of Plant Sciences* 161, S121–S153.

Doyle, J.A., Donoghue, M.J. and Zimmer, E. (1994) Integration of morphological and ribosomal RNA data on the origin of angiosperms. *Botanical Review* 52, 321–431.

Eames, A.J. (1931) The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. *American Journal of Botany* 18, 147–188.

Egea-Cortines, M., Saedler, H. and Sommer, H. (1999) Ternary complex formation between the MADS-box proteins *SQUAMOSA*, *DEFICIENS* and *GLOBOSA* is involved in the control of floral architecture in *Antirrhinum majus*. *EMBO Journal* 18, 5370–5379.

Endress, P.K. (1980) Ontogeny, function and evolution of extreme floral construction in Monimiaceae. *Plant Systematics and Evolution* 134, 79–120.

Endress, P.K. (1986) Floral structure, systematics and phylogeny in Trochodendrales. *Annals of the Missouri Botanical Garden* 73, 297–324.

Endress, P.K. (1987) The early evolution of the angiosperm flower. *Trends in Ecology and Evolution* 2, 300–304.

Endress, P.K. (1994a) *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge University Press, Cambridge.

Endress, P.K. (1994b) Floral structure and evolution of primitive angiosperms: recent advances. *Plant Systematics and Evolution* 192, 79–97.

Endress, P.K. (2001) The flowers in extant basal angiosperms and inferences on ancestral flowers. *International Journal of Plant Sciences* 162, 1111–1140.

Endress, P.K. (2003) Early floral development in Eupomatiaceae. *International Journal of Plant Sciences* 164, 489–503.

Endress, P.K. and Igersheim, A. (2000) Gynoecium structure and evolution in basal angiosperms. *International Journal of Plant Sciences* 161, S211–S223.

Endress, P.K. and Sampson, F.B. (1983) Floral structure and relationships of the Trimeniaceae (Laurales). *Journal of the Arnold Arboretum* 64, 447–473.

Endress, P.K., Igersheim, A., Sampson, F.B. and Schatz, G.E. (2000) Floral structure of *Takhtajania* and its systematic position in Winteraceae. *Annals of the Missouri Botanical Garden* 87, 347–365.

Engler, A. and Prantl, K. (eds) (1887–1915) *Die Natürlichen Pflanzenfamilien*, 1st edn. W. Engelmann, Leipzig.

Engler, A. and Prantl, K. (eds) (1959) *Die Natürlichen Pflanzenfamilien*, 2nd edn. W. Engelmann, Leipzig.

Enright, A.J., Van Dongen, S. and Ouzounis, C.A. (2002) An efficient algorithm for large scale detection of protein families. *Nucleic Acids Research* 30, 1575–1584.

Erbar, C. and Leins, P. (1981) Zur Spirale in Magnolienbluten. *Beitrage zur Biologie der Pflanzen* 56, 225–241.

Erbar, C. and Leins, P. (1983) Zur Sequenz von Blutenorganen bei enigen Magnoliiden. *Botanische Jahrbucher für Systematik* 103, 433–449.

Farras, R., Ferrando, A., Jasik, J., Kleinow, T., Okresz, L., Tiburcio, A., Salchert, K., Del Pozo, C., Schell, J. and Koncz, C. (2001) SKP1–SnRK protein kinase interactions mediate proteasomal binding of a plant SCF ubiquitin ligase. *EMBO Journal* 20, 2742–2756.

Ferrario, S., Immink, R.G., Shchennikova, A., Busscher-Lange, J. and Angenent, G.C. (2003) The MADS box gene *FBP2* is required for the *SEPALLATA* function in *Petunia*. *Plant Cell* 15, 914–925.

Franks, R.G., Wang, C., Levin, J.Z. and Liu, Z. (2002) SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. *Development* 129, 253–263.

Friis, E.M., Pedersen, K.R. and Crane, P.R. (1994) Angiosperm floral structures from the Early Cretaceous of Portugal. *Plant Systematics and Evolution, Supplement* 8, 31–49.

