

# ***Lilium longiflorum* and Molecular Floral Development: the ABCDE Model**

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## **Abstract**

Because lily (*Lilium longiflorum* Thunb.) is an important cut-flower crop, molecular characterisation of genes that are involved in flower morphology could help breeders to develop novel floral architectures in this species. The early ABC model for flower development emerged more than 10 years ago from studies with *Arabidopsis thaliana*, *Antirrhinum majus* and petunia. Since then, floral identity genes have been studied in many other species as well and the studies evolved to the so-called ABCDE model. However, the lily ABCDE genes have only been studied in the last four years. Here, we review the current status of the molecular model of flower development in model species and lily, and present two homeotic floral mutants that we have found in *Lilium* spp., one of which is a newly identified phenotype, *festiva*, never reported earlier, not even in the model species *Arabidopsis*. This phenotype shows homeotic conversion of stamens into petals while keeping the carpel identity unchanged. Further characterisation of more homeotic floral genes in lily and studies of the genetic configuration of lily mutants such as *festiva* may help to develop new tools for molecular breeding of this species.

## **INTRODUCTION**

Lily (*Lilium* spp.) is among the most traditional and beloved ornamental flowers worldwide. The genus *Lilium* comprises almost one hundred species, among which is the primary subject of our research, the species *Lilium longiflorum* (Thunb.), known as trumpet lily or Easter lily.

Lily flowers can reach up to 20 cm in length before anthesis. They are structured in concentric whorls. Six showy, petal-like organs form the first and second outermost whorls, consisting of three organs per whorl. These similar organs are denominated tepals, instead of the sepals and the petals found in the first and second whorls in model dicot species. Six stamens are displayed in two adjacent whorls (usually designated as only one whorl for the sake of simplicity), and a pistil, formed by three fused carpels, is located in the innermost whorl.

Knowing and understanding the mechanisms that coordinate the molecular interactions converting a vegetative meristem into a floral meristem and subsequently determining floral organ development is the key for manipulating floral architecture. Our studies are focussed at this moment in characterising functional lily genes that trigger floral organ development.

## **Flower Development in Model Species and the ABCDE Model**

A typical eudicotyledoneous flower is arranged in four concentric whorls with defined organs (sepals, petals, stamens and carpels from outer to innermost positions). The great variation in floral organ shapes and inflorescence architectures in plant species inspired Carl Linnaeus in the 18th century to propose his 'Systema Naturae' binomial classification.

In the same 18th century, Johann Wolfgang von Goethe, based on anatomical studies of plant development, proposed that every floral organ was in fact a modified leaf (1790). However, it was not before the early 1990s that a molecular model could be formulated to explain the conversion of leaves into floral organs. The early ABC model for flower development, conceived by Coen and Meyerowitz (1991) when studying floral mutants of *Arabidopsis* and *Antirrhinum*, is an elegant and neat model, which they poetically referred to as “the war of the whorls”.

This model was described as the expression of overlapping genetic functions in two adjacent whorls, leading to the composition of the different floral organs. When only type A genes are expressed, sepals are generated; when type A plus type B genes are expressed, petals arise; concomitant expression of type B and type C genes leads to stamen formation; and when only type C genes are expressed, carpels develop. Additionally, type A and C genes are antagonists, with the A domain in the two outermost whorls and the C domain in the two innermost whorls.

Another important aspect is that the C functional gene is also responsible for the determinacy of the flower and, when the C function is not active, in addition to the homeotic change of stamens into petals, a new flower emerges in the fourth whorl in a reiterated fashion. The model itself does not explain this characteristic but, since this function was revealed by type C mutants, the loss of determinacy of the flower was adopted as a C functional trait.

A D function was added to the early ABC model as a result of studies of homeotic MADS-box genes involved in *Petunia* ovule development (Angenent et al., 1995; Angenent and Colombo, 1996; Colombo et al., 1995), making this species another model for flower development at a genetic and molecular level (Immink et al., 1999; Immink and Angenent, 2002; Kapoor et al., 2002).

Two hundred years after the insightful proposition of Goethe stating that the ground state of every floral organ is a vegetative leaf, Bowman et al. (1991) could predict and produce a triple ABC loss-of-function mutant in which each floral organ resembled the ground state, a cauline-like leaf. Conversely, a leaf could not be transformed into a floral organ with the genetic toolkit available at that time, since the ABC genes proved to be recognisably necessary but not sufficient for this homeotic conversion.

The question could only be answered with the discovery of the ‘Sepallata’ function in *Arabidopsis*. The production of a *sepallata* (*sep*) 1, 2 and 3 triple mutant led to the ground state (sepal-like organs in each whorl) even when the ABC genes were functionally active (Pelaz et al., 2000). Moreover, the metamorphosis of leaves into petals has been proven possible now, i.e. when a *SEP* gene is ectopically expressed together with the other A and B genes (Honma and Goto, 2001; Pelaz et al., 2001). This clearly confirms the hypothesis that a petal is indeed a modified leaf. The *SEP* function was then recognised as the mysterious missing factor to the homeotic conversion of a leaf into a petal, being added to the model for flower development as the E function, designating the model as ABCDE from then on (Theissen, 2001) (Fig. 1A, B).

