

# Floral Organ Determination and Ontogenetical Patterns during Angiosperm Evolution

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## ABSTRACT

Since the late 1980's/early 1990's enormous progress had been made in understanding the genetic and molecular regulation of flower development. The genetic ABCDE model describes five classes of genes that are responsible for the specification of floral organ identity in a combinatorial manner. The molecular quartet model advances the genetic ABCDE model by describing the presumed interactions between floral MADS-domain proteins. Although the basic developmental program appears to be quite conserved, in non-core eudicots modifications such as "sliding-boundary" and "fading borders" models had to be established. The genetic models proposed as yet predict the organ quality, but do not explain how the number of organs or the spatial pattern in which organ primordia appear (e.g. spiral or whorled) is regulated. With Apiaceae and Brassicaceae two families are presented which contrary to their uniform flower construction show fairly diverse patterns in organ initiation. Cleomaceae, sister to Brassicaceae, are even more diverse as regards stamen number as well as initiation patterns. The other members of the core Brassicales add further diversity to the androecial initiation pattern. Multistaminate androecia stand for a further interesting aspect as the stamen primordia are initiated either spirally directly on the floral apex or on so-called primary androecial primordia (fascicle primordia) in centrifugal or centripetal succession. Since the identity of floral organs is strictly dependent on the activity of the MADS-box genes, duplication and diversification within these genes must have been key processes in flower evolution. Hence, insights into the phylogeny of the floral homeotic genes may help to better understand the evolution of flowers ("evo-devo"). The unique nectary organs of the Ranunculaceae are presented as example for duplication and new functions in the B class genes.

**Keywords:** ABCDE model, floral evolution, floral organ determination, flower development, flower ontogeny, ontogenetical patterns

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## INTRODUCTION

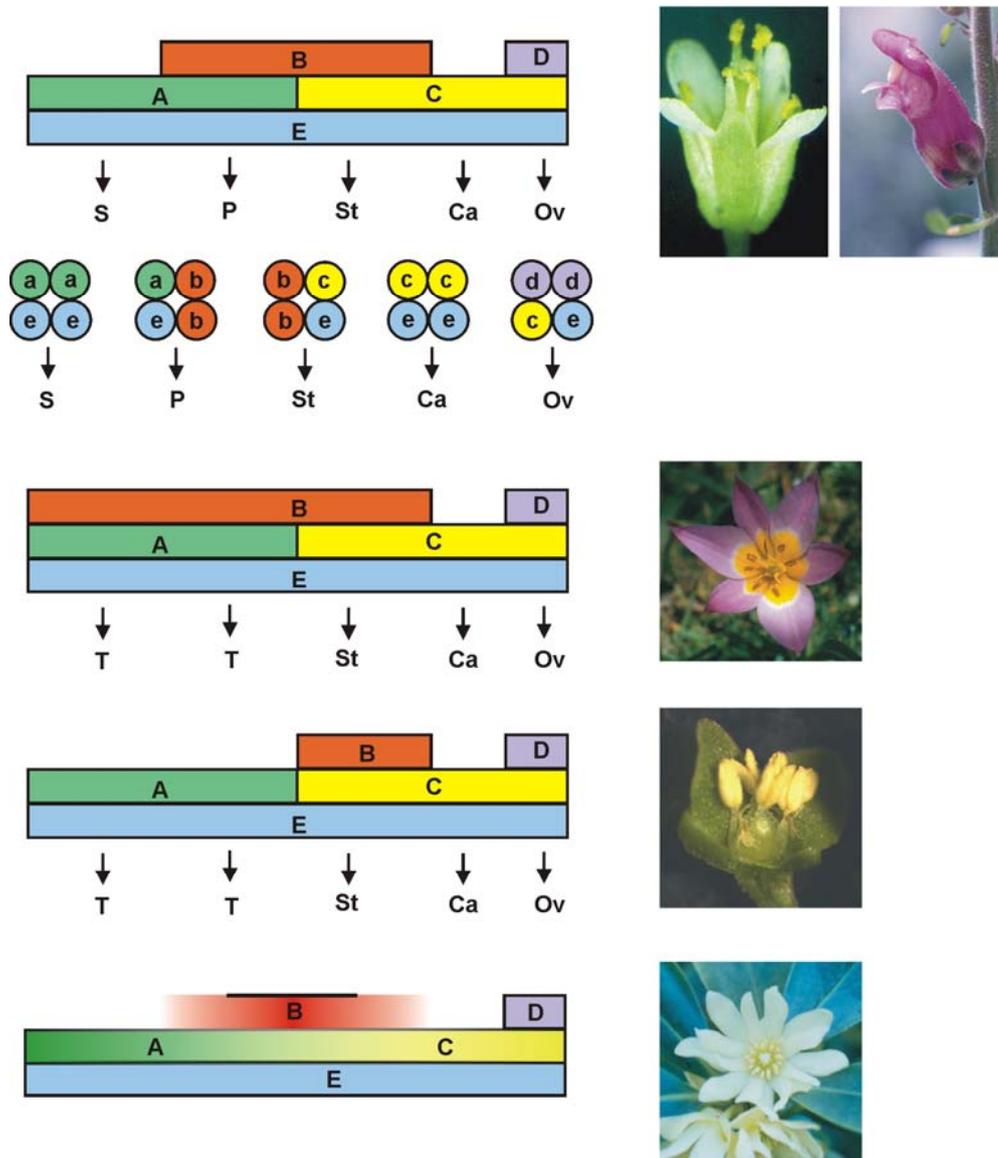
Shortly after their "sudden" appearance, the angiosperms diversified quickly and had an explosive evolutionary success and it is not surprising that Charles Darwin (in his letter to Joseph Dalton Hooker, 22 July 1879; edited as letter 395 by Francis Darwin and Albert C. Seward in 1903) referred to the origin and rapid radiation of the angiosperms as an "abominable mystery" (but see on this topic Friedman, 2009, who explicates Darwin's thoughts as referring to the possibility that evolution could be both rapid and potentially even saltational). Stuessy (2004) tried to resolve part of the problems by proposing the "transitional-combinational theory" for the origin of the angiosperms. This theory suggests that the angiosperms evolved slowly from seed ferns in the Jurassic beginning first with the carpel, followed later by double fertilization, and lastly by the appearance of flowers, a process that may have taken more than 100 million years (Stuessy 2004). Only the final combination of all three important features provided the opportunity for explosive evolutionary diversification, especially in response to selection from insect pollinators and predators as well as in compatibility and breeding systems. However,

several key questions remain. For example, what is the genetic basis of the different evolutionary innovations?

An extant complete angiosperm flower is composed of the perianth (undifferentiated or differentiated in sepals and petals), the microsporangia bearing, pollen producing stamens (androecium) and the carpels (gynoecium) enclosing the ovules (megasporeangia), all arising from the floral axis of determinate growth. There are about 260,000-300,000 species of extant angiosperms with an enormous diversity of size, shape, number of organs, contributing to flower complexity.

Genetic studies of homeotic<sup>1</sup> mutants in *Arabidopsis thaliana* (Brassicaceae; see Fig. 1B) and *Antirrhinum majus* (Plantaginaceae, formerly Scrophulariaceae; see Fig. 1C) have shown that during the formation of the flower the determination of organ identity is controlled by homeotic genes. The different analyses have led to the classical ABC model of Coen and Meyerowitz (1991), in which three regions in a floral primordium are the domains of action of

<sup>1</sup> This term is derived from "homeosis". It refers to the wrong position of a floral organ: A floral organ is found in a place where organs of another type are normally found, e.g. carpels instead of stamens in the *pistillata-2* mutant of *Arabidopsis*.



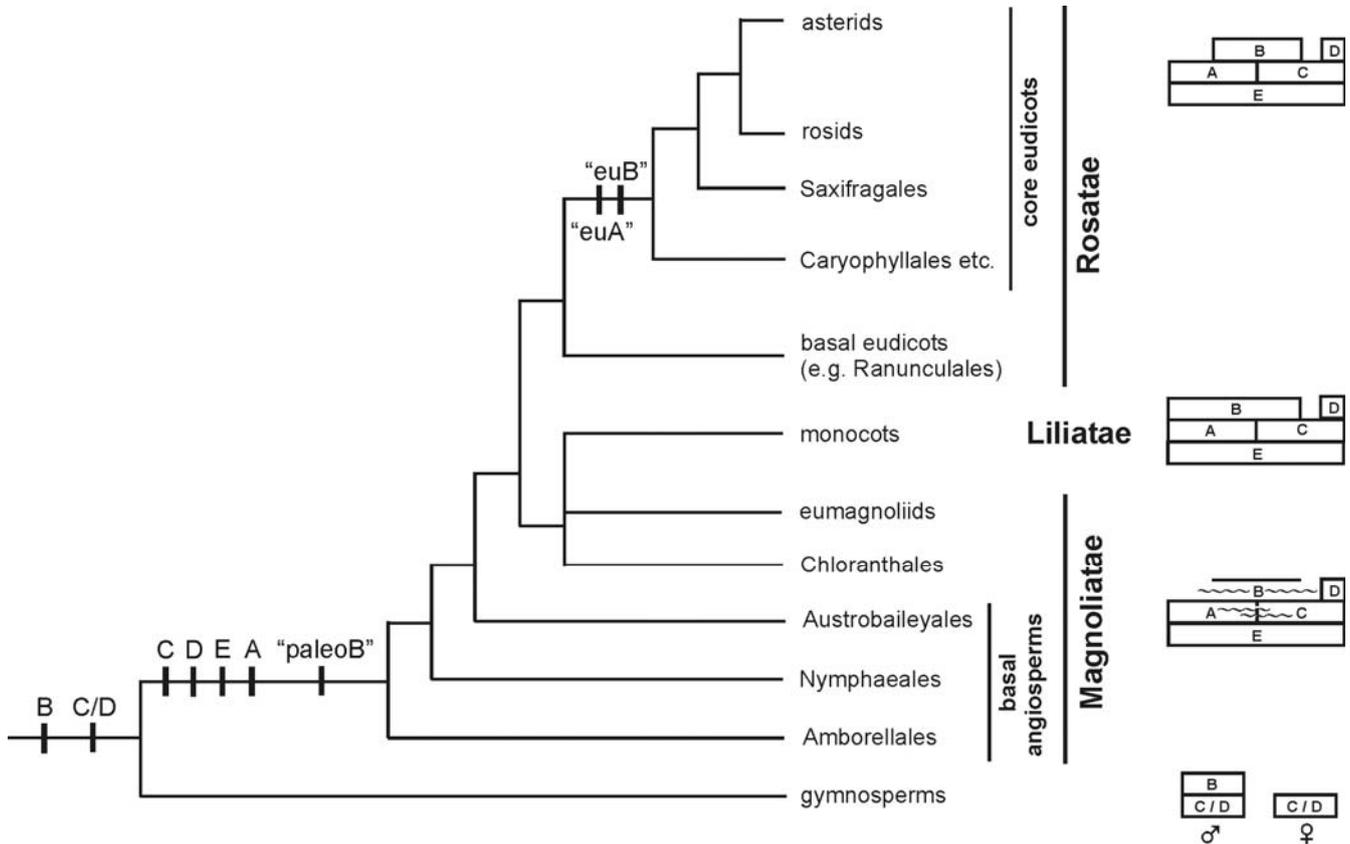
**Fig. 1 Genetic model of organ identity.** (A) ABCDE model of floral organ identity in core eudicots; (B) flower of *Arabidopsis thaliana* (Brassicaceae); (C) flower of *Antirrhinum majus* (Plantaginaceae); (D) “floral quartet” model of organ specification (in *Arabidopsis*, modified after Theissen and Saedler 2001; Zahn *et al.* 2005a); this model directly links floral organ identity to the presumed action of five different tetrameric transcription factor complexes of MADS-box proteins (termed as a, b, c, d, e); (E+G) modifications of the ABC model: “sliding-boundary-model”; (E) modified ABC model for most monocots; (F) flower of *Tulipa bakeri* (Liliaceae); (G) modified ABC model for *Rumex acetosa* (Polygonaceae); (H) flower of *Rumex acetosa* (Polygonaceae); (I) ABCDE model for basal angiosperms: “fading-borders” model; (J) flower of *Illicium anisatum* (Illiciaceae). – A, B, C, D, E: domains of action of the floral homeotic genes; a, b, c, d, e: interaction of the proteins (transcription factors); Ca = carpel, P = petal, S = sepal, St = stamen, Ov = ovule.

three classes of homeotic genes, either acting alone or in combination. Expression of class A genes specifies sepal formation. The combination of class A and B genes specifies the formation of petals. Class B and C genes specify stamen formation and expression of the class C gene alone determines the formation of carpels. A second major tenet of the ABC model is that A and C activities are mutually repressive. In 2001, the classical ABC model was extended to the ABCDE model (Theissen 2001; Fig. 1A). D function genes are required for ovule development (e.g. Colombo *et al.* 1995, Angenent and Colombo 1996) and at least one of the E genes is required for the organ identity in all five organ categories (e.g. Pelaz *et al.* 2000; Zahn *et al.* 2005a, 2005b; for further citations see Erbar 2007).

The interaction of these five different gene classes is shown in the so-called floral quartet model. The genes A, B, C, D, and E encode transcription factors, i.e. proteins that recognize specific DNA motifs of other genes and influence their transcription. Most ABCDE genes are members of a transcription factor family that has a characteristic conserved DNA binding domain structure, termed the MADS

box.<sup>2</sup> The resulting ABCDE proteins bind to the target genes in complexes larger than a dimer (Egea-Cortines *et al.* 1999; Honma and Goto 2001). Thereby, they activate or repress target gene expression as appropriate for the development of the different floral organ identities. Thus the “quartet model” of floral organ specification (Fig. 1D) postulates that five different tetrameric transcription factor complexes composed of MADS proteins specify floral organ identities (Theissen 2001; Theissen and Saedler 2001; Ferrario *et al.* 2003; Kaufmann *et al.* 2005; Zahn *et al.* 2005a). The “quartet model” is a molecular model – although in some aspects still highly hypothetical – that advances the genetic ABCDE model and directly links floral organ identity to the action of MADS-domain proteins.

<sup>2</sup> MADS is an acronym derived from the founding four members of this transcription factor family: MCM1 from yeast, AGAMOUS from *Arabidopsis*, DEFICIENS from *Antirrhinum* and SRF from human. Function A is partially carried out by members of the APETALA2 transcription factor family.



