

**PLENARNA PREDAVANJA**

**PLENARY LECTURES**

# The flowering of the angiosperms: insights from developmental genetics and genomics into the origin and diversification of the flowering plants

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## Abstract

In a now famous quotation, Darwin referred to the origin of the angiosperms as “an abominable mystery.” Through research in diverse disciplines, including paleobotany, phylogenetics, and classical developmental biology, enormous progress has been made in the past decade in elucidating the origin and early diversification of the angiosperms. Furthermore, in just the past several years, additional major contributions have been provided by genomics approaches and evolutionary developmental genetics (evo-devo). We summarize these recent contributions to the angiosperm paradigm. Evidence from genes that control floral initiation and development coupled with recent genomics data suggest that several rounds of genome-wide duplication played a major role in floral, as well as angiosperm evolution: one such event may have occurred just prior to the origin of the angiosperms or early in their history; a second event may have coincided with the origin of the core eudicots.

Recent research has demonstrated that the pattern of expression of floral organ identity genes is broader in basal angiosperms than in well-known eudicot models, such as *Arabidopsis* and *Antirrhinum*. The ABC model of floral organ identity explains the regular, sequential development of sepals, petals, stamens, and carpels in eudicot flowers. This general model, based on studies of the derived eudicots *Arabidopsis* and *Antirrhinum*, may apply to nearly all eudicots, most of which are characterized by discrete whorls of floral organs. However, floral morphology of basal angiosperms is typically characterized by variable numbers of floral parts and gradual transitions among floral organs, and it is unclear that the ABC model applies to such flowers. For example, B-class genes, which are restricted to stamens and petals in eudicots, are expressed throughout the perianth, as well as in stamens and carpels, of many basal angiosperms. This broader pattern of gene expression is ancestral. Expression data for homologs of ABC genes for basal angiosperms indicate that the ABC model represents an evolutionarily derived regulatory network that arose through spatial restriction of regulatory gene expression. Comparative genomic data also indicate that early angiosperms possessed a diverse tool kit of floral genes; many of the gene families shown to control floral development in *Arabidopsis* were already present in basal angiosperm lineages.

## 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. Recent progress in understanding angiosperm phylogeny (reviewed in Soltis and Soltis 2004) provides a solid framework for evaluating evolutionary innovation, and identifies the taxa that provide the best insights into key innovations. 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.

Basal angiosperms as identified in recent phylogenetic analyses, which include Amborella, Nymphaeaceae, and Austrobaileya (the successive sisters to all other extant flowering plants), as well as the large magnoliid clade, exhibit considerable diversity in the type, arrangement, and number of floral parts (Endress, 1994, 2001). Understanding the genetics of floral development in basal

angiosperms in general and of novel features in particular should provide key insights into the early evolution of the flower (Soltis et al., 2002).

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). 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.

The ABC model of floral organ identity is based on studies of *Arabidopsis* and *Antirrhinum*, both of which are highly derived eudicots. Most of the genes required for the ABC functions in *Arabidopsis* and *Antirrhinum* are members of the MADS-box gene family, and their orthologs are present in all major angiosperm lineages. Although the eudicots comprise 75% of all angiosperms, most of the diversity in arrangement and number of floral parts is actually found among basal angiosperm lineages, for which little is known about the genes that control floral development.

We review evidence from genes that control floral initiation and development (including the well-know A, B, C genes), to examine the floral genetic architecture of floral organ identity in basal angiosperms and compare these results with those for well-known models in the core eudicots (*Arabidopsis* and *Antirrhinum*).

## **Materials and methods**

We investigated a suite of basal angiosperms, including *Amborella* (Amborellaceae), *Nuphar* and *Nymphaea* (Nymphaeaceae), *Magnolia* and *Liriodendron* (Magnoliaceae), *Persea* (Lauraceae). We used two general methods to isolate and characterize floral organ identity genes. The first method used ESTs obtained as part of the Floral Genome Project (Soltis et al., 2002; Albert et al., 2005; <http://fgp.bio.psu.edu/cgi-bin/fgpmine/index.cgi>). The second method involved isolating RNA from floral buds and developing flowers, making cDNA, and using RT-PCR following the general methods of Kim et al. (2004, 2005). To verify the subfamily identities of newly isolated genes from basal angiosperms and to address their orthology to previously reported genes, we added our sequences to a large data set of sequences representing all subfamilies of MIKCC-type MADS genes (Becker and Theissen 2003). Amino acid and nucleotide alignment was conducted and then the data analyzed using maximum parsimony (MP).