Friis, E.M., Pedersen, K.R. and Crane, P.R. (2000) Reproductive structure and organization of basal angiosperms from the early Cretaceous (Barremian or Aptian) of western Portugal. *International Journal of Plant Science* 161, 5169–5182.

Friis, E.M., Pedersen, K.R. and Crane, P.R. (2001) Fossil evidence of waterlilies (Nymphaeales) in the Early Cretaceous. *Nature* 410, 357–360.

Friis, E.M., Doyle, J.A., Endress, P.K. and Leng, Q. (2003) *Archaefructus* – angiosperm precursor or specialized early angiosperm? *Trends in Plant Science* 8, 369–373.

Frohlich, M.W. (2001) A detailed scenario and possible tests of the Mostly Male theory of flower evolutionary origins. In: Zelditch, M.L. (ed.) *Beyond Heterochrony: the Evolution of Development.* Wiley-Liss, New York, pp. 59–104.

Frohlich, M.W. (2002) The Mostly Male theory of flower origins: summary and update regarding the Jurassic pteridosperm *Pteroma*. In: Cronk, Q.C.B., Bateman, R.M. and Hawkins, J.A. (eds) *Developmental Genetics and Plant Evolution*, Systematics Association Special Volume Series 65. Taylor & Francis, New York, pp. 85–108.

Frohlich, M.W. (2003) An evolutionary scenario for the origin of flowers. *Nature Reviews Genetics* 4, 559–566.

Frohlich, M.W. and Parker, D.S. (2000) The Mostly Male theory of flower evolutionary origins: from genes to fossils. *Systematic Botany* 25, 155–170.

Ganoth, D., Borstein, G., Ko, Y.K., Larsen, B., Tyers, M., Pagano, M. and Hershko, A. (2001) The cell-cycle regulatory protein Cks1 is required for SCF (Skp2)-mediated ubiquitinylation of p27. *Nature Cell Biology* 3, 321–324.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B.M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W.L., Chen, L., Cooper, B., Park, S., Wood, T.C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R.M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A. and Briggs, S. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296, 92–100.

Graham, S. and Olmstead, R. (2000) Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *American Journal of Botany* 87, 1712–1730.

Hahn, G.W. and Sommerville, C.R. (1988) Genetic control of morphogenesis in *Arabidopsis*. *Developmental Genetics* 9, 73–89.

Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. *Annual Review of Biochemistry* 67, 425–479.

Hiepko, P. (1965) Vergleichend-morphologische und entwicklungs-geschichtliche Untersuchungen über das Perianth bei den Polycarpicae. *Botanische Jarbucher für Systematik* 84, 359–508.

Hilu, K.W., Borsch, T., Muller, K., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M., Alice, L., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T., Rohwer, J. and Catrou, L. (2003) Inference of angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* 90, 1758–1776.

Honma, T. and Goto, K. (2000) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* 409, 525–529.

Hughes, N.F. (1994) *The Enigma of Angiosperm Origins.* Cambridge University Press, Cambridge.

Irish, V.F. (2003) The evolution of floral homeotic gene function. *BioEssays* 25, 637–646.

Johansen, B., Pedersen, L.B., Skipper, M. and Frederiksen, S. (2002) MADS-box gene evolution–structure and transcription patterns. *Molecular Phylogenetics and Evolution* 23, 458–480.

Kanno, A., Saeki, H., Kameya, T., Saedler, H. and Theißen, G. (2003) Heterotropic expression of class B floral homeotic genes supports a modified ABC model for tulip (*Tulipa gesneriana*). *Plant Molecular Biology* 52, 831–841.

Keck, E., McSteen, P., Carpenter, R. and Coen, E. (2003) Separation of genetic functions controlling organ identity in flowers. *EMBO Journal* 22, 1058–1066.

Kim, I. and Kimmel, A.R. (2000) GSKs, a master switch regulating cell-fate specification and tumorigenesis. *Current Opinion in Genetics and Development* 10, 508–514.

Kim, S., Yoo, M.-J., Albert, V.A., Farris, J.S., Soltis, P.S. and Soltis, D.E. (2004) Phylogeny and diversification

of B-function MADS-box genes in angiosperms: evolutionary and functional implications of a 260-million-year-old duplication. *American Journal of Botany* 91 (in press).