**Fig. 2** A generalized phylogenetic tree of seed plants (based on APG II 2003, Stevens 2001 onwards) onto which different extended ABC models as well as the occurrence of floral homeotic genes and main duplication events in the class B genes are plotted. “euA” = duplication results in euAP1 and euFUL, “paleoB” = duplication produces paleo AP3 + PI lineages, “euB” = duplication produces euAP3 und TM6 lineages; modified after Erbar 2007.

Based on the known genetic and molecular data, this review will discuss three aspects:

1. Modifications of the classical ABCDE model in flowers that do not have the typical whorled construction. 2. Organ quality versus organ position and sequence of organ initiation. 3. The origin of the flowers and their enormous diversity.

### MODIFICATIONS OF THE CLASSICAL ABCDE MODEL OF FLORAL ORGAN DETERMINATION

Since in all investigated angiosperms homologues of the ABCDE genes<sup>3</sup> are present (e.g. Kim *et al.* 2004; Zahn *et al.* 2005b), we can assume that the determination of the organ identity is generally conserved (e.g. Ma and Pamphilis 2000; Buzgo *et al.* 2005), but variation is also expected particularly with regard to the perianth.

The ABCDE model proposed so far refers to flowers that can be found in most eudicots (= Rosatae; see Fig. 2). Nearly 74% of all angiosperms belong to this large clade that can be further distinguished into the core eudicots and the basal eudicots. Most eudicots have the floral organs

arranged in five or four whorls, a fixed number of organs in each whorl and a perianth of distinct sepals and petals. In the morphological terminology an androecium is defined as consisting of two whorls if an outer and an inner whorl of stamens is formed alternating and sequentially (see e.g. Leins and Erbar 2008, 2010). In the genetic approaches the term “whorl” unfortunately is used in a “broad” sense covering the domain of action of the homeotic genes so that this operational definition is quite different (and rather wrong) from the morphological one (see e.g. Bowman *et al.* 1989; Coen 1991). It is much better to use the term “organ category” instead of “whorl” in the genetic models.

### DETERMINATION OF ORGAN IDENTITY IN MONOCOTS

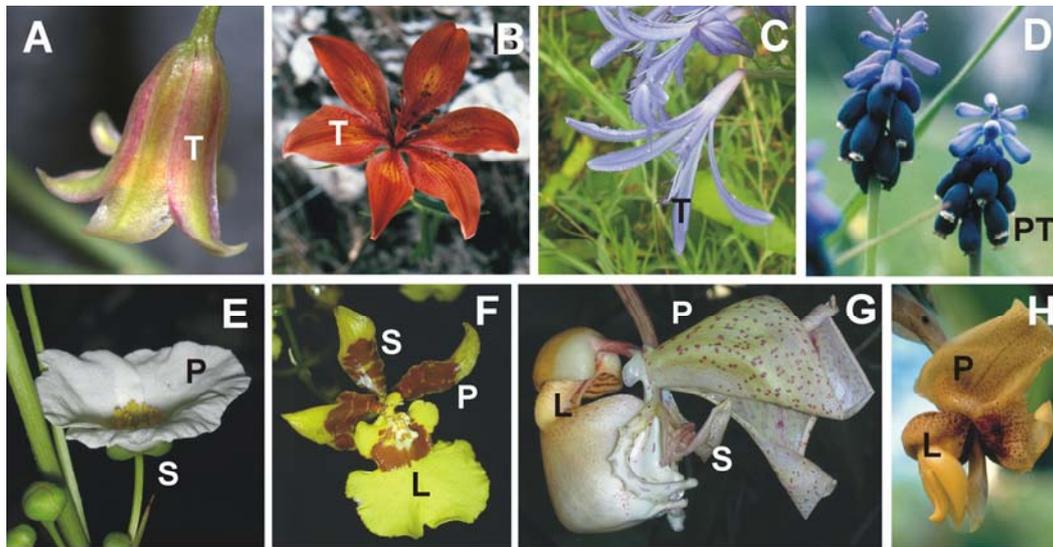
In contrast to the well-differentiated sepal and petal whorls of eudicots, the two outer floral whorls in many members of the monocots, which comprise 22% of angiosperm species, are identical in morphology and called tepals, forming a perigone. How has the ABCDE model to be modified in these cases?

In *Asparagus* (Asparagaceae, Fig. 3A), the developmental pattern in the perigone with almost identical tepals resembles the classical ABC model for flowers with differentiated perianth (Park *et al.* 2003, 2004) and thus contrasts all other monocots described below.<sup>4</sup>

In *Lilium* (Liliaceae, Fig. 3B; see Tzeng and Yang 2001), *Tulipa* (Liliaceae, Fig. 1F; see Kanno *et al.* 2003), *Agapanthus* (Alliaceae or Agapanthaceae, Fig. 3C; see

<sup>3</sup> So far, class A mutants were not found in other species except for the model organisms *Arabidopsis* and *Antirrhinum* (e.g. Drews *et al.* 1991; Ferrario *et al.* 2004; Litt 2007). The A function remains somewhat ambiguous since it has been shown that the A gene AP1 is implicated in the specification of sepal and petal identity as well as of floral meristem identity (see Bowman *et al.* 1993, Litt and Irish 2003). The exact role and broad applicability of the A class model in the regulation of sepal development remains unclear (Preston and Kellog 2006; Zanis 2007). Consistent with its two roles, AP1 is expressed throughout the flowers, but becomes restricted to the A domain during later stages. However, the role of A-genes in the control of C expression appears to be more universal (Drews *et al.* 1991; Motte *et al.* 1998; Theissen *et al.* 2000). Perhaps, a BC model is sufficient and a discrete perianth identity gene function is not required (Schwarz-Sommer *et al.* 1990; Soltis *et al.* 2007a).

<sup>4</sup> Park *et al.* (2003) suggest that the class B gene in *Asparagus* is probably not required for tepal identity and that the expression of the B gene homologue is involved in sex determination in the unisexual flowers. Alternatively, the contrasting results in *Tulipa/Lilium* and *Asparagus* may be due to the examination of different stages with different techniques (Kramer and Jaramillo 2005).



**Fig. 3 Diversity in the monocot flowers.** (A) *Asparagus officinalis* (Asparagaceae): the developmental pattern resembles the classical ABC model; (B-H), monocot flowers in which B class expression is found in both perianth whorls; (B) *Lilium bulbiferum* (Liliaceae) and (C) *Agapanthus praecox* (Alliaceae or Agapanthaceae) represent typical trimerous pentacyclic monocot flowers with undifferentiated perigone; (D) *Muscari racemosum* (Hyacinthaceae) with syntepalous perigone; (E), *Echinodorus cordifolius* (Alismataceae) with a perianth differentiated in sepals and petals; (F) *Oncidium baueri* (Orchidaceae); (G) *Coryanthes leferenziorum* and (H) *Stanhopea comata* (Orchidaceae) with a highly elaborated labellum. – L = labellum, P = petalum, PT = perigone tube, S = sepalum, St = stamen, T = tepalum.

Nakamura *et al.* 2005) as well as in the syntepalous *Muscari* (Hyacinthaceae, **Fig. 3D**; see Nakada *et al.* 2006) class B genes are expressed in both tepal whorls as well as in the stamens. This transference of B function seems also to be present in *Sagittaria* (Alismataceae; Kramer and Irish 2000), although adult flowers of *Sagittaria* (as well as in *Echinodorus*, **Fig. 3E**) have a perianth differentiated in sepals and petals. The expansion of function B genes into the outer whorl also has been shown in orchids (*Oncidium*: Hsu and Yang 2002, **Fig. 3F**, *Dendrobium*: Xu *et al.* 2006). These results are astonishing since later sepals, petals and the elaborated lip can be distinguished (**Figs. 3G-H**). Xu *et al.* (2006) suggest that gene duplications followed by divergence in expression patterns or regulatory mechanisms may be responsible for the unique floral morphology of the orchid flower (compare **Figs. 3F-H**). Recently it has been shown (Mondragón and Theissen 2008, 2009; Mondragón *et al.* 2009), that gene duplications indeed occurred during early orchid evolution and that these gene duplications are followed by a complex series of sub- and neofunctionalization events (see also paragraph on Ranunculaceae further down as regards gene duplication and subfunctionalization).

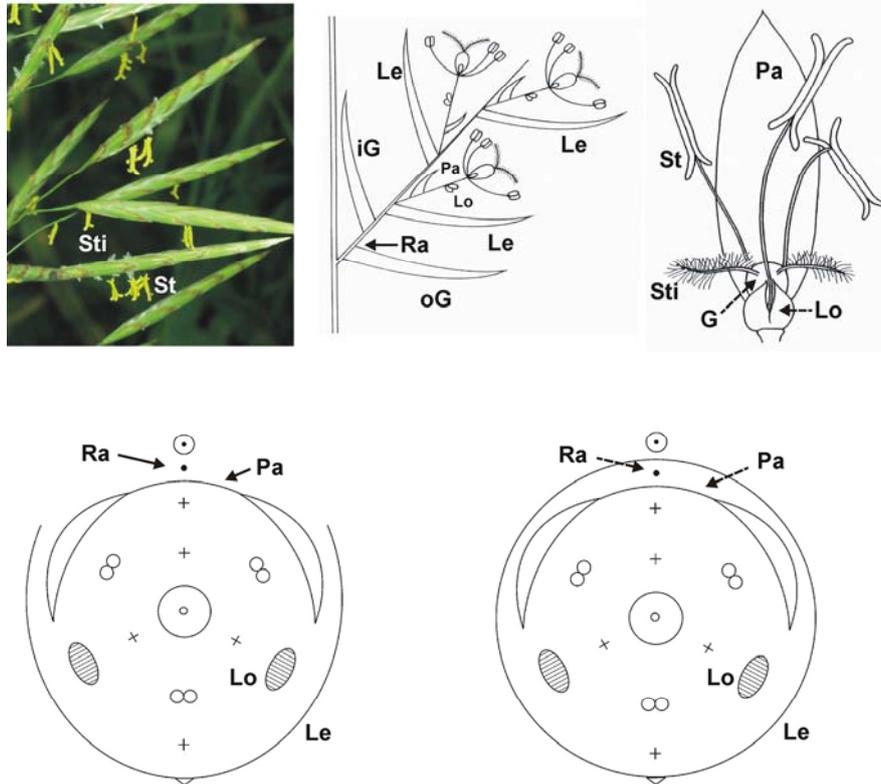
Compared to the ABCDE model of the eudicots (**Fig. 1A**) the B function is extended outwards in most monocots studied so far (**Fig. 1E**). It should be mentioned at this point, that within the eudicots (more precisely within the “basal core eudicots”) another possibility of organ identity specification in the perianth has been demonstrated: The perigone is realized without the B function in *Rumex acetosa* (Polygonaceae, see **Fig. 1H**; see Ainsworth *et al.* 1995); the B function is confined to the stamens (**Fig. 1G**).

The modifications mentioned above can be described by the “sliding-boundary” model (or “shifting-boundary” model) of the B domain (Bowman 1997; Albert *et al.* 1998; Kramer *et al.* 2003): The (two-whorled) perigone<sup>5</sup> is achieved either by outward shift (expansion) or by inward shift (contraction) of the outer boundary of gene B function (**Figs. 1E-G**).

Within Poaceae (**Figs. 4A-C**), in which the floral organs partly are highly modified especially as regards the outer (peripheral) parts, some results are available from maize

(Ambrose *et al.* 2000), rice (Fornara *et al.* 2003; Nagasawa *et al.* 2003) and a basal grass *Streptochaeta* (Whipple *et al.* 2007) showing that function B is expressed in the lodicules (see also Cui *et al.* 2010). This suggests that these organs are homologues to the two abaxial members of the inner perianth whorl. Further data indicate that the (mostly two-keeled) palea correspond to the outer perianth whorl, namely to the two adaxial members (Ambrose *et al.* 2000; Nagasawa *et al.* 2003). It is further suggested by Ambrose *et al.* (2000) and Nagasawa *et al.* (2003) that possibly also the lemma is part of the outer perianth whorl. Lodicules, palea and lemma are grass-specific organs that have been variously interpreted. The basic unit of the grass inflorescence is a spikelet (**Fig. 4A, 4B**) comprising one or more flowers and having (often) two subtending bracts (= glumes). Each floral axis arises on the spikelet axis (= rachilla) in the axil of the lemma and usually bears in adaxial position a two-keeled palea. Two (or three) lodicules, three (or six) stamens and the pistil complete the spikelet. During anthesis the lodicules swell to open the flower thereby pushing the lemma and palea aside so that stamens and stigmas can be presented. Considering the lodicules as modified members of the second (inner) perianth whorl and the palea as those of the first (outer) whorl is conform to earlier interpretations (see, e.g., Schuster 1910; see **Fig. 4D**). Comparing the lemma with part of the outer perianth whorl is critical from the morphological/ontogenetical point of view. Firstly, the primordia of the lemma and the palea arise at different levels on the floral axis, that of the lemma distinctly further down and somewhat overlapping the palea (see figures in Sattler 1973; Nagasawa *et al.* 2003); the different position contradicts the assumption as members of one whorl. Secondly, the floral ontogenetical studies in barley (*Hordeum vulgare*, Sattler 1973) show that the lemma primordium girdles the bud and even encloses the rachilla (**Fig. 4E**). Since there are within the Poaceae gene duplications in the A-like gene (Litt and Irish 2003; Preston and Kellogg 2006), there may be other genes or transcription factors that specify the organ identity of lemma and palea (Zanis 2007). Summarizing, we can state that interpreting lodicules and palea as parts of the perianth is congruent with morphological/ontogenetical data and genetic data. From the morphological/ontogenetical point of view it is better interpreting the lemma as the scarious subtending bract of the flower.

<sup>5</sup> It has to be mentioned, however, that number and arrangement of tepals (as well as stamens) is quite variable within the Polygonaceae (Galle 1977; Leins and Erbar 2008).



**Fig. 4 Poaceae.** (A) Spikelets of *Bromus inermis*; (B) (three-flowered) spikelet in an extremely schematic vertical section; for a better understanding the floral axes are drawn as elongated lines; (C) generalized flower (lemma dissected); (D) floral diagram of the majority of Poaceae; (E) floral diagram of barley (*Hordeum vulgare*). – iG = inner (upper) glume, Le = lemma, Lo = lodicule, oG = outer (lower) glume, Pa = palea, Ra = rachilla, St = stamen, Sti = stigma.