To quantify levels of gene expression, we used Relative-Quantitative-Reverse Transcriptase Polymerase Chain Reaction (R-Q-RT PCR) and real-time PCR. Methods followed those used previously (Kim et al., 2005).

## **Results**

Our results from multiple methods (relative-quantitative-RT PCR, real-time PCR, and RNA in situ hybridization) revealed that expression patterns of floral MADS-box genes in basal angiosperms are broader than those found in eudicots and monocots. In particular, 1) AP1 homologs are generally expressed in all floral organs and leaves, 2) AP3/PI homologs are generally expressed in all floral organs, and 3) AG homologs are expressed in stamens and carpels of most basal angiosperms, in agreement with the expectations of the ABC model; however, an AG homolog is also expressed in tepals of *Illicium*. The broader range of strong expression of AP3/PI homologs is inferred to be the ancestral pattern for all angiosperms and is also consistent with the gradual morphological intergradations often observed between adjacent floral organs in basal angiosperms.

## Discussion

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. 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. 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). In *Amborella*, which has a spirally arranged perianth with parts that are not differentiated into sepals and petals, a similar pattern is observed, with B class genes expressed throughout the perianth, as well as in the stamens. 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. 2004, 2005). 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). 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 staminodes (Kim et al., in prep.). 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 (Kim et al., 2005; Soltis et al., 2005) following the original 'BC model' idea put forth by Schwarz-Sommer et al. (1990; see also Albert et al. 1998). 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.

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. Other important changes in floral genes similarly appear to coincide with the origin of core eudicots, including duplication of *AP3* yielding the euAP3 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. 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).

## 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., Soltis, D.E., Carlson, J.E., Farmerie, W.G., Wall, P.K., Ilut, D.C., Solow, T.M., Mueller, L.A., Landherr, L.L., Hu, Y., Buzgo, M., Kim, S., Yoo, M.-J., Frohlich, M.W., Perl-Treves, R., Schlarbaum, S. E., Bliss, B.J., Zhang, X., Tanksley, S., Oppenheimer, D.G., Soltis, P.S., Ma, H., dePamphilis, C.W., and Leebens-Mack, J.H. Floral gene resources from basal angiosperms for comparative genomics research. *BMC Plant Biology* 5: 1-15.
- 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.
- Coen, E.S. and Meyerowitz, E.M. (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353: 31–37.
- 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.
- Endress, P.K. (1994) 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.
- 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.
- Kim, S., H. Ma, M. Buzgo, P. Soltis, D. Soltis. 2005. Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. *Plant Journal* 43: 724-744.
- 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: evolution and functional implications of a 260-million-year-old duplication. *Amer. J. Bot.* 91: 2102-2118.
- 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.
- 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.
- 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.
- Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P.J., Hansen, R., Tetens, F., Lonngig, W.E., Saedler, H., and Sommer, H. (1992) Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *EMBO J.* 11: 251-263.
- 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., V. A. Albert, S. Kim, M.-J. Yoo, P. S. Soltis, M. W. Frohlich, J. Leebens-Mack, H. Kong, K. Wall, H. Ma, C. dePamphilis. 2005. Evolution of the Flower. In R. Henry (ed.) *Diversity and Evolution of Plants*, pp 165-200. CABI Publishers.
- Soltis, P. S. and D. E. Soltis. (2004) Phylogeny and evolution of the angiosperms. *American Journal of Botany* 91: 1614-1626.
- 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.
- Zhao, D., Ni, W., Feng, B., Han, T., Petrusek, M.G. and Ma, H. (2003) Members of the *Arabidopsis-SKPI*-like gene family exhibit a variety of expression patterns and may play diverse roles in *Arabidopsis*. *Plant Physiology* 133: 203–217.