Kong, H., Leebens-Mack, J., Ni, W., DePamphilis, C.W. and Ma, H. (2004) Highly heterogeneous rates of evolution in the SKP1 gene family in plants and animals: functional and evolutionary implications. *Molecular Biology and Evolution* 21, 117–128.

Kosuge, K. (1994) Petal evolution in Ranunculaceae. *Plant Systematics and Evolution* 152, 185–191.

Kotilainen, M., Elomaa, P., Uimari, A., Albert, V.A., Yu, D. and Terri, T.H. (2000) GRCD1, an AGL2-like MADS box gene, participates in the C function during stamen development in *Gerbera hybrida*. *Plant Cell* 12, 1893–1902.

Kramer, E.M. and Irish, V.F. (1999) Evolution of genetic mechanisms controlling petal development. *Nature* 399, 144–148.

Kramer, E.M. and Irish, V.F. (2000) Evolution of the petal and stamen developmental programs: evidence from comparative studies of the lower eudicots and basal angiosperms. *International Journal of Plant Sciences* 161, S29–S40.

Kramer, E.M., Dorit, R.L. and Irish, V.F. (1998) Molecular evolution of genes controlling petal and stamen development: duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. *Genetics* 149, 765–783.

Kramer, E.M., Di Stillio, V.S. and Schluter, P.M. (2003) Complex patterns of gene duplication in the *Apetala3* and *Pistillata* lineages of the Ranunculaceae. *International Journal of Plant Sciences* 164, 1–11.

Kubitzki, K. (1987) Origin and significance of trimerous flowers. *Taxon* 36, 21–28.

Lamb, R.S. and Irish, V.F. (2003) Functional divergence within the *APETALA3/PISTILLATA* floral homeotic gene lineages. *Proceedings of the National Academy of Sciences USA* 100, 6558–6563.

Leins, P. and Erbar, C. (1985) Ein Beitrag zur Blutenentwicklung der Aristolochiaceen, einer Vermittlergruppe zu den Monokotylen. *Botanische Jahrbucher für Systematik* 107, 343–368.

Litt, A. and Irish, V.F. (2003) Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development. *Genetics* 165, 821–833.

Lu, R., Martin-Hernandez, A.M., Peart, J.R., Malcuit, I. and Baulcombe, D.C. (2003) Virus-induced gene silencing in plants. *Methods* 30, 296–303.

Ma, H. (1994) The unfolding drama of flower development: recent results from genetic and molecular analyses. *Genes and Development* 8, 745–756.

Ma, H. (1998) To be, or not to be, a flower – control of floral meristem identity. *Trends in Genetics* 14, 26–32.

Ma, H. and dePamphilis, C. (2000) The ABCs of floral evolution. *Cell* 101, 5–8.

Maddison, W.P. and Maddison, D.R. (1992) *MacClade: analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Mathews, S. and Donoghue, M. (1999) The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286, 947–950.

Meyerowitz, E.M., Bowman, J.L., Brockman, L.L., Drews, G.N., Jack, T., Sieburth, L.E. and Weigel, D. (1991) A genetic and molecular model for flower development in *Arabidopsis thaliana*. *Development Supplement* 1, 157–167.

Moon, Y.H., Chen, L., Pan, R.L., Chang, H.S., Zhu, T., Maffeo, D.M. and Sung, Z.R. (2003) EMF genes maintain vegetative development by repressing the flower program in *Arabidopsis*. *Plant Cell* 15, 681–693.

Münster, T., Pahnke, J., Di Rosa, A., Kim, J.T., Martin, W., Saedler, H. and Theißen, G. (1997) Floral homeotic genes were recruited from homologous MADS-box genes preexisting in the common ancestor of ferns and seed plants. *Proceedings of the National Academy of Sciences USA* 94, 2415–2420.

Münster, T., Wingen, L.U., Faigl, W., Werth, S., Saedler, H. and Theißen, G. (2001) Characterization of three *GLO-BOSA*-like MADS-box genes from maize: evidence for ancient parology in one class of floral homeotic B-function genes of grasses. *Gene* 262, 1–13.