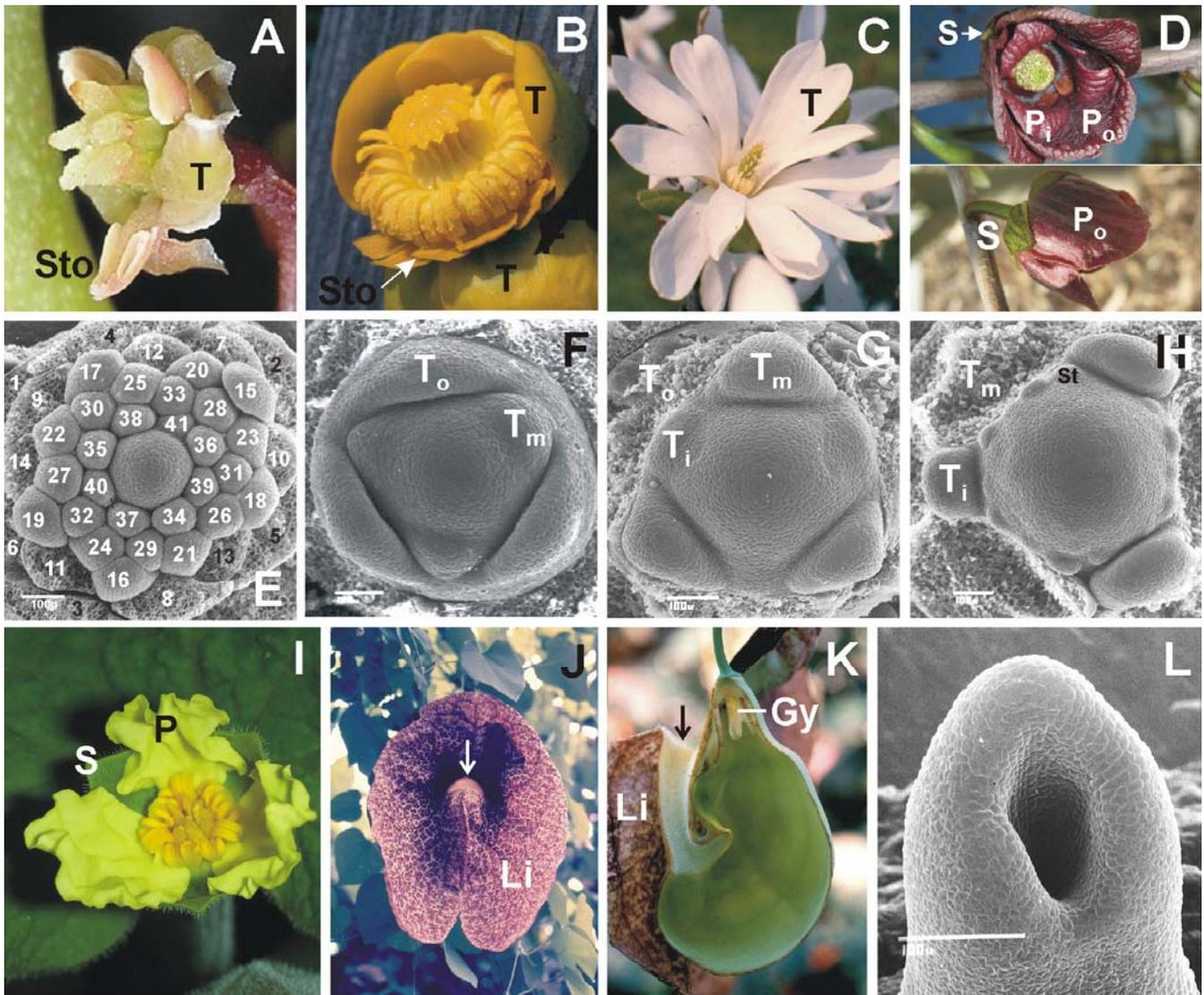
## DETERMINATION OF ORGAN IDENTITY IN NON-CORE EUDICOTS

About 4% of angiosperm species belong to basal lineages (= Magnoliatae; **Fig. 2**). The basal-most group of Amborellales, Nymphaeales and Austrobaileyales is followed by Chloranthales and the large magnoliid clade. Despite the small number of species, the Magnoliatae exhibit a great diversity in floral form and structure. The flowers vary in size, number of floral parts, and arrangement of the floral organs (spirals or whorls). In the studied representatives (mainly *Amborella*, *Nuphar*, *Illicium*, *Magnolia*, *Calycanthus*, *Eupomatia*; examples see **Figs. 1J, 5A-H**), genes of the B class are expressed in spiral and whorled perianths as well as in stamens and, if existing, in staminodes (Kramer and Irish 2000; Kim *et al.* 2005a, 2005b). However, the details of the expression patterns in the perianth vary considerably, spatially and temporally. In contrast, in *Asimina* (Annonaceae) with a differentiated perianth (three sepals in one whorl, six petals in another two whorls, **Fig. 5D**), the class B genes (AP3 and PI) were expressed in petals and stamens, but were either not or only weakly expressed in sepals (Kim *et al.* 2005b). Quite different is the situation in Aristolochiaceae (Jaramillo and Kramer 2004). In *Saruma* (**Fig. 5I**) with a two-whorled perianth of outer sepals and inner petals, the expression pattern of B class genes is in general similar to what is observed in the model species of eudicots. In the genus *Aristolochia* (**Figs. 5J-L**) the perianth is one-whorled with the organs fused to form a peculiar tubular structure, functioning as pollinator trap, and a limb, being coloured and attractive to pollinators. Stamens and carpels together form a gynostemium (Leins and Erbar 1985; Gonzales and Stevenson 2000a, 2000b). In *Aristolochia manshuriensis*, class B genes are expressed in a dynamic and unique pattern: The AP3 homologue is not expressed during early stages of perianth development (but in the stamens), and the PI homologue is restricted to only a portion of the developing perianth and during development the place of expression changes. The role of the class B

genes in the perianth of *Aristolochia* seems not to be in determining the organ identity but rather in promoting late aspects of cell differentiation (for details see Jaramillo and Kramer 2004).

Despite the peculiarities, the results in general indicate that most of the homologues of floral genes from basal angiosperms are expressed in those floral organs that are functionally and/or morphologically similar to those in the model organisms of the eudicots: In basal angiosperms, the class B homologues are expressed in the perianth and stamens, class C homologues in stamens and carpels and the class E homologues are expressed in all floral organs (Kim *et al.* 2005b). However, in the basal angiosperms the expression pattern of the B class genes seems to be neither uniform nor constant during the perianth development (in the eudicots B expression is constant throughout all stages of petal and stamen development). Likewise, expression of A and C homologues is broader across the floral apex in basal angiosperms than in eudicot models studied to present. These aspects are taken into account in the “fading borders” model (Buzgo *et al.* 2004, 2005) that posits that organ identities in basal angiosperms are regulated by broad and overlapping expression of floral genes, although with weaker expression at the limits of their expression (**Fig. 1I**).

Within eudicots, the ABCDE model with **fixed borders of gene expression** (**Fig. 1A**) is only applicable in the core eudicots and seems to be the end point of an evolutionary series with transitional and overlapping gene domains (**Fig. 1I**). In other words: The broad pattern of gene expression of ABC homologues may represent the ancestral and the pattern with fixed borders of gene expression the derived condition (see **Fig. 2**). In the flower whorls of the monocots (see above) studied so far, the borders of gene expression also are fixed (**Fig. 1E**). From a phylogenetic perspective, broad expression of B-function genes is considered to be the ancestral condition for angiosperms (e.g. Soltis *et al.* 2007a).



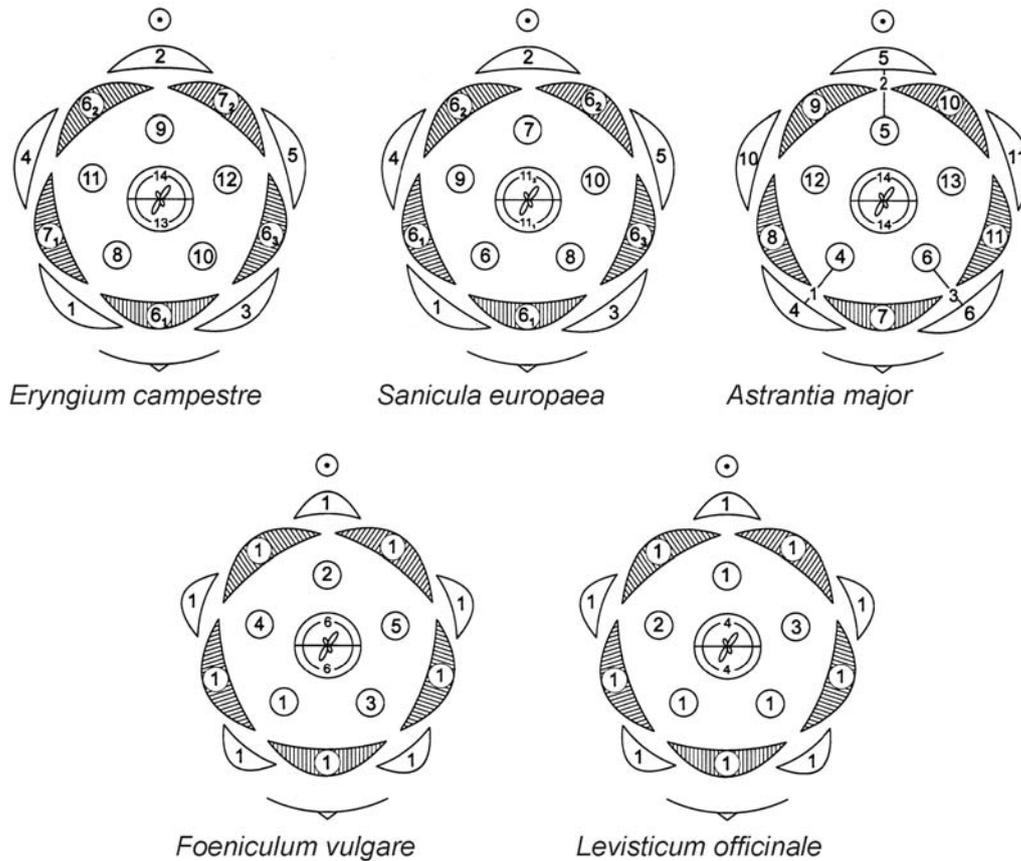
**Fig. 5** Flowers of basal angiosperms (A-H) with genes of class B expressed in spiral and whorled perianths as well as stamens and staminodes and Aristolochiaceae (I-L). (A) *Amborella trichopoda* (Amborellaceae); female flower with staminodes between perianth and carpels; (B) *Nuphar lutea* (Nymphaeaceae); between tepals and stamens staminodes are present; (C) *Magnolia stellata* (Magnoliaceae) with polymery in all organ categories and with a prevailing spiral; (D) *Asimina triloba* (Annonaceae); the outer (green coloured) members of the 3+3+3 perianth are reflexed and may be named as calyx; as usual in the Annonaceae the two inner perianth whorls (here named petals) are of different shape; (E) SEM image of *Illicium anisatum* (Illiciaceae) with a regular spiral inception of all floral organs according to the limiting divergence (“golden divergence angle”); the primordia 34-41 are carpel primordia; (F-H) SEM images of *Magnolia denudata* (Magnoliaceae); the whorled perianth (3+3+3) shows more or less spiral sequence within a whorl, but there is a relatively long interval between the subsequent whorls; (I) *Saruma henryi* (Aristolochiaceae) with a differentiated perianth and expression pattern of B class genes similar to the classical model; (J-L), *Aristolochia grandiflora* (Aristolochiaceae) with highly modified perianth (first whorl); the limb (Li) surrounds the entrance (arrow) to the siphon-like tubular part at which end the gynostemium (Gy) can be found. From Leins and Erbar 2008; (L), Early ontogeny of the perigone tube. – P = petal, P<sub>i</sub> = petal of the inner whorl, P<sub>o</sub> = petal of the outer whorl, St = stamen, Sto = staminodium, T = tepal, T<sub>i</sub> = tepal of the inner whorl, T<sub>m</sub> = tepal of the middle whorl, T<sub>o</sub> = tepal of the outer whorl.

## ORGAN QUALITY VERSUS ORGAN POSITION AND SEQUENCE OF ORGAN INITIATION

Flower formation is a series of consecutive developmental steps. The first step, the floral induction, is the switch from a vegetative to an inflorescence and/or floral meristem controlled by internal as well as external environmental signals (such as day length, temperature, etc.). Afterwards, meristem identity genes that specify floral identity get activated. The floral meristem then generates by cell divisions floral organ primordia which appear in an acropetal sequence at its flanks and have a distinct structure and function in the mature flower. Since Payer (1857) the different patterns of organ sequence have been shown in numerous ontogenetical studies. Despite their later structure and function, all different floral organs start their ontogeny as little bulges, mostly hemispherical, of meristematic cells generated by periclinal and anticlinal cell divisions. One ques-

tion was how these undifferentiated cells and their derivatives find their position within the developing flower and differentiate into the appropriate cell types.

The genetic models proposed as yet predict the organ quality, but they do not explain how the number of organs or the spatial pattern in which organ primordia appear (e.g. spiral or whorled) is regulated. Different ontogenetical pathways lead to a relatively invariant mature floral morphology (Erbar and Leins 1997a). Detailed studies have shown that a floral apex can produce more than one organ category at the same time and the sequence of whorls must not be strictly acropetal. Genetic and molecular studies have shown that MADS-box transcription factors control the identity of the floral organs. Up to now, however, it is largely unsolved how number and position of the floral organs are regulated within the regulatory network at a hierarchy level above that controlled by the MADS box genes. Perhaps glutaredoxins (= oxidoreductases) besides others are candidates to



**Fig. 6 Developmental diagrams of some members of the Umbelliferae (Apiaceae).** Numbering of the primordia reflects the sequence of initiation; indices on the numerals indicate a very rapid sequence (at the limit of observation!); in *Astrantia major* at three sites sepals and stamens develop from a common primordium, indicated by the connecting lines 1-3. From Erbar and Leins 1985, modified.

play a role in this scenario. Known as a key component of plant antioxidant defence, they recently came into focus as they seem to be involved in different processes of floral development due to their capability to modify protein activity posttranslationally (Xing *et al.* 2006). It is further noteworthy that there is a subgroup of glutaredoxins being specific for angiosperms. Genetic and molecular studies have shown that MADS-box transcription factors control the identity of the floral organs. Although the underlying developmental mechanisms at present remain largely elusive, there are genetic data suggesting that regulatory factors contribute to a stable and uniform development of flowers (see, e.g., Chen *et al.* 2003; Prunet *et al.* 2008). In *Arabidopsis*, it has been shown that the number of organs is affected by the gene *PERIANTHIA* (Running and Meyerowitz 1996). This transcription factor acts as a direct regulator of the class C gene *AGAMOUS* (Maier *et al.* 2009).