### **The ABCDE Model in the Liliaceae Family**

As soon as the early ABC model was proposed, van Tunen et al. (1993) postulated that, as the flowers of Liliaceae family members show indistinct organs in the first and second whorls called tepals, instead of sepals and petals of dicots, Liliaceae species could contain a distinct gene expression pattern of some genes of the model. This hypothesis regarded mainly the B function, of which expression would have expanded towards the first whorl in order to provide petal appearance also to the first whorl organs.

Lily species (*Lilium* spp.), despite their importance as ornamental flowers, had the first report about a molecular study on floral genes no earlier than the year 2000, when Theissen et al. (2000) sequenced several MADS-box genes from *L. regale* and located them phylogenetically among other genes derived from several species. As most of the homeotic transcription factors involved in the ABCDE model encompass a MADS domain, this was the beginning of the flower development history in lily at the molecular

level.

Following this first event, the molecular genetics of lily has progressed at a rapid pace. Several functional orthologues from *L. longiflorum* have been identified in the last years, including a B type (*LMADS1*; Tzeng and Yang, 2001), a C type (*LLAG1*; under investigation in our lab), a putative D type (*LMADS2*; Tzeng et al., 2002) and a putative E type gene (*LLSEP3*; also being currently investigated in our group).

Fig. 1 shows the current state of the ABCDE model for flower development in *L. longiflorum* in comparison with the most studied reference, *Arabidopsis*. It can be noticed that the A function is still elusive in lily.

Some double flowers, i.e. *agamous*-like homeotic lily mutants, are commercially available and the correlated phenotype is found in several species (Fig. 2). It is clear that it involves the C function of the ABCDE model (Roeder and Yanofsky, 2001), especially due to its coupled abnormalities (homeotic mutation and loss of floral determinacy). It would be very interesting to investigate at what level this function is misregulated. It is highly plausible that the *LLAG1* gene is involved in the process, since it shows a C function when expressed in *Arabidopsis* (Benedito et al., in prep.)

### **Heterologous Genetic Characterization in Model Species**

Despite the differences found in the ABCDE model between dicot and monocot species and the huge flower variability among the Angiospermae species, this model shows consistency throughout evolution.

As stated before, the ABCDE model was established using the model species *Arabidopsis* and *Antirrhinum*, and later, *Petunia*. In monocot species, such as rice and maize, many MADS-box genes have been studied, but the complexity involved in the floral anatomy of these species creates difficulties to set up a definitive and general ABCDE model for monocots so far.

However, despite the dicot-monocot evolutionary divergence that happened circa 120-180 million years ago (Wolfe et al., 1989), MADS-domain proteins derived from both classes are still able to cross interact in yeast in a proper fashion (Favaro et al., 2002), indicating the importance of their functions throughout evolution.

MADS-box transcription factors are involved in many aspects of plant development, including meristem identity, flowering time, flower determination, floral organ identity, pollen fertility, ovule development, fruit identity and development (Causier et al., 2002), and lateral root elongation (Zhang and Forde, 1998). Expression of MADS-box genes was also found in leaf guard cells and trichomes (Alvarez-Buylla et al., 2000). This gene family encompasses 107 members in the *Arabidopsis* genome (Parenicová et al., 2003).

The evolutionary strength of the ABCDE model for flower development facilitates functional studies of orthologous genes, allowing to use model species as heterologous systems in order to speed up the genetic characterisation. This circumvents the analysis of the species of interest that usually present significant drawbacks, such as lack of null phenotypes for the genes in study, long generation time, difficulties to generate transgenic plants and so on.

*Arabidopsis* has been by far the most used heterologous system in developmental biology. Many species have their MADS-box genes functionally characterized in *Arabidopsis* by complementing mutants or overexpressing heterologous genes, or producing dominant negative phenotypes. Examples of heterologous characterization of MADS-box genes can be found with genes derived from lily (Tzeng and Yang, 2001; Tzeng et al., 2002; Benedito et al., in prep), hyacinth (Li et al., 2002), lisianthus (Tzeng et al., 2002) and orchid (Hsu and Yang, 2002), to name just some ornamentals.

### **Lily Challenges the Floral Development Model one More Time**

After the establishment of the early ABC model as a general model for flower development, the Liliaceae family challenged the model for the first time, since its flowers do not show clear distinction among the organs in the first and second whorls.

The problem was solved, by proposing a modified ABC model for Liliaceae members, in which the B function is extended to the first whorl (van Tunen and Angenent, 1993; Theissen et al., 2000). The current modified ABCDE model for Liliaceae has shown to be suitable, at least up to a certain extent, and a genetic analysis of C and E functions is performed in our work at Plant Research International.

Surprisingly, we recently found a natural lily mutant with a complete homeotic change in only one floral organ type: each stamen turned into a tepal, whereas its pistil is completely normal (Fig. 2). We named this mutant *festiva* and there is no report of a correlated phenotype in *Arabidopsis*.

This mutant shows interesting features for both fundamental research and breeding perspectives, since insertion of this homeotic mutation would certainly be of high value in many commercial varieties of *Lilium* spp. Our group has intentions of starting to study this mutant at the molecular level in order to comprehend the genetic nature of this homeotic change.

Studies on genetic characterisation of the ABCDE functions in *Lilium* are significant in both fundamental and applied fields. Evolutionary and developmental biologists can use this information to assess the aspects of floral biology in monocots, and in the Liliaceae family particularly. On the other hand, molecular breeders can take advantage of the knowledge acquired on lily flower genetics and attempt to manipulate its floral morphology and flowering time, in order to generate novel and commercially interesting phenotypes.

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## Figures