Nam, J., dePamphilis, C.W., Ma, H. and Nei, M. (2003) Antiquity and evolution of the MADS-box gene family controlling flower development in plants. *Molecular Biology and Evolution* 20, 1435–1447.

Nayak, S., Santiago, S.F., Jin, H., Lin, D., Schedl, T. and Kipreos, E.T. (2002) The *Caenorhabditis elegans Skp1*-related gene family: diverse functions in cell proliferation, morphogenesis, and meiosis. *Current Biology* 12, 277–287.

Ni, W., Li, W., Zhang, X., Hu, W., Zhang, W., Wang, G., Han, T., Zahn, L.M., Zhao, D. and Ma, H. (2004) Genetic control of reproductive development in flowering plants. In: Sharma, R.P. (ed.) *Molecular Plant Physiology*. Haworth Press, New York (in press).

Parkinson, C.L., Adams, K.L. and Palmer, J.D. (1999) Multigene analyses identify the three earliest lineages of extant flowering plants. *Current Biology* 9, 1485–1488.

Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E. and Yanofsky, M.F. (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature* 405, 200–203.

Pelaz, S., Tapia-Lopez, R., Alvarez-Buylla, E.R. and Yanofsky, M.F. (2001) Conversion of leaves into petals in *Arabidopsis*. *Current Biology* 11, 182–184.

Purugganan, M.D. (1997) The MADS-box floral homeotic gene lineages predate the origin of seed plants: phylogenetic and molecular clock estimates. *Journal of Molecular Evolution* 45, 392–396.

Purugganan, M.D., Sounsley, S.D., Schmidt, R.J. and Yanofsky, M.F. (1995) Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family. *Genetics* 140, 345–356.

Qiu, Y.-L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Chen, Z., Savolainen, V. and Chase, M.W. (1999) The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402, 404–407.

Riechmann, J.L. and Meyerowitz, E.M. (1997) MADS domain proteins in plant development. *Biological Chemistry* 378, 1079–1101.

Riechmann, J.L., Krizek, B.A. and Meyerowitz, E.M. (1996) Dimerization specificity of *Arabidopsis* MADS domain homeotic proteins Apetala1, Apetala3, Pistillata and Agamous. *Proceedings of the National Academy of Sciences USA* 93, 4793–4798.

Ronse De Craene, L.P., Louis, P., Soltis, P.S. and Soltis, D.E. (2003) Evolution of floral structure in basal angiosperms. *International Journal of Plant Sciences* 164, S329–S363.

Sablowski, R.W. and Meyerowitz, E.M. (1998) A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. *Cell* 92, 93–103.

Samach, A., Klenz, J.E., Kohalmi, S.E., Risseeuw, E., Haughn, G.W. and Crosby, W.L. (1999) The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. *Plant Journal* 20, 433–445.

Schmid, M., Uhlenhaut, N.H., Godard, F., Demar, M., Bressan, R., Weigel, D. and Lohmann, J.U. (2003) Dissection of floral induction pathways using global expression analysis. *Development* 130, 6001–6012.

Schmidt, R.J. and Ambrose, B.A. (1998) The blooming of grass flower development. *Current Opinion in Plant Biology* 1, 60–67.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H. and Sommer, H. (1990) Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science* 250, 931–936.

Smith, G.H. (1928) Vascular anatomy of Ranalian flowers. II. Ranunculaceae (continued), Menispermaceae, Calycanthaceae, Annonaceae. *Botanical Gazette* 85, 152–177.

Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M. (1990) Early flower development in *Arabidopsis*. *Plant Cell* 2, 755–768.

Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C. and Farris, J.S. (2000) Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* 133, 381–461.

Soltis, D.E., Soltis, P.S., Albert, V.A., dePamphilis, C.W., Frohlich, M., Ma, H. and Theißen, G. (2002) Missing links: the genetic architecture of the flower and floral diversification. *Trends in Plant Sciences* 7, 22–30.