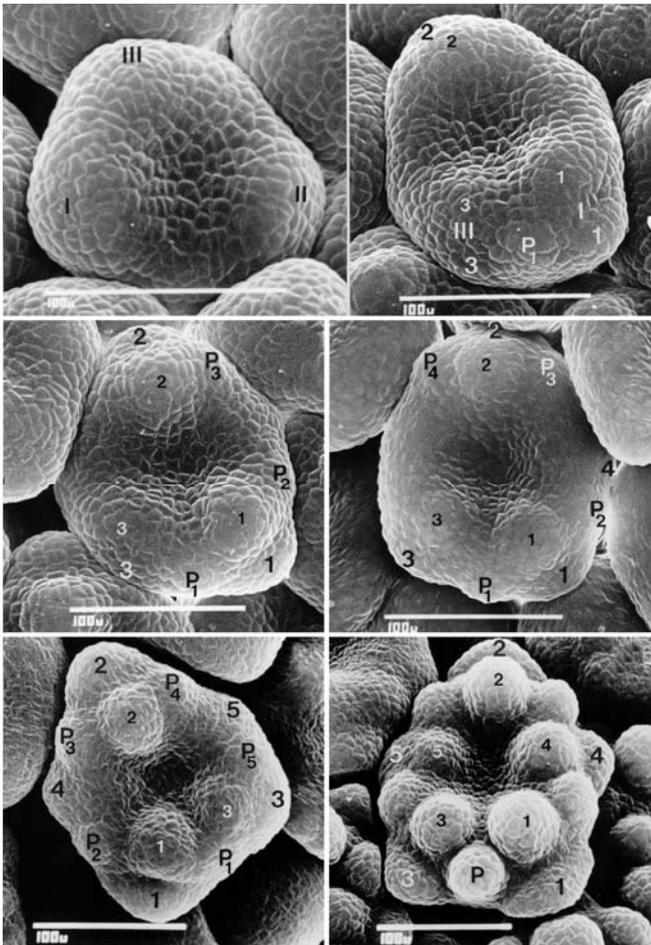
Against this background, two families will be presented which are characterized by a uniform floral diagram, namely the crucifers (Brassicaceae, rosids) and the umbellifers (Apiaceae, asterids), but contrary to their uniform flower construction show fairly diverse patterns in organ initiation (see Erbar and Leins 1985, 1997; Leins and Erbar 2004).

The flowers of the Apiaceae are tetracyclic, with pentamerous calyx, corolla and stamen whorl and a dimerous carpel whorl. The stamens alternate with the petals. Contrary to their uniform flower construction the Apiaceae show fairly diverse patterns in organ initiation (Fig. 6). The terminal flowers of *Eryngium campestre*, for example, show an almost continuous spiral sequence of all organs, with the restriction though, that sepals, petals, and stamens nearly alternate. Merely within the corolla the plastochrons (the time intervals between subsequent organ primordia on the floral apex) are very short, they even tend towards zero! In *Foeniculum vulgare*, the primordia of calyx, corolla and the

first stamen originate simultaneously and in *Levisticum officinale*, even three stamens arise simultaneously with the sepals and petals. In the flowers of *Sanicula*, after the spiral inception of five sepal primordia in spiral sequence and of four petals successively in two pairs, the last petal is formed simultaneously with the first stamen in front of sepal 1. The remaining stamen primordia follow in a more or less distinct spiral sequence.

The flower of *Astrantia major* (greater masterwort) exhibits an exceptional developmental pattern (Figs. 6, 7). The floral development starts with the successive formation (divergence about  $2/5$ ) of three big protuberances (Fig. 7A). These protuberances then each differentiate in the same sequence into a sepal primordium and a stamen primordium (Figs. 7B-C). Immediately after the splitting of the three “stamen-sepal primordia” the five petals are initiated following a  $1/5$ -spiral, starting with the first petal between the first and third sepals (Figs. 7B-E). During the further initiation of the corolla the fourth petal originates almost synchronously with the fourth sepal (Fig. 7D). Finally, the last petal originates at the same time as the last sepal (Fig. 7E). It is not until now that the spiral initiation of the androecium continues with the initiation of the last two stamen primordia (Fig. 7F). Finally, with notable delay, the gynoecium starts its development.

If we transfer the temporal overlaps during the initiation of the organ whorls in *Astrantia* onto the ABC gene class model of organ determination – on condition that the appearance of the organ primordia temporally coincides with the expression of the homeotic genes – we may assume that in three sectors (successively!) the expression of gene A (for determination of three sepals) and of genes B/C (for determination of three stamens in front of them) is taking place simultaneously. After the subsequent expression of genes A/B determining three petals, once more two sepals are determined by gene A and simultaneously the other two



**Fig. 7** Early flower development in *Astrantia major* (Apiaceae). (A) Protrusion of three common stamen-sepalum-primordia (I-III); (B) differentiation of each of the three stamen-sepalum-primordia into a sepal (large numerals 1-3) and a stamen primordium (small numerals 1-3); the first petal primordium ( $P_1$ ) becomes visible; (C-E) continuation of the spiral inception of the petals ( $P_2$ - $P_5$ ) in a  $1/5$ -spiral and completion of the sepal whorl (E) according  $2/5$ ; (F) continuation of the  $2/5$ -spiral in the androecium (stamen primordia labeled with smaller numerals); the spirals in organ inception run either in clockwise or anticlockwise direction.

petals by genes A/B, followed by the expression of genes B/C determining the last two stamens (Leins and Erbar 2008, 2010).

The crucifers, to which the model organism *Arabidopsis* belongs, are another family that is characterized by a uniform floral diagram but great variability in the initiation sequence of the floral organs (Erbar and Leins 1997a). Most members of the Brassicaceae have four sepals, four petals, two outer shorter and four inner longer stamens as well as a pistil composed of two carpels. Different possibilities of initiation sequence of the floral organs are shown in the floral developmental diagrams (Fig. 8). In this family, too, the sequence of the whorls is not strongly acropetal in all species (as in *Iberis* and *Isatis*, for example, Figs. 8, 9A-B), namely in the pattern shown by *Fibigia clypeata* and *Arabidopsis thaliana* (Figs. 8, 9C-D). In the latter species, the sequence of the two androecial whorls is reversed: The four inner stamen primordia are formed before the two outer ones in transversal position. This sequence (4 sepals  $\rightarrow$  4 petals  $\rightarrow$  4 inner stamens  $\rightarrow$  2 outer stamens  $\rightarrow$  2 carpels) can be found in some other members, too (e.g. *Arabis*, *Barbarea*, see Erbar and Leins 1997a). As in some species of the Apiaceae, members of more than one organ category can be initiated at the same time: Either the four inner stamens (as in *Brassica napus*, Figs. 9E-F) or all six stamens (as in *Cardaria*, Figs. 9G-H) are initiated simultaneously with the petals. It is interesting that in the tribe

Aethionemeae, which is sister to all other Brassicaceae (Koch and Al Shehbaz 2009), likewise petals and all stamens are formed simultaneously (namely in *Aethionema grandiflorum*, Nigrelli 2008). However, this pattern is also found in other tribes.

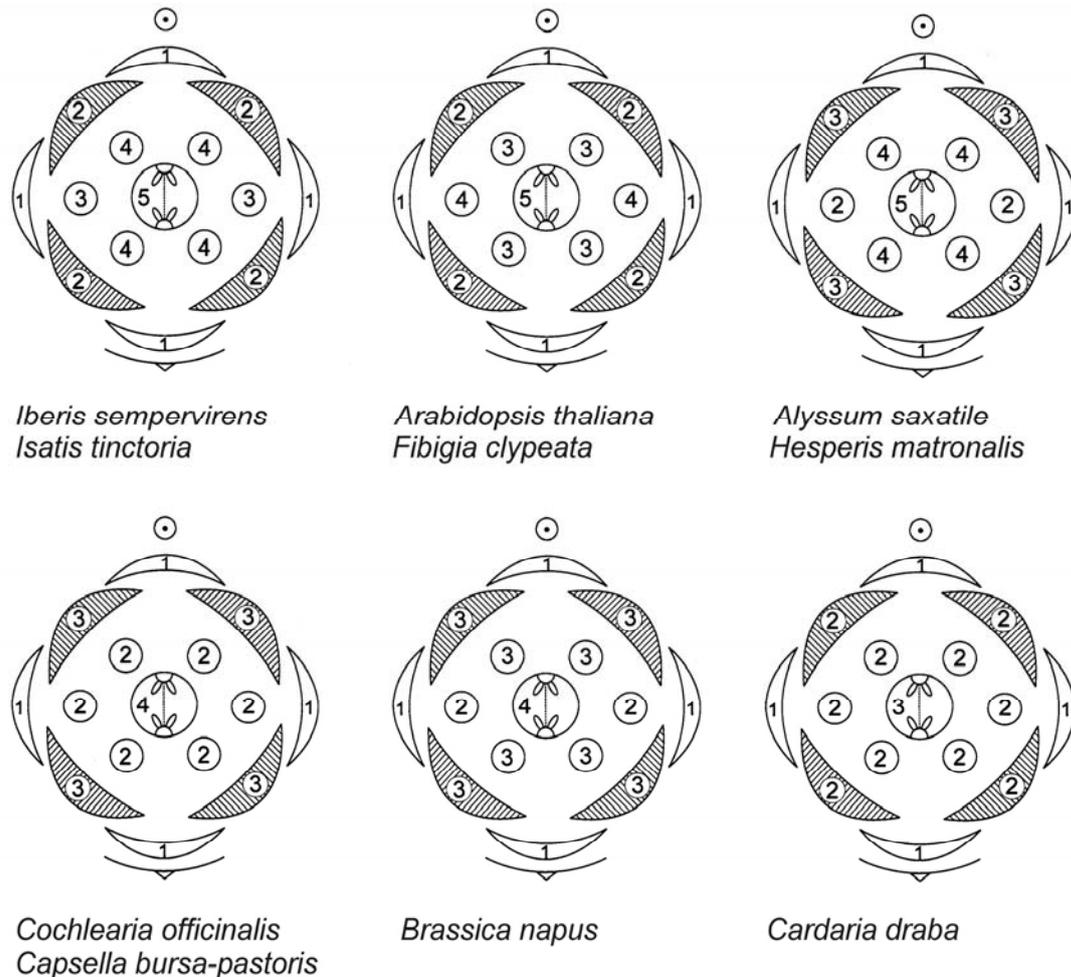
We can conclude that organ position and sequence of organ initiation on the one hand and organ identity on the other hand are regulated on different genetic levels. The ABCDE model only predicts the organ quality and does not explain how floral organs appear in appropriate positions and appropriate number (see above). *Arabidopsis* is remarkable in this context because the two stamen whorls are formed basipetally, i.e. the inner stamen whorl is formed before the outer one. Further genetic investigations should take into consideration the different temporal patterns of stamen initiation in Brassicaceae.

The typical 2+4 pattern in the androecium of the Brassicaceae can also be found in flowers of the Cleomaceae, which are sister to Brassicaceae. *Cleome spinosa* (Figs. 10A-B) shows the same sequence in the androecium as some Brassicaceae, for example *Iberis* (4 sepals  $\rightarrow$  4 petals  $\rightarrow$  2 transversal stamens  $\rightarrow$  4 stamens in pairs in front of the median sepals  $\rightarrow$  2 carpels). In *Cleome violacea* (Figs. 10C-D), however, with the same number and position of stamens, the initiation pattern is quite different. The stamen inception takes place in a unidirectional order: The initiation of the stamens starts abaxially in front of the (subtending) bract and continues toward the opposite side, namely the adaxial side.

In this context it should be mentioned that, in *Polanisia*, for example, another member of the Cleomaceae, and in *Capparis* (Capparaceae; see Figs. 11E-F), a family which is sister to Cleomaceae and Brassicaceae, the number of stamens is higher. In *Polanisia dodecandra*, 9-18 stamen primordia are initiated sequentially starting on the adaxial side of the floral apex (Fig. 10E-F). Most stamen primordia (range between 9 and 16) arise in one row; sometimes a few additional are formed above the basic row (positioned on a higher level). In species of the Capparaceae with a multistaminate androecium (see next paragraph), the stamens are initiated in a centrifugal sequence on a primary primordium (Leins and Metzner 1979; further citations see Erbar and Leins 1997b).

Apart from Brassicaceae, Cleomaceae and Capparaceae, also Gyrostemonaceae, Resedaceae, Pentadiplandraceae and Tovariaceae belong to the core Brassicales (Hall *et al.* 2002, 2004) and add further diversity to the androecial initiation pattern. In the (6-8)(-9)-merous flowers of Tovariaceae the alternipetalous stamen primordia are initiated simultaneously in a single whorl (Fisel and Weberling 1990). The male flowers of Gyrostemonaceae differ in number of stamens and number of stamen whorls. Most members of the family have a one-whorled androecium with 7-12 stamens. Only in a few species the androecium consists of more whorls (with up to 20-60 stamens; Hufford 1996). Ontogenetical studies, however, are lacking. Two whorls of five stamens are formed in a rapid sequence in the Pentadiplandraceae. Frequently, the number of stamens is higher (11-12 stamens, sometimes there are also more petals; Ronse Decraene 2002). Resedaceae show considerable variation in stamen number. In *Reseda luteola*, the stamens originate on four antepetalous primary primordia (Sobick 1983; Fig. 10G) and not in 3-4 whorls as mentioned by Stevens 2001 onwards). In most cases, however, the stamens arise in one row on a primordial ring (*Reseda alba*, Fig. 10H, *Reseda odorata*, *Caylusea*, *Sesamoides*); in *Oligomeris*, only the adaxial part of such a primordial ring is present on which the three stamens are formed (Sobick 1983).