## The green roof of the globe in a changing atmosphere

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Elevated CO<sub>2</sub> can affect plant photosynthesis and thus the biosphere directly. Given that close to 90 % of the planet's biomass is tied up in trees, it is key to know how this large life carbon pool will respond to rising CO<sub>2</sub>. For practical reasons the scientific exploration of this aspect of global change in large remained limited to experimental approaches using young, vigorously growing test systems. In this lecture, I will focus on growth, biomass responses and water relations of older trees. I will demonstrate the overarching significance of developmental processes and biodiversity. Elevated CO<sub>2</sub> is likely to enhance forest dynamics, rather than increase carbon storage. Forests carbon storage does and will depend on the size of land area they cover and on their successional stage. The slower carbon turns over and the closer forests are to a steady state carbon balance, the greater the amount of carbon they store. Thus, a global, carbon storage oriented policy will care for the protection of old growth forests, with pulp and fiber largely produced in fast rotation plantations. Their productivity can profit from elevated CO<sub>2</sub>, provided soil nutrients permit. Science 309:1360-1362.

The lecture will first re-assess the principles of the CO<sub>2</sub> response of plant photosynthesis and its translation into growth (Fig. 1). The question whether a growth response translates into a carbon stocking response (C sequestration) will be discussed by referring to principles of forest dynamics (Fig. 2; see also Körner 2003a,b). The lecture will present various cases which evidence the significance of species identity and abundance for CO<sub>2</sub> effects at ecosystem scale.

## The fate of carbon in plants

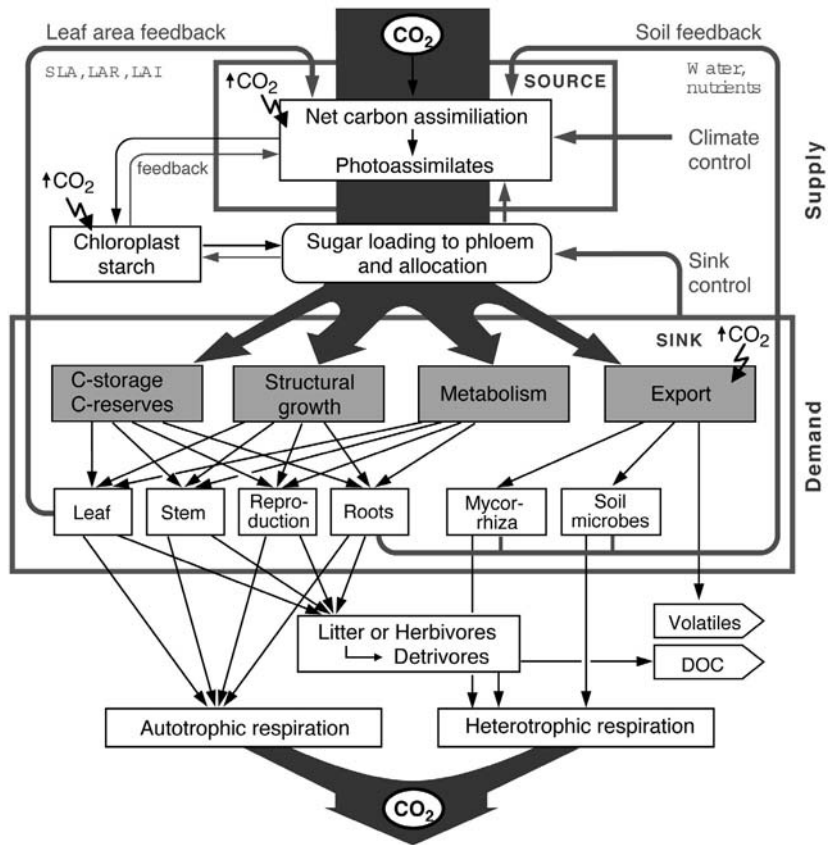


Figure 1: The fate of carbon in plants. A schematic presentation of uptake, allocation and export of carbon, with examples of feedback responses. (With permission from Blackwell Publishers, from Körner, 2003c).