Soltis, D.E., Senters, A.E., Zanis, M.J., Kim, S., Thompson, J.D., Soltis, P.S., Ronse De Craene, L.P., Endress, P.K. and Farris, J.S. (2003) Gunnerales are sister to other core eudicots: implications for the evolution of pentamery. *American Journal of Botany* 90, 463–470.

Soltis, D.E., Endress, P.K., Chase, M.W. and Soltis, P.S. (2004) *Phylogeny and Evolution of Angiosperms*. Smithsonian Institution Press, Washington, DC.

Soltis, P.S., Soltis, D.E. and Chase, M.W. (1999) Angiosperm phylogeny inferred from multiple genes as a research tool for comparative biology. *Nature* 402, 402–404.

Stebbins, G.L. (1974) *Flowering Plants: Evolution Above the Species Level*. Belknap Press, Cambridge, Massachusetts.

Sun, G., Li, Q., Dilcher, D.L., Zheng, S., Nixon, K.C. and Wang, X. (2002) Archaefructaceae, a new basal angiosperm family. *Science* 296, 899–904.

Sundström, J., Carlsecker, A., Svensson, M.E., Svenson, M., Johanson, U., Theißen, G. and Engström, P. (1999) MADS-box genes active in developing pollen cones of Norway spruce (*Picea abies*) are homologous to the B-class floral homeotic genes in angiosperms. *Developmental Genetics* 25, 253–266.

Takhtajan, A. (1969) *Flowering Plants: Origin and Dispersal*. Smithsonian Institution Press, Washington, DC.

Takhtajan, A. (1997) *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

Teeri, T.H., Albert, V.A., Elomaa, P., Hämäläinen, J., Kotilainen, M., Pöllänen, E. and Uimari, A. (2002) Involvement of non-ABC MADS-box genes in determining stamen and carpel identity in *Gerbera*

hybrida (Asteraceae). In: Cronk, Q.C.B., Bateman, R.M. and Hawkins, J.A. (eds) *Developmental Genetics and Plant Evolution*. Taylor & Francis, London, pp. 220–232.

Theißen, G. (2001) Development of floral organ identity: stories from the MADS house. *Current Opinion in Plant Biology* 4, 75–86.

Theißen, G., Becker, A., Di Rosa, A., Kanno, A., Kim, J.T., Münster, T., Winter, K.-U. and Saedler, H. (2000) A short history of MADS-box genes in plants. *Plant Molecular Biology* 42, 115–149.

Theißen, G., Becker, A., Winter, K.-U., Münster, T., Kirchner, C. and Saedler, H. (2002) How the land plants learned their floral ABCs: the role of MADS box genes in the evolutionary origin of flowers. In: Cronk, Q.C.B., Bateman, R.M. and Hawkins, J.A. (eds) *Developmental Genetics and Plant Evolution*, Systematics Association Special Volume Series 65. Taylor & Francis, New York, pp. 173–206.

Tucker, S.C. (1960) Ontogeny of the floral apex of *Michelia fuscata*. *American Journal of Botany* 47, 266–277.

Tucker, S.C. and Douglas, A.W. (1996) Floral structure, development, and relationships of Paleoherbs: *Saruma, Cabomba, Lactoris,* and selected Piperales. In: Taylor, D.W. and Hickey, L.J. (eds) *Flowering Plant Origin, Evolution, and Phylogeny*. Chapman & Hall, New York, pp. 141–175.

Van Tunen, A.J., Eikelboom, W. and Angenet, G.C. (1993) Floral organogenesis in *Tulipa*. *Flower Newsletter* 16, 33–38.

Von Balthazar, M. and Endress, P.K. (2002) Development of inflorescences and flowers in Buxaceae and the problem of perianth interpretation. *International Journal of Plant Sciences* 163, 847–876.

Weigel, D. (1995) The genetics of flower development: from floral induction to ovule morphogenesis. *Annual Review Genetics* 29, 19–39.

Weigel, D. and Meyerowitz, E.M. (1994) The ABCs of floral homeotic genes. *Cell* 78, 203–209.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* 69, 843–859.

Winter, K.-U., Becker, A., Münster, T., Kim, J., Saedler, H. and Theißen, G. (1999) MADS-box genes reveal that gnetophytes are much more closely related to conifers than to flowering plants. *Proceedings of the National Academy of Sciences USA* 96, 7342–7347.