The families Cleomaceae and Capparaceae lead over to multistaminate androecia (Fig. 11) that add a further interesting aspect. In Magnoliatae (e.g. Illiciaceae, Magnoliaceae, Calycanthaceae) and basal eudicots (e.g. Ranunculaceae), stamens originate directly on the floral apex in a regular spiral sequence (Fig. 11B). In the core eudicots actually



**Fig. 8 Floral developmental diagrams of some members of Brassicaceae.** The numbers refer to the order of initiation in corolla and androecium; in each case the calyx is initiated first (variations in sepal sequences are not considered) and the gynoecium last. From Erbar and Leins 1997a, modified.

characterized by a canalized floral structure of five or four whorls, nevertheless, in all major clades except euasterids multistaminate androecia occur in a considerable number of families (e.g. Aizoaceae, Paeoniaceae, Myrtaceae, Fabaceae-Mimosoideae, Capparaceae, Malvaceae, Theaceae; see Fig. 66 in Leins and Erbar 2008, 2010). The stamen primordia originate on primary primordia in centrifugal (**Fig. 11D**) or centripetal (**Fig. 11H**) succession resulting in fascicled androecia. In other cases, an androecial ring primordium with centrifugal stamen initiation can be observed (for example in *Capparis spinosa*, Leins and Metzenauer 1979, **Fig. 11F**). Sometimes, the primary primordia are initiated in a spiral sequence, namely in *Paeonia* (Paeoniaceae, Leins and Erbar 1991, **Fig. 11J**) and *Stewartia* (Theaceae, Erbar 1986, **Fig. 11L**). What is the genetic basis for these ontogenetical pathways? At what hierarchical level of the regulatory network of gene functions (see, e.g., diagrams of regulatory cascade of flower development in Theissen 2001; Soltis *et al.* 2002; Kaufmann *et al.* 2005) happens the switch to the new pattern of organ initiation?

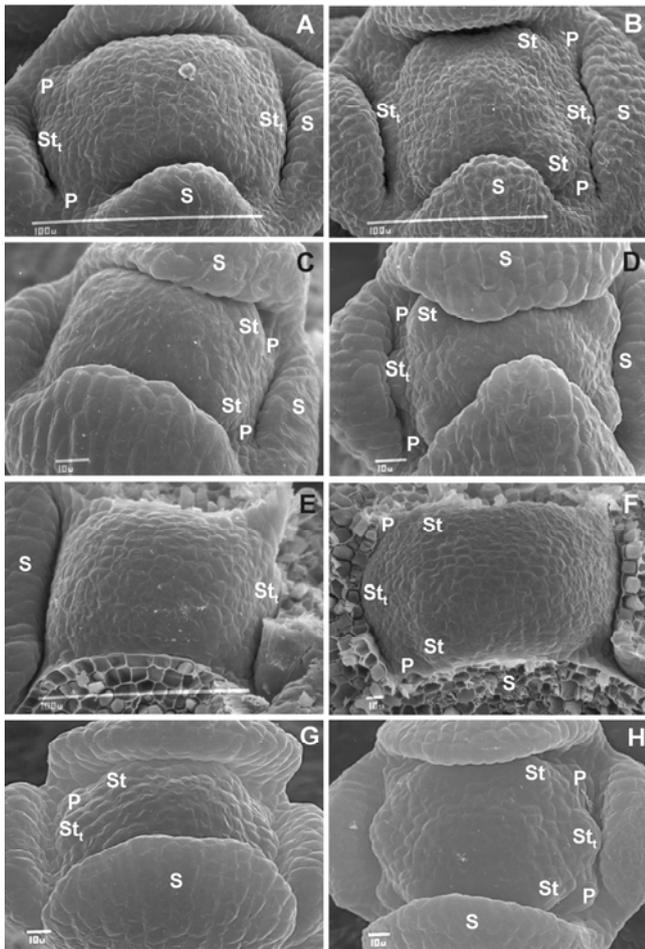
In summary, it is necessary to point out once more that the ABCDE model of floral development in its actual version has neither purpose nor potential to explain all floral diversities.

## THE ORIGIN OF THE ANGIOSPERM FLOWERS AND THEIR ENORMOUS DIVERSITY

The phylogeny of seed plants seems at least on the family-level to be well-established (e.g. APG II 2003, APG III 2009, Stevens 2001 onwards). Gymnosperms are sister to the angiosperms. Within the angiosperms, the monocots (= Liliatae) are embedded in the Magnoliatae, a group that

simplifying can be divided in the basal angiosperms (originally termed the ANITA group with Amborellales, Nymphaeales, and Illiciaceae, Trimeniaceae and Austrobaileyaceae in Austrobaileyales, the group has recently been named ANA from Amborellales, Nymphaeales and Austrobaileyales; Frohlich and Chase 2007) and the magnoliids. The largest monophyletic clade, the eudicots or Rosatae (united morphologically by a single synapomorphy: triaperturate pollen), can be further distinguished into the basal eudicots (e.g. Ranunculales) and the core eudicots. Major clades of the core eudicots are the Saxifragales and Caryophyllales (together with some smaller orders named here as “basal core eudicots”) as well as the rosids and asterids (**Fig. 2**). The model organism *Arabidopsis* (Brassicaceae) belongs to the rosids and *Antirrhinum* (Plantaginaceae, formerly Scrophulariaceae) to the asterids.

However, the origin of the flower (a recent bisexual flower is made up of perianth, androecium, gynoecium and receptacle) could not be clarified even with the actual, highly accurate phylogeny. As regards morphology (i.e. homology between reproductive organs), there is a gap between the angiosperms and their sister group, the extant gymnosperms. Also data from paleobotany cannot solve the problem truly satisfying (mainly due to incomplete fossil record). The oldest unequivocal angiosperm macrofossil is the genus *Archaeofructus* (125 million years old; Sun *et al.* 2002), despite the fact that the taxon might be regarded as more specialized than rather basal (Friis *et al.* 2003). In addition, earlier microfossils (mostly pollen) are dated to be about 140-130 million years old (Crane *et al.* 2004; Friis *et al.* 2005). Molecular data, however, suggest a distinct pre-Cretaceous origin of the angiosperms. In context of molecular clock hypotheses, the datings have given widely dif-

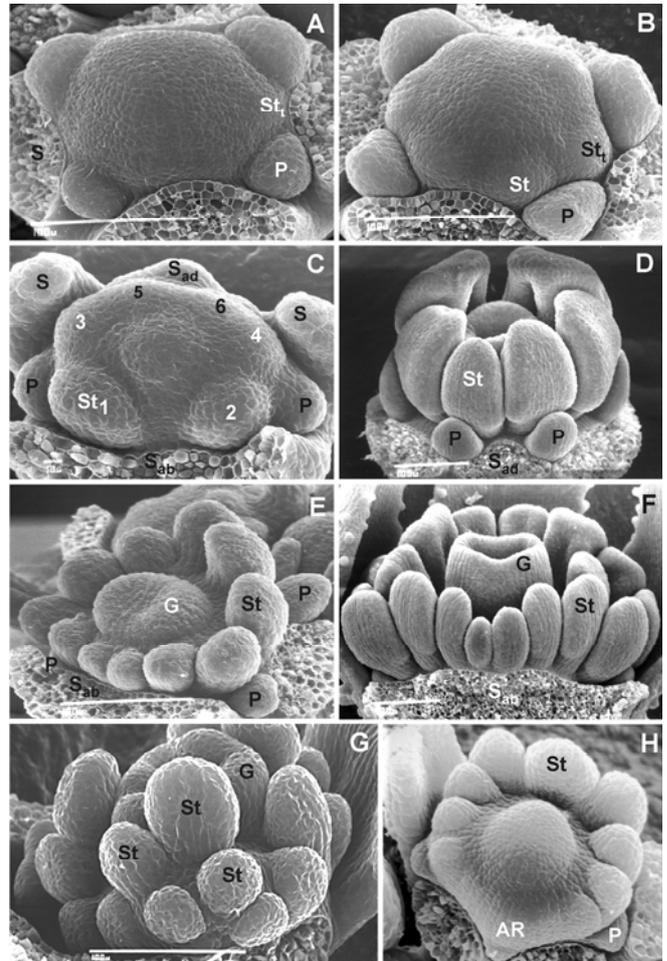


**Fig. 9 Floral ontogeny in Brassicaceae.** The uniform floral phyllotaxis is brought about by sequences that vary in detail. (A-B) *Isatis tinctoria*; the two transversal stamens (St<sub>t</sub>) are visible before the four inner stamen primordia arise in front of the sepals; (C-D) *Arabidopsis thaliana*; formation of the four inner stamens in diagonal position earlier than the initiation of the; (E-F) *Brassica napus*; petals and the four inner stamen primordia arise simultaneously but after the transversal stamens (St<sub>t</sub>); (G-H) *Cardaria draba*; petals and all six stamens arise simultaneously. – P = petal, S = sepal, St = stamen, St<sub>t</sub> = stamen in transversal position; from Erbar and Leins 1997a, modified.

ferent results, although more recently the datings tend to converge on similar ages, indicating the crown node of the angiosperms from 145-208 mya (Sanderson *et al.* 2004; see also Kim *et al.* 2004; Anderson *et al.* 2005). The key characters that distinguish angiosperms from gymnosperms are not primarily the bisexual flowers but also the carpels (enclosing the ovules) and the double fertilization.

Changes in morphology during evolution and thus also morphological innovations are due to changes in developmental control genes, since development is largely under genetic control. Especially changes in the structure and functions of the MADS-box genes appear to be correlated with large-scale changes in the morphologies of flowers in different lineages (Theissen *et al.* 2000; Irish 2003; Theissen 2005). The “evolutionary developmental genetics” (“evo-devo”) tries to get insights into the phylogeny of the floral homeotic genes and thus to help to better understand the evolution of flowers and how the divergence of the MADS-box genes contributed to the evolution of new characters in flowers.

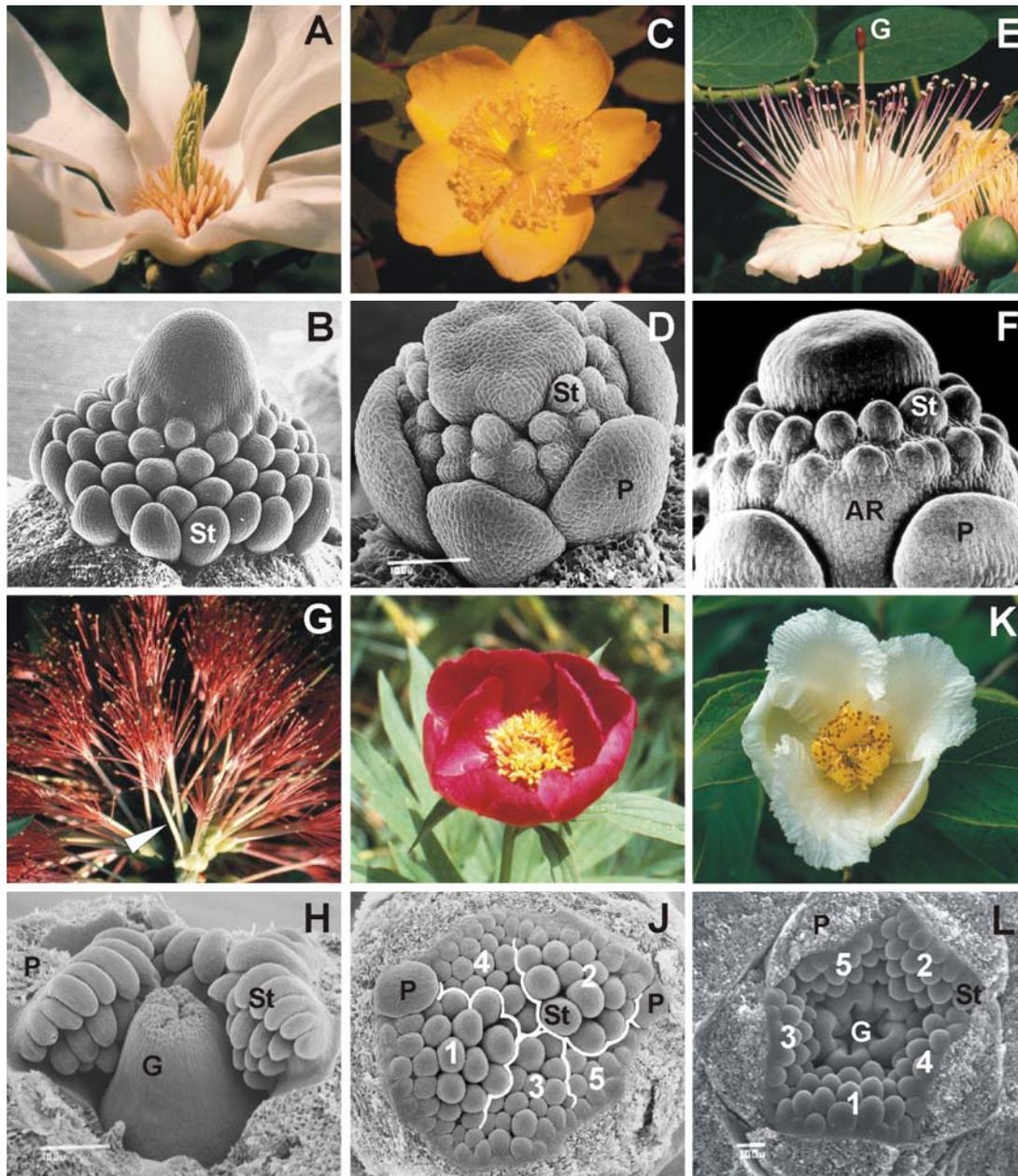
Multiple gene duplications have occurred within floral MADS-box gene subfamilies. One of the best studied gene class is the B class which controls petal and stamen identity. B class genes comprise the homologues of the genes *APETALA3* (AP3) and *PISTILLATA* (PI), forming two subclades. The B genes in basal angiosperms, monocots as well



**Fig. 10 Floral ontogeny in core Brassicales with diverse patterns in the initiation of the androecium.** (A-B) *Cleome spinosa* (Cleomaceae) with an androecial pattern as some Brassicaceae, e.g. *Isatis* (compare with Figs. 9A-B); (C-D) *Cleome violacea* (Cleomaceae) with a unidirectional order of six stamen primordia; (E-F) *Polanisia dodecandra* (Cleomaceae) with numerous stamens initiated unidirectionally in one row; (G), *Reseda luteola* (Resedaceae); centrifugal inception of stamen primordia on distinct primary primordia; (H), *Reseda alba* (Resedaceae); stamens arise in solely one row on a narrow androecial ring primordium (AR). G+H from Sobick 1983. – G = gynoecium, P = petal, S = sepal, S<sub>ab</sub> = sepal in abaxial position, S<sub>ad</sub> = sepal in adaxial position, St = stamen, St<sub>t</sub> = stamen in transversal position.

as basal eudicots (e.g. in Ranunculales which are sister to all other eudicots; Fig. 2) belong to the so-called “paleo-lineages” of the different homologous B genes (Kramer and Irish 1999; Kramer *et al.* 2003; Stellari *et al.* 2004; Zahn *et al.* 2005a; Kramer *et al.* 2006). These exhibit, as already mentioned, a spatial and temporal expression pattern that implies more complex functions and interactions than those underlying the more fixed and (presumably) uniform ABCDE model of the core eudicots. The paleo-lineages (paleo-AP3 as well as paleo-PI; termed for short “paleoB” in Fig. 2) result from a gene duplication event after the split between extant gymnosperms and extant angiosperms well before the further diversification (Kim *et al.* 2004; Zahn *et al.* 2005a). Also after the duplication that produced the separate paleo-AP3 and paleo-PI-lineages gene duplications providing multiple potential opportunities for functional divergence occurred at every phylogenetic level.