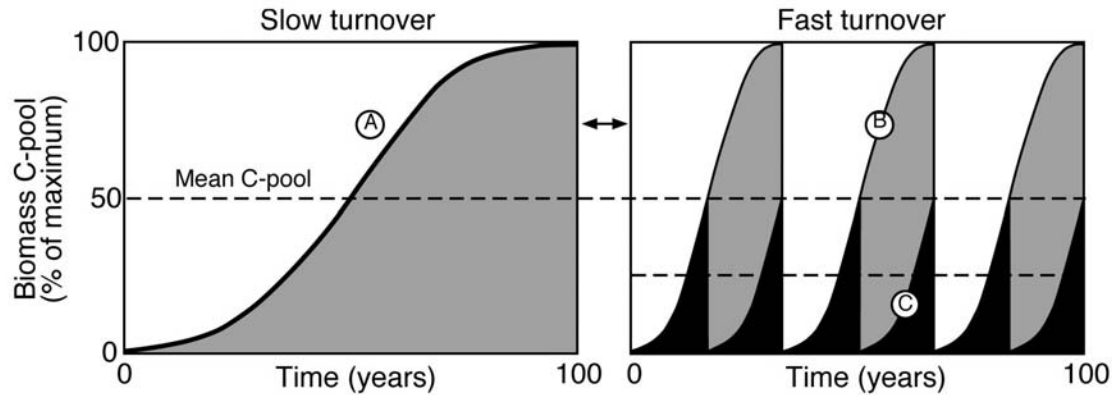


Figure 2: Idealized growth curves of trees growing at slow (a), three and six times as fast (b and c) rates. In a and b, trees grow to equal individual mass, in c trees are harvested in accordance to economic maximum yield scenarios (rapid rotation plantation). The horizontal dashed line indicates the mean biomass storage over the whole life cycle, which is not different in a and b, but lower in c. These single tree growth curves also apply to equal aged stands. In a commercial forest landscape, all tree/stand age classes ideally would cover equal fractions of land area (sustainable forestry). In a pristine natural forest all age classes may be randomly mixed or occur patch-wise, depending on disturbance regimes.

## References

- Körner Ch. (2003a). *Journal of Ecology* 91, 4-17.  
 Körner Ch. (2003b). *Science* 300, 1242-1243.  
 Körner Ch. (2003c). *New Phytologist* 159, 537-538.  
 Körner Ch., Asshoff R., Bignucolo O., Hättenschwiler S., Keel S.G., Pelaez-Riedl S., Pepin S., Siegwolf R.T.W., Zotz G. (2005). *Science* 309, 1360-1362

## Physical methods in elemental analysis of biological probes.

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### Abstract

Characteristic X rays have been used in elemental analysis since their discovery in 1914 (Moseley law). But their broad use in the material-science research including biology started with the introduction of the solid-state detectors in the X-ray spectroscopy. The characteristic X rays can be excited by photons, electrons, protons or heavier projectiles.

An alternative method, in particular if not only an elemental but rather an isotopic analysis of the samples is required, is mass spectroscopy. Pulsed heavy ion beams or pulsed lasers are used to evaporate and to ionize the atoms of the sample. The ions are accelerated to the same energy and by measuring the time of flight they are sorted according to their velocities. This method is suitable to analyze the surfaces in micro volumes.

For the detection of trace elements in micro volumes of solid-state as well as of biological samples the proton induced X-ray excitation and the X-ray fluorescent method are best suited. The first commercially available micro beam was the electron beam. Because of the high background of the bremsstrahlung produced by the electron beam only the major elements can be detected. In contrast to electrons proton induced X-ray spectra are almost background free and allow an excellent determination of the trace elements. Proton beams focused to ~1 micron became available in late seventies when powerful magnetic lenses able to focus 2 MeV protons were developed (Bosch et al., 1978). The micro-pixe has been successfully used in botany measuring the Cd and salt contamination in plants (Scheloske et al., 2004).

The X-ray fluorescent method has also a high potential for the detection of trace elements in micro volumes. At present, however, the lenses for the X rays are in the development phase. But the monochromatic X rays from the free electron lasers in conjunction with the X-ray lenses may eventually be used not only for the elemental analysis in micro volumes but also for determination of the molecular structure to which the trace element is bound.

### References

- Moseley law, Philos.Mag. 27:703 (1914)
- Bosch et al., Nucl.Meth.Instr. 149:665 (1978)
- Scheloske et al., Protoplasma 223:183 (2004)