Winter, K.-U., Weiser, C., Kaufmann, K., Bohne, A., Kirchner, C., Kanno, A., Saedler, H. and Theißen, G. (2002) Evolution of class B floral homeotic proteins: obligate heterodimerization originated from homodimerization. *Molecular Biology and Evolution* 19, 587–596.

Yamanaka, A., Yada, M., Imaki, H., Koga, M., Ohshima, Y. and Nakayama, K. (2002) Multiple *Skp1*-related proteins in *Caenorhabditis elegans*: diverse patterns of interaction with Cullins and F-box proteins. *Current Biology* 12, 267–275.

Yang, M., Hu, Y., Lodhi, M., McCombie, W.R. and Ma, H. (1999) The *Arabidopsis SKP1-LIKE1* gene is essential for male meiosis and may control homolog separation. *Proceedings of the National Academy of Sciences USA* 96, 11416–11421.

Yanofsky, M.F. (1995) Floral meristems to floral organs: genes controlling early events in *Arabidopsis* flower development. *Annual Review of Plant Physiology and Plant Molecular Biology* 46, 167–188.

Yoo, M.-J., Albert, V.A., Soltis, P.S. and Soltis, D.E. (2005) Diversification of glycogen synthase kinase 3/*SHAGGY-like* kinase genes in plants. *BMC Evolutionary Biology* (submitted).

Yu, D.Y., Kotilainen, M., Pollanen, E., Mehto, M., Elomaa, P., Helariutta, Y., Albert, V.A. and Teeri, T.H. (1999) Organ identity genes and modified patterns of flower development in *Gerbera hybrida*. *Plant Journal* 17, 51–62.

Yu, J., Hu, S., Wang, J., Wong, G.K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Li, J., Liu, Z., Qi, Q., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Zhao, W., Li, P., Chen, W., Zhang, Y., Hu, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Tao, M., Zhu, L., Yuan, L. and Yang, H. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296, 79–92.

Zanis, M.J., Soltis, D.E., Soltis, P.S., Mathews, S. and Donoghue, M.J. (2002) The root of the angiosperms revisited. *Proceedings of the National Academy of Sciences USA* 99, 6848–6853.

Zanis, M.J., Soltis, P.S., Qiu, Y.-L., Zimmer, E. and Soltis, D.E. (2003) Phylogenetic analyses and perianth evolution in basal angiosperms. *Annals of the Missouri Botanical Garden* 90, 129–150.

Zhao, D., Yang, M., Solava, J. and Ma, H. (1999) The *ASK1* gene regulates development and interacts with the *UFO* gene to control floral organ identity in *Arabidopsis*. *Developmental Genetics* 25, 209–223.

Zhao, D., Yu, Q., Chen, C. and Ma, H. (2001a) Genetic control of reproductive meristems. In: McManus, M.T. and Veit, B. (eds) *Meristematic Tissues in Plant Growth and Development.* Sheffield Academic Press, Sheffield, pp. 89–142.

Zhao, D., Yu, Q., Chen, C. and Ma, H. (2001b) The *ASK1* gene regulates B function gene expression in cooperation with *UFO* and *LEAFY* in *Arabidopsis. Development* 128, 2735–2746.

Zhao, D., Ni, W., Feng, B., Han, T., Petrasek, M.G. and Ma, H. (2003) Members of the *Arabidopsis-SKP1*-like gene family exhibit a variety of expression patterns and may play diverse roles in *Arabidopsis. Plant Physiology* 133, 203–217.

Zheng, N., Schulman, B.A., Song, L., Miller, J.J., Jeffrey, P.D., Wang, P., Chu, C., Koepp, D.M., Elledge, S.J., Pagano, M., Conaway, R.C., Conaway, J.W., Harper, J.W. and Pavletich, N.P. (2002) Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. *Nature* 416, 703–709.

Zik, M. and Irish, V.F. (2003) Flower development: initiation, differentiation and diversification. *Annual Review of Cell and Developmental Biology* 19, 119–140.