After gene duplication the copies can have different fates: 1. maintenance of function in one copy and non-functionalization of the other; 2. neo-functionalization (i.e., one or both copies adopt a novel function); 3. sub-functionalization (both copies acquire complementary loss-of-function mutations such that both genes are required to produce the full functions of the single ancestral gene (Becker and

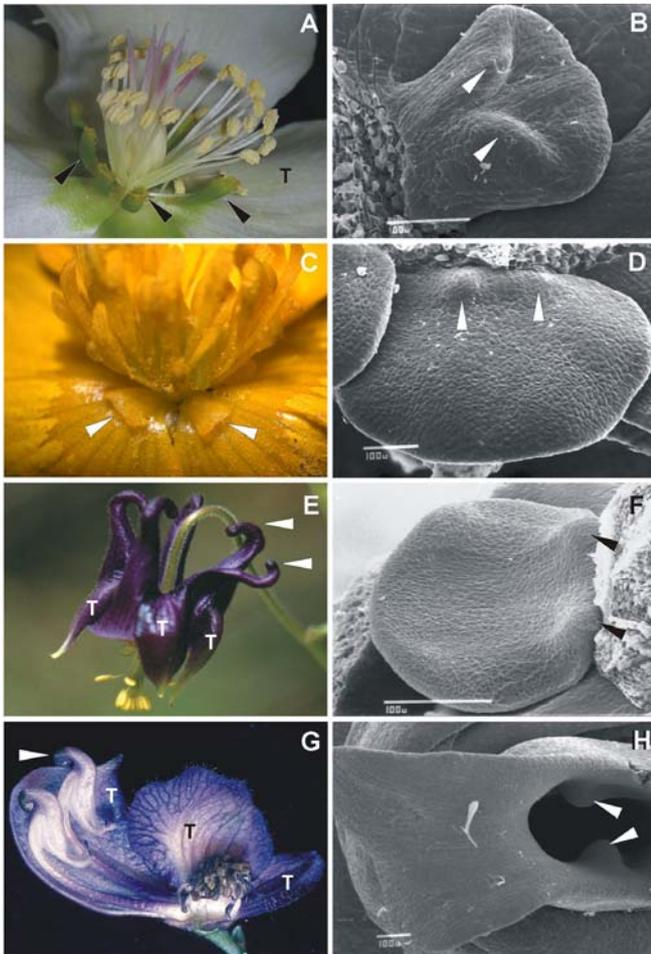


**Fig. 11 Flowers with polymerous androecia and their formation pattern.** (A-B), *Magnolia denudata* (Magnoliaceae) with spiral inception of the stamens directly on the floral axis; (C-D), *Hypericum hookerianum* (Hypericaceae): the individual stamens are part of fascicled androecium; on primary primordia the stamen primordia arise in centrifugal direction (the oldest stamen primordium at the top of the primary primordium); (E-F), *Capparis spinosa* (Capparaceae) with centrifugal stamen initiation on an androecial ring primordium (AR); (G-H) in *Melaleuca hypericifolia* (Myrtaceae) the individual stamen primordia originate in centripetal direction on the fascicle primordia (the oldest stamen primordium at the base of the primary primordium); in adult flowers the primary primordia have elongated stalk-like (arrow points to an individual stamen fascicle); (I-J) *Paeonia officinalis* (Paeoniaceae) with a prevailing spiral sequence in the flower with limiting divergence; in contrast to the magnolias not the individual stamens but stamen fascicles, five in number, continue the spiral of the perianth; P = both innermost petals; (K-L), *Stewartia pseudocamellia* (Theaceae); as in *Paeonia*, five stamen fascicles continue the spiral sequence of the petals; the spiral, however, is based on a  $2/5$ -divergence; consequently, the five spirally initiated stamen fascicles obtain an antepetalous position. – G = gynoecium, P = petal, St = stamen, T = tepal; from Leins and Erbar 2008, 2010, modified.

Theissen 2003).

One example for duplication and new functions in the B class genes has been already mentioned in the perianth elaboration of the Orchidaceae (see above). A further example comes from the Ranunculaceae (Kramer and Irish 1999; Kramer *et al.* 2003), whose B genes belong to the so-called paleo-lineage (see above). The unique nectary organs (Fig. 12) are either tube-shaped or flat (petaloid with basal nectary scales) or spurred (Kosuge 1994; Erbar *et al.* 1999; Tucker and Hodges 2005). Kramer *et al.* (2003) outlined a hypothesis that duplications in the paleo-AP3 (and paleo-PI) lineages may have contributed to the evolution of separate petal identity programs (or more precisely: unique “nectary organ identity programs”). Differential expression or functional specialization of particular homologues could

provide the information needed to distinguish between the different types of nectary organs. In addition, distinct AP3/PI interaction could occur in the petal identity programs. A further possibility could be that the nectary organs may express B class genes alone (Kramer *et al.* 2003). However, in the Ranunculales as well as in the basal angiosperms the gene expression pattern is neither uniform nor constant during organ development in contrast to the core eudicots. A third possibility is that the nectary organs are determined by overlapping gene functions in a broad range, i.e. genes of the classes A, B and C are expressed in combination (Erbar *et al.* 1999; see also Albert *et al.* 1998). A difficult aspect in this hypothesis is that A and C functions are thought to be mutually exclusive in the classical ABC model as they negatively regulate each other, i.e. a concurrent function of



**Fig. 12** Different nectary organs in the Ranunculaceae and their early ontogenetical stages (SEM images). (A) *Helleborus niger* with tubular nectary organs (arrows); (B) nectary organ primordium of *Helleborus foetidus*; (C) *Ranunculus repens* with petal-like nectary organs with the nectary scale (arrows) at their bases; (D) nectary organ primordium of *Ranunculus ficaria*; (E) *Aquilegia atrata*; five blue corollinic tepals alternate with five showy blue spurred nectary organs (arrows); (F) nectary organ primordium of *Aquilegia vulgaris*; (G) in the zygomorphic flowers of *Aconitum napellus*, the only two, long-stalked, spurred nectary organs (arrow points to one of them) are hidden in the hooded adaxial tepal (perigone partly removed); (H) nectary organ primordium of *Aconitum napellus*. – In the SEM images the arrows point to the two bulges which arise laterally at the ventral side of the primordium; the ventral bulges remain separate in *Aquilegia* and *Aconitum*; in other genera, they become connected and grow up to a tubular part (*Helleborus*) or a scale (*Ranunculus*). – T = tepal.

A and C is excluded (Coen and Meyerowitz 1991). However, it has been shown in *Arabidopsis* that the ability of C genes to suppress the expression of A genes depends on the concentration: Low amounts of C proteins can provide organ identity but are not sufficient to repress A function (Mizukami and Ma 1995; see also Kramer *et al.* 2003). It is also imaginable that the antagonistic operation between A and C function is neutralized through a temporally delayed C expression. However, our suggestion (Erbar *et al.* 1999) that some level of C-genes expression, which normally promotes stamen identity, could contribute to the development of the organs, is not observed in *Aquilegia* (Kramer *et al.* 2007).

From the floral ontogenetical point of view, it is conspicuous that the occurrence of two bulges at the ventral base of the flat primordium is a shared feature (compare **Figs. 12B, 12D, 12F, 12H**). Whereas the ventral protuberances remain more or less separate from each other in the spurred nectary organs (*Aquilegia*, *Aconitum*), they expand towards the median plane of the primordium and fuse in the tubular

(*Helleborus*) and petaloid (*Ranunculus*) ones. In the buttercup or crowfoot (*Ranunculus*), the fusion product grows out independently from the flat part of the primordium and forms a scale covering a nectar-secreting pit. In the hellebore (*Helleborus*), the ventral protuberances are continuous with the margins of the primordium already from the beginning. Further, the growth of the ventral part is very intensive finally resulting in a tubular or cup-like structure. The nectary tissue lies at the base of this tube. In spurred nectary organs, the nectar is secreted at the end of the spur. In the columbine (*Aquilegia*), there are five of them, which additionally contribute to the attraction of the pollinators through their showiness. In the monkey hood (*Aconitum*), there are two spurred organs that lie side by side and are hidden in the hooded adaxial tepal. The spur itself develops by locally enhanced surface growth which starts quite early in the ontogeny (for a more detailed description of the ontogeny see Erbar *et al.* 1999).

The nectary organs in the Ranunculaceae are often regarded as homologous to stamens and usually the tubular shape of some nectary organs is cited as a homology criterion (e.g. Kosuge 1994). However, the detailed ontogenetical investigations revealed that the stamens are by no means tubular (peltate) structures (see **Fig. 12**; Leins and Erbar 2008, 2010). Nevertheless, the two initially free ventral protuberances of the nectary organ primordia, which can be interpreted as rudimentary (sterile) adaxial pollen sacs, may be an indication for an ontogenetical (!) connection between nectary organs and stamens (Fig. 58 in Erbar *et al.* 1999). However, this presumed relation of nectary organs and stamens does not compellingly imply that the nectary organs have phylogenetically developed directly from initially fertile stamens. They may be just as well phylogenetically interpreted as new organs that developed during the early evolution of “nectar-offering flowers”. The genetic programs of the tepals and the organs, that succeed within the flower, the stamens, overlap in these new organs (see above). Our hypothesis does not contradict a model supposed by Rasmussen *et al.* (2009) to consider position, early developmental patterns and presence of nectaries as evidence of a commonly inherited syndrome. In summary, many aspects of nectary organ evolution in Ranunculaceae are unresolved.

In the context of ranunculaceous nectary organs it is noteworthy that, unlike in core eudicots, no expression of CRABS CLAW<sup>6</sup>, a gene required for nectaries (e.g. in *Arabidopsis*<sup>7</sup>, *Cleome*, *Nicotiana*, *Petunia*) was found in the nectary spur of *Aquilegia* (Lee *et al.* 2005a, 2005b). Nectaries are nectar glands that may occur anywhere in the flower. First results in a limited number of taxa examined indicate that irrespective of the position within the flower the CRABS CLAW gene is essential for nectary development (Bowman and Smyth 1999; Lee *et al.* 2005a, 2005b). Its expression is mostly limited to carpels (here it is involved in suppressing early radial growth of the gynoecium and in promoting its later elongation) and nectaries (Bowman and Smyth 1999; Fourquin *et al.* 2005).

Another major event of gene duplication (in the AP3 lineage, which yielded the TM6 and euAP3 lineages; for short termed “euB” in **Fig. 2**), is proposed to have occurred

<sup>6</sup> CRABS CLAW belonging to the so-called YABBY gene family encodes a putative transcription factor (Bowman and Smyth 1999).

<sup>7</sup> In Brassicaceae, the nectaries (various in shape) are receptacular, i.e. arise from the floral axis. Their position can be described as basal to the filaments or as around the filament bases (see, e.g., Appel and Al-Shebaz 2003). In Cleomaceae as well the receptacle differentiates the nectaries (see Leins and Metzner 1979; Kers 2003). Hence, it should be avoided to associate the nectaries of these families with the stamens and this implying that they are formed by the latter (Lee *et al.* 2005a, 2005b). Inexact formulations may lead to misunderstandings and may cause some troubles since the site and histology of nectary tissue can differ and thus may contribute to our understanding of systematic relationships (see, e.g. Bernaldo 2007; Erbar and Leins 2010).

just prior to the diversification of the core eudicots (Kramer *et al.* 1998; Kramer and Irish 2000; Kim *et al.* 2004). This may have contributed to the canalization of the core eudicot flower structure and the petal-specific function of the B genes (Zahn *et al.* 2005a; Kramer *et al.* 2006; Hileman and Irish 2009). In general, the paleo-lineages are expressed in the stamen primordia, but show only little (or no) expression in the perianth organs. Thus the ancestral function of paleoAP3 and PI genes was likely restricted to specifying male reproductive development (Kramer and Irish 2000; Irish 2003). In other words, the stamen identity program was established before the radiation of the angiosperms, whereas the petal identity program remains at first plastic and becomes fixed only along the lineage leading to the higher eudicots (Kramer and Irish 2000). Ancestry of B gene function in specifying male organ identity is also corroborated by studies in gymnosperms (e.g., Winter *et al.* 1999).

As already mentioned, the MADS-box genes have undergone a significant amount of gene duplication in plants, and it is this increase in numbers and diversification of MADS-box genes, as well as the recruitment of these genes to new roles, that are likely to have contributed to the evolution of new plant morphologies. Some functions of the MADS-box genes also are found in the gymnosperms. Homologues of the floral homeotic genes of classes B, C and D are present in diverse gymnosperms (Münster *et al.* 1997; Winter *et al.* 1999; Theissen 2001; Theissen *et al.* 2002; Jager *et al.* 2003). Since B homologues are expressed in male reproductive organs only, we can assume that the ancestral function of B genes was to distinguish male (B gene expression “on”) and female (B gene expression “off”) reproductive organs (Theissen *et al.* 2000; Theissen 2001; Theissen and Becker 2004; Theissen 2005; alternative theories reviewed in Erbar 2007; see also Scutt *et al.* 2006; Soltis *et al.* 2007b; Melzer *et al.* 2010). Thus the “ABCDE system” of floral organ identity can be derived from an older but functionally related “BC/D system”, already present in the last common ancestor of all extant seed plants (Fig. 2). The B genes might encode transcription factors that control target genes required for male or female organ identity. At the molecular level, it is therefore only a relatively simple switch to create a bisexual system. But this knowledge does not help in understanding the innovation of the carpel! It is the “invention” of angiospermy and the consequences that follow the enclosure of the ovules (e.g. stigmatic tissue instead of pollination drop, improved self-incompatibility systems, possibility of pollen tube competition in stigma and style, establishing mechanisms that ensure allogamy and/or reduce autogamy, compitum in the coenocarpous gynoecium optimizing pollination events) that are crucial for the great success of the flowering plants. The enclosure of the ovules, however, seems to be triggered by the destructive behaviour of the first pollinators (e.g. Leins and Erbar 1994, 2008, 2010). But what are the underlying genetic control mechanisms?

During the angiosperm evolution, the B function must have undergone substantial changes: primary only controlling sex-determination, the B genes acquired a new, additional role in that they specify distinct petals. Whereas the paleoAP3 members play variable, not yet fixed roles in petal identity, the function was canalized at the base of the core eudicots where the euAP3 lineages clearly specify petals in the second whorl of the flowers.

Class C genes of flowering plants specify stamens and carpels, and we can speculate about the function of homologues of these floral identity genes in taxa that do not form flowers with stamens and carpels (Theissen *et al.* 2002). The ancestral function of class C/class D homologues in gymnosperms might be to distinguish between reproductive organs (expression “on” → male sporophylls and ovuliferous scales) and non-reproductive organs (expression “off”), and to specify ovules as judged from expression studies (Theissen *et al.* 2000, 2002). An ancient gene duplication event (before the radiation of extant angiosperms) might

have resulted in the fixation of two different genes: the ovule-specific D lineage and the C lineage promoting stamen and carpel identity. Possibly, diversification (sub-functionalization in the terms of genetics) between the C and D lineages decoupled megasporophyll and ovule development and facilitated evolutionary modifications of both structures (Theissen *et al.* 2000; Kramer *et al.* 2004; Theissen and Melzer 2007). In addition to the class C genes YABBY genes (see above) are involved in the carpel morphogenesis (in the model organism *Arabidopsis* controlling width and elongation of the ovary and development of carpel margins and the tissues that arise from them; their expression has been also demonstrated in basal angiosperms; Bowman and Smyth 1999; Fourquin *et al.* 2005). Further investigations will show, if these genes are also expressed in the reproductive structures of the gymnosperms as well. In any case, the results will help to understand carpel evolution (see Scutt *et al.* 2006).

Class A as well as class E genes have not been identified in any gymnosperm investigated so far and seem to have originated later than the B, C and D genes (Theissen *et al.* 2000; Theissen 2005; Zahn *et al.* 2005a). Class A genes may be derived from floral meristem identity function distinguishing floral from vegetative tissue (Theissen *et al.* 2000, 2002). The ancestral function of A genes in the specification of floral meristems may be reflected by the broad expression of A in the perianthless flower of *Chloranthus*. B function in *Chloranthus*, however, is exclusively expressed in the stamens, providing evidence that B genes have ancestral function in differentiation between male and female reproductive organs (Li *et al.* 2005). Obviously, the perianth originated later than the bisexuality of flowers (Theissen and Melzer 2007). Like the B class genes, the A class genes have undergone multiple duplication events followed by sequence divergence. Non-core eudicot species have only sequences similar to those of the core eudicots (euAP1 and euFUL; termed for short “euA” in Fig. 2). Thus at the base of the core eudicots not only the duplication event in the AP3 lineage (B class) but also a similar event may have occurred in the AP1/FUL lineage producing the euAP1 genes.

Data support an early duplication of the E genes before the diversification of the angiosperms (Zahn *et al.* 2005b). Since especially the class E genes are required for the identities of all floral organs and thus are flower-specific, these E genes seem to have a key function in the origin of bisexual flowers (Zahn *et al.* 2005b).

The oracle whether the angiosperms arose via rapid accumulation of the synapomorphies that characterize flowering plants (the carpel, double fertilization, flower) or through gradual accumulation of these traits over longer time (the “transitional-combinational theory”, Stuessy 2004) remains unanswered although the results from “evo-devo” research support the possibility of a more or less sudden flower origin. The coincidence between the origin and diversification of the class E genes, the duplication event in the class B genes, the decoupling of C and D function and the origin of the angiosperms (Fig. 2) suggests that these genes are involved in the processes that made possible the morphological invention of the flower.

## REFERENCES

- Ainsworth C, Crossley S, Buchanan-Wollaston V, Thangavelu M, Parker J (1995) Male and female flowers of the dioecious plant sorrel show different patterns of MADS box gene expression. *The Plant Cell* 7, 1583-1598
- Albert VA, Gustafsson MHG, Di Laurenzio L (1998) Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. In: Soltis PS, Soltis DE, Doyle JJ (Eds) *Molecular Systematics of Plants* (Vol II), Kluwer Academic Publishers, Boston, pp 349-374
- Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RJ (2000) Molecular and genetic analysis of the Silky1 gene reveal conservation in floral organ specification between eudicots and monocots. *Molecular Cell* 5, 569-579
- Anderson CL, Bremer K, Friis EM (2005) Dating phylogenetically basal eudicots using *rbcL* sequences and multiple fossil reference points. *American*

- Journal of Botany* **92**, 1737-1748
- Angenent GC, Colombo L** (1996) Molecular control of ovule development. *Trends in Plant Sciences* **1**, 228-232
- APG II** (Angiosperm Phylogeny Group) (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141**, 399-436
- APG III** (Angiosperm Phylogeny Group) (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**, 105-121
- Appel O, Al-Shebaz IA** (2003) Cruciferae. In: Kubitzki K, Bayer C (Eds) *The Families and Genera of Vascular Plants* (Vol V), Springer, Berlin, pp 75-174
- Becker A, 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
- Bernadello G** (2007) A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E (Eds) *Nectaries and Nectar*, Springer, Dordrecht, pp 19-128
- Bowman JL** (1997) Evolutionary conservation of angiosperm flower development at the molecular and genetic levels. *Journal of Biosciences* **22**, 515-527
- Bowman JL, Alvarez J, Weigel D, Meyerowitz EM, Smyth DR** (1993) Control of flower development in *Arabidopsis thaliana* by APETALA1 and interacting genes. *Development* **119**, 721-743
- Bowman JL, Smyth DR** (1999) CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**, 2387-2396
- Buzgo M, Soltis PS, Kim S, Soltis DE** (2005) The making of the flower. *Biologist* **52**, 149-154
- Buzgo M, Soltis PS, Soltis DE** (2004) Floral developmental morphology of *Amborella trichopoda* (Amborellaceae). *International Journal of Plant Sciences* **165**, 925-947
- Chen J-G, Willard FS, Huang J, Liang J, Chasse SA, Jones AM, Siderovski DP** (2003) A seven-transmembrane RGS protein that modulates plant cell proliferation. *Science* **310**, 1728-1731
- Coen ES** (1991) The role of homeotic genes in flower development and evolution. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 241-279
- Coen ES, Meyerowitz EM** (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* **353**, 31-37
- Colombo L, Franken J, Koetje E, van Went J, Dons HJM, Angenent GC, van Tunen AJ** (1995) The Petunia MADS box gene FBP11 determines ovule identity. *The Plant Cell* **7**, 1859-1868
- Crane PR, Herendeen PS, Friis EM** (2004) Fossils and plant phylogeny. *American Journal of Botany* **91**, 1683-1699
- Cui R, Han J, Zhao S, Su K, Wu F, Du X, Xu Q, Chong K, Theissen G, Meng Z** (2010) Functional conservation and diversification of class E floral homeotic genes in rice (*Oryza sativa*). *The Plant Journal* **61**, 767-781
- Darwin F, Seward AC** (1903) More letters from Charles Darwin: A record of his work in a series of hitherto unpublished letters (Vol 2), Appelton, New York
- Dreus GN, Bowman JL, Meyerowitz EM** (1991) Negative regulation of the *Arabidopsis* homeotic gene AGAMOUS by the APETALA2 product. *Cell* **65**, 991-1002
- Egea-Cortines M, Saedler H, 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*. *The EMBO Journal* **18**, 5370-5379
- Erbar C** (1986) Untersuchungen zur Entwicklung der spiraligen Blüte von *Stewartia pseudocamellia* (Theaceae). *Botanische Jahrbücher für Systematik* **106**, 391-407
- Erbar C** (2007) Current opinions in flower development and the evo-devo approach in plant phylogeny. *Plant Systematics and Evolution* **269**, 107-132
- Erbar C, Kusma S, Leins P** (1999) Development and interpretation of nectary organs in Ranunculaceae. *Flora* **194**, 317-332
- Erbar C, Leins P** (1985) Studien zur Organsequenz in Apiaceen-Blüten. *Botanische Jahrbücher für Systematik* **105**, 379-400
- Erbar C, Leins P** (1997a) Different patterns of floral development in whorled flowers, exemplified by Apiaceae and Brassicaceae. *International Journal of Plant Sciences* **158** (6 Suppl.), S49-S64
- Erbar C, Leins P** (1997b) Studies on the early floral development in Cleomoidae (Capparaceae) with emphasis on the androecial development. *Plant Systematics and Evolution* **206**, 119-132
- Erbar C, Leins P** (2010) Nectaries in Apiales and related groups. *Plant Diversity and Evolution (Botanische Jahrbücher für Systematik)* **128**, 269-295
- Ferrario S, Immink RGH, Angenent GC** (2004) Conservation and diversity in flower land. *Current Opinion in Plant Biology* **7**, 84-91
- Ferrario S, Immink RGH, Shchennikova A, Busscher-Lange J, Angenent GC** (2003) The MADS box gene FBP2 is required for SEPALLATA function in *Petunia*. *The Plant Cell* **15**, 914-925
- Fisel K J, Weberling F** (1990) Untersuchungen zur Morphologie und Ontogenie der Blüten von *Tovaria pendula* Ruiz and Pavón und *Tovaria diffusa* (Macfad.) Fawcett and Rendle (Tovariaceae). *Botanische Jahrbücher für Systematik* **111**, 365-387
- Fornara F, Marziani G, Mizzi L, Kater M, Colombo L** (2003) MADS-box genes controlling flower development in rice. *Plant Biology* **5**, 16-22
- Fourquin C, Vinauger-M, Fogliani B, Dumas C, Scutt CP** (2005) Evidence that CRABS CLAW and TOUNLED have conserved their roles in carpel development since the ancestor of the extant angiosperms. *Proceedings of the National Academy of Sciences USA* **102**, 4649-4654
- Friedman WE** (2009) The meaning of Darwin's "abominable mystery". *American Journal of Botany* **96**, 5-21
- Friis EM, Doyle JA, Endress PK, Leng Q** (2003) *Archaeofructus* - angiosperm precursor or specialized early angiosperm? *Trends in Plant Sciences* **8**, 369-373
- Friis EM, Pedersen KR, Crane PR** (2005) When earth started blooming: insights from the fossil record. *Current Opinion in Plant Biology* **8**, 5-12
- Frohlich MW, Chase MW** (2007) After a dozen years of progress the origin of angiosperms is still a great mystery. *Nature* **450**, 1184-1189
- Galle P** (1977) Untersuchungen zur Blütenentwicklung der Polygonaceen. *Botanische Jahrbücher für Systematik* **98**, 449-489
- Gonzales F, Stevenson DW** (2000a) Perianth development and systematics of *Aristolochia*. *Flora* **195**, 370-391
- Gonzales F, Stevenson DW** (2000b) Gynostemium development in *Aristolochia* (Aristolochiaceae). *Botanische Jahrbücher für Systematik* **122**, 249-291
- Hall JC, Iltis HH, Sytsma KJ** (2004) Molecular phylogenetics of core Brassicales, placement of orphan genera *Emblingia*, *Forchhammeria*, *Tirania*, and character evolution. *Systematic Botany* **29**, 654-669
- Hall JC, Sytsma KJ, Iltis HH** (2002) Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *American Journal of Botany* **89**, 1826-1842
- Hileman LC, Irish VF** (2009) More is better: The use of developmental genetic data to reconstruct perianth evolution. *American Journal of Botany* **96**, 83-95
- Honma T, Goto K** (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* **409**, 525-529
- Hsu H-F, Yang C-H** (2002) An orchid (*Oncidium* Gower Ramsey) AP3-like MADS gene regulates floral formation and initiation. *Plant and Cell Physiology* **43**, 1198-1209
- Hufford L** (1996) Developmental morphology of female flowers of *Gyrostemon* and *Tersonia* and floral evolution among Gyrostemonaceae. *American Journal of Botany* **83**, 1471-1487
- Irish VF** (2003) The evolution of floral homeotic gene function. *BioEssays* **25**, 637-646
- Jager M, Hassanin A, Manuel M, Guyader HL, Deutsch J** (2003) MADS-box genes in *Ginkgo biloba* and the evolution of the AGAMOUS family. *Molecular Biology and Evolution* **20**, 842-854
- Jaramillo MA, Kramer EM** (2004) APETALA3 and PISTILLATA homologs exhibit novel expression patterns in the unique perianth of *Aristolochia* (Aristolochiaceae). *Evolution and Development* **6**, 449-458
- Kanno A, Saeki H, Kameya T, Saedler H, Theissen G** (2003) Heterotopic expression of class B floral homeotic genes supports a modified ABC model for tulip (*Tulipa gesneriana*). *Plant Molecular Biology* **52**, 831-841
- Kaufmann K, Melzer R, Theissen G** (2005) MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. *Gene* **347**, 183-198
- Kers LE** (2003) Capparaceae. In: Kubitzki K, Bayer C (Eds) *The Families and Genera of Vascular Plants* (Vol V), Springer, Berlin, pp 36-56
- Kim S, Koh J, Ma H, Hu Y, Endress PK, Hauser BA, Buzgo M, Soltis PS, Soltis DE** (2005a) Sequence and expression studies of A-, B-, and E-class MADS-box homologues in *Eupomatia* (Eupomatiaceae): support for the bracteate origin of the calyptra. *International Journal of Plant Sciences* **166**, 185-198
- Kim S, Koh J, Yoo M-J, Kong H, Hu Y, Ma H, Soltis PS, Soltis DE** (2005b) Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. *The Plant Journal* **43**, 724-744
- Kim S, Yoo M-J, Albert VA, Farris JS, Soltis PS, Soltis DE** (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**, 2102-2118
- Koch MA, Al Shebaz IA** (2009) Molecular Systematics and Evolution of "wild" crucifers (Brassicaceae or Cruciferae). In: Gupta S (ED) *Biology and Breeding of Crucifers*, London, Taylor and Francis Group, pp 1-18
- Kosuge K** (1994) Petal evolution in Ranunculaceae. *Plant Systematics and Evolution* **8** (Suppl.), 185-191
- Kramer EM, Holappa L, Gould B, Jaramillo MA, Setnikov D, Santiago PM** (2007) Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot *Aquilegia*. *The Plant Cell* **19**, 750-766
- Kramer EM, di Stilio VS, Schlüter PM** (2003) Complex patterns of gene duplication in the APETALA3 and PISTILLATA lineages of the Ranunculaceae. *International Journal of Plant Sciences* **164**, 1-11
- Kramer EM, Dorit RL, Irish VF** (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 EM, Irish VF** (1999) Evolution of genetic mechanisms controlling petal development. *Nature* **399**, 144-148
- Kramer EM, Irish VF** (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** (Suppl.), S29-S40
- Kramer EM, Jaramillo MA** (2005) Genetic basis for innovations in floral organ identity. *Journal of Experimental Zoology (Molecular and Developmental Evolution)* **304B**, 526-535
- Kramer EM, Jaramillo MA, di Stilio V** (2004) Patterns of gene duplication and functional evolution during the diversification of the AGAMOUS subfamily of MADS box genes in angiosperms. *Genetics* **166**, 1011-1023
- Kramer EM, Su H-J, Wu C-C, Hu J-M** (2006) A simplified explanation for the frameshift mutation that created a novel C-terminal motif in the APETALA3 gene lineage. *BMC Evolutionary Biology* **6**, 30-47
- Lee J-Y, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL** (2005) Activation of CRABS CLAW in the nectaries and carpels of *Arabidopsis*. *The Plant Cell* **17**, 25-36
- Lee J-Y, Baum SF, Oh S-H, Jiang C-Z, Chen J-C, Bowman JL** (2005) Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* **132**, 5021-5032
- Leins P, Erbar C** (1985) Ein Beitrag zur Blütenentwicklung der Aristolochiaceae, einer Vermittlergruppe zu den Monokotylen. *Botanische Jahrbücher für Systematik* **107**, 343-368
- Leins P, Erbar C** (1991) Fascicled androecia in Dilleniidae and some remarks on the *Garcinia* androecium. *Botanica Acta* **104**, 336-344
- Leins P, Erbar C** (1994) Flowers in Magnoliidae and the origin of flowers in other subclasses of the angiosperms. II. The relationships between flowers of Magnoliidae, Dilleniidae and Caryophyllidae. *Plant Systematics and Evolution* **8** (Suppl.), 209-218
- Leins P, Erbar C** (1995) Das frühe Differenzierungsmuster in den Blüten von *Saruma henryi* Oliv. (Aristolochiaceae). *Botanische Jahrbücher für Systematik* **117**, 365-376
- Leins P, Erbar C** (2004) Floral organ sequences in Apiales (Apiaceae, Araliaceae, Pittosporaceae). *South African Journal of Botany* **70**, 469-475
- Leins P, Erbar C** (2008) Blüte und Frucht. Aspekte der Morphologie, Entwicklungsgeschichte, Phylogenie, Funktion und Ökologie. 2. Aufl. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart
- Leins P, Erbar C** (2010) *Flower and Fruit. Morphology, Ontogeny, Phylogeny, Function and Ecology*, Schweizerbart Science Publisher, Stuttgart, 439 pp (English version of German text)
- Leins P, Metzner G** (1979) Leins, P. & Metzner, G. 1979: Entwicklungsgeschichtliche Untersuchungen an *Capparis*-Blüten. *Botanische Jahrbücher für Systematik* **100**, 542-554
- Li G-S, Meng Z, Kong H-Z, Chen Z-D, Theissen G, Lu A-M** (2005) Characterization of candidate class A, B and E floral homeotic genes from the perianthless basal angiosperm *Chloranthus spicatus* (Chloranthaceae). *Development, Genes and Evolution* **215**, 437-449
- Litt A** (2007) An evaluation of A function evidence from the APETALA1 and APETALA2 gene lineages. *International Journal of Plant Sciences* **168**, 73-79
- Litt A, Irish VF** (2003) Duplication and diversification in the APETALA1/FRUITFULL floral homeotic gene lineage: implications for the evolution of floral development. *Genetics* **165**, 821-833
- Ma H, dePamphilis C** (2000) The ABCs of floral evolution. *Cell* **101**, 5-8
- Maier AT, Stehling-Sun S, Wollmann H, Demar M, Hong RL, Haubeiß S, Weigel D, Lohmann JU** (2009) Dual roles of the bZIP transcription factor PERANTHIA in the control of floral architecture and homeotic gene expression. *Development* **136**, 1613-1620
- Melzer R, Wang Y-Q, Theissen G** (2010) The naked and the dead: The ABCs of gymnosperm reproduction and the origin of the angiosperm flower. *Seminars in Cell and Developmental Biology* **21**, 118-128
- Mizukami Y, Ma H** (1995) Separation of AG function in floral meristem determination from that in reproductive organ identity by expressing antisense AG RNA. *Plant Molecular Biology* **28**, 767-784
- Mondragón-Palomino M, Hiese L, Haerter A, Koch M, Theissen G** (2009) Positive selection and ancient duplications in the evolution of class B floral homeotic genes of orchids and grasses. *BMC Evolutionary Biology* **9**, 81
- Mondragón-Palomino M, Theissen G** (2008) MADS about the evolution of orchid flowers. *Trends in Plant Science* **13**, 51-59
- Mondragón-Palomino M, Theissen G** (2009) Why are orchid flowers so diverse? Reduction of evolutionary constraints by paralogues of class B floral homeotic genes. *Annals of Botany* **104**, 583-594
- Motte P, Saedler H, Schwarz-Sommer Z** (1998) STYLOSA and FISTULATA: regulatory components of the homeotic control of *Antirrhinum* floral organogenesis. *Development* **125**, 71-84
- Münster T, Pahnke J, di Rosa A, Kim JT, Martin W, Saedler H, 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
- Nagasawa N, Miyoshi M, Sano Y, Satoh H, Hirano H, Sakai H, Nagato Y** (2003) SUPERWOMAN1 and DROOPING LEAF genes control floral organ identity in rice. *Development* **130**, 705-718
- Nakada M, Komatsu M, Ochiai T, Ohtsu K, Nakazono M, Nishizawa NK, Nitta K, Nishiyama R, Kameya T, Kanno A** (2006) Isolation of *MaDEF* from *Muscari armeniacum* and analysis of its expression using laser microdissection. *Plant Science* **170**, 143-150
- Nakamura T, Fukuda T, Nakano M, Hasebe M, Kameya T, Kanno A** (2005) The modified ABC model explains the development of the petaloid perianth of *Agapanthus praecox* ssp. *orientalis* (Agapanthaceae). *Plant Molecular Biology* **58**, 435-445
- Nigrelli L** (2008) Zeitliche und räumliche Entwicklung des Androeceums in der Familie Brassicaceae BURN. (Cruciferae JUSS.) Diploma thesis, University of Heidelberg, 91 pp
- Park J-H, Ishikawa Y, Kanno A, Kameya T** (2003) Expression of AODEF, a B-functional MADS-box gene, in stamens and inner tepals of the dioecious species *Asparagus officinalis* L. *Plant Molecular Biology* **51**, 867-875
- Park J-H, Ishikawa Y, Ochiai T, Kanno A, Kameya T** (2004) Two GLO-BOSA-like genes are expressed in second and third whorls of homochlamydeous flowers in *Asparagus officinalis* L. *Plant and Cell Physiology* **45**, 325-332
- Payer J-B** (1857) *Traité d'organogénie comparée de la fleur*, Masson, Paris
- Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF** (2000) B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature* **405**, 200-203
- Preston JC, Kellogg EA** (2006) Reconstructing the evolutionary history of paralogous APETALA1/FRUITFULL-like genes in grasses (Poaceae). *Genetics* **174**, 421-437
- Prunet N, Morel P, Thierry A-M, Eshed Y, Bowman JL, Negrutiu I, Trehin C** (2008) REBELOTE, SQUINT, and ULTRAPETALA1 function redundantly in the temporal regulation of floral meristem termination in *Arabidopsis thaliana*. *The Plant Cell* **20**, 901-919
- Rasmussen DA, Kramer EM, Zimmer EA** (2009) One size fits all? Molecular evidence for a commonly inherited petal identity program in Ranunculales. *American Journal of Botany* **96**, 96-109
- Running MP, Meyerowitz EM** (1996) Mutations in the PERANTHIA gene of *Arabidopsis* specially alter floral organ number and initiation pattern. *Development* **122**, 1261-1269
- Ronse Decraene LP** (2002) Floral development and anatomy of *Pentadiplandra* (Pentadiplandraceae): a key genus in the identification of floral morphological trends in the core Brassicales. *Canadian Journal of Botany* **80**, 443-459
- Sanderson MJ, Thorne JL, Wikström N, Bremer K** (2004) Molecular evidence on plant divergence times. *American Journal of Botany* **91**, 1656-1665
- Sattler R** (1973) *Organogenesis in Flowers*, Toronto, University of Toronto Press, 207 pp
- Schuster J** (1910) Über die Morphologie der Grasblüte. *Flora* **100**, 213-266
- Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H** (1990) Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science* **250**, 931-936
- Scutt CP, Vinauger-Douard M, Fourquin C, Finet C, Dumas C** (2006) An evolutionary perspective on the regulation of carpel development. *Journal of Experimental Botany* **57**, 2143-2152
- Sobick U** (1983) Blütenentwicklungsgeschichtliche Untersuchungen an Resedaceen unter besonderer Berücksichtigung von Androeceum und Gynoeceum. *Botanische Jahrbücher für Systematik* **104**, 203-248
- Soltis DE, Chanderbali AS, Kim S, Buzgo M, Soltis PS** (2007a) The ABC model and its applicability to basal angiosperms. *Annals of Botany* **100**, 155-163
- Soltis DE, Ma H, Frohlich MW, Soltis PS, Albert VA, Oppenheimer DG, Altman NS, dePamphilis C, Leebens-Mack J** (2007b) The floral genome: an evolutionary history of gene duplication and shifting patterns of gene expression. *Trends in Plant Science* **12**, 358-365
- Soltis DE, Soltis PS, Albert VA, Oppenheimer DG, dePamphilis CW, Ma H, Frohlich MW, Theissen G** (2002) Missing links: the genetic architecture of flower and floral diversification. *Trends in Plant Science* **7**, 22-31
- Stellari GM, Jaramillo MA, Kramer EM** (2004) Evolution of the APETALA3 and PISTILLATA lineages of MADS-box-containing genes in the basal angiosperms. *Molecular Biology and Evolution* **21**, 506-519
- Stevens PF** (2001 onwards): Angiosperm Phylogeny Website. Version 9, June 2008. Available online: <http://www.mobot.org/MOBOT/research/APweb/>
- Stuessy TF** (2004) A transitional-combinational theory for the origin of angiosperms. *Taxon* **53**, 3-16
- Sun G, Ji Q, Dilcher DL, Zheng S, Nixon KC, Wang X** (2002) Archaeofrutaceae, a new basal angiosperm family. *Science* **296**, 899-904
- Theissen G** (2001) Development of floral organ identity: stories from the MADS house. *Current Opinion in Plant Biology* **4**, 75-85
- Theissen G** (2005) On the origin of flowers: molecular views. *Nova Acta Leopoldina NF 92* **342**, 209-219
- Theissen G, Becker A** (2004) Gymnosperm orthologues of class B floral homeotic genes and their impact on understanding flower origin. *Critical Reviews in Plant Sciences* **23**, 129-148
- Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Münster T, Winter K-U, 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, 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, Bateman R, Hawkins J (Eds) *Developmental Genetics and Plant Evolution*, Taylor and Francis, London, pp 173-205

- Theissen G, Melzer R** (2007) Molecular mechanisms underlying origin and diversification of the angiosperm flower. *Annals of Botany* **100**, 603-619
- Theissen G, Saedler H** (2001) Floral quartets. *Nature* **409**, 469-471
- Tucker SC, Hodges SA** (2005) Floral ontogeny of *Aquilegia*, *Semiaquilegia*, and *Enemion* (Ranunculaceae). *International Journal of Plant Sciences* **166**, 557-574
- Tzeng T-Y, Yang C-H** (2001) A MADS box gene from lily (*Lilium longiflorum*) is sufficient to generate dominant negative mutation by interacting with PISTILLATA (PI) in *Arabidopsis thaliana*. *Plant Cell Physiology* **42**, 1156-1168
- Whipple CJ, Zanis MJ, Kellogg EA, Schmidt RJ** (2007) Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. *Proceedings of the National Academy of Sciences USA* **104**, 1081-1096
- Winter K-U, Becker A, Münster T, Kim JT, Saedler H, Theissen G** (1999) MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. *Proceedings of the National Academy of Sciences USA* **96**, 7341-7347
- Xing S, Lauri A, Zachgo S** (2006) Redox regulation and flower development: a novel function for glutaredoxins. *Plant Biology* **8**, 547-555
- Xu Y, Teo LL, Zhou J, Kumar PP, Yu H** (2006) Floral organ identity genes in the orchid *Dendrobium crumenatum*. *Plant Journal* **46**, 54-68
- Zahn LM, Kong H, Leebens-Mack JH, Kim S, Soltis PS, Landherr LL, Soltis DE, dePamphilis CW, Ma H** (2005a) The evolution of the SEPALLATA subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. *Genetics* **169**, 2209-2223
- Zahn LM, Leebens-Mack J, dePamphilis CW, Ma H, Theissen G** (2005b) To B or Not to B a flower: The role of DEFICIENS and GLOBOSA orthologs in the evolution of the angiosperms. *Journal of Heredity* **96**, 225-240
- Zanis MJ** (2007) Grass spikelet genetics and duplicate gene comparisons. *International Journal of Plant Sciences* **168**, 93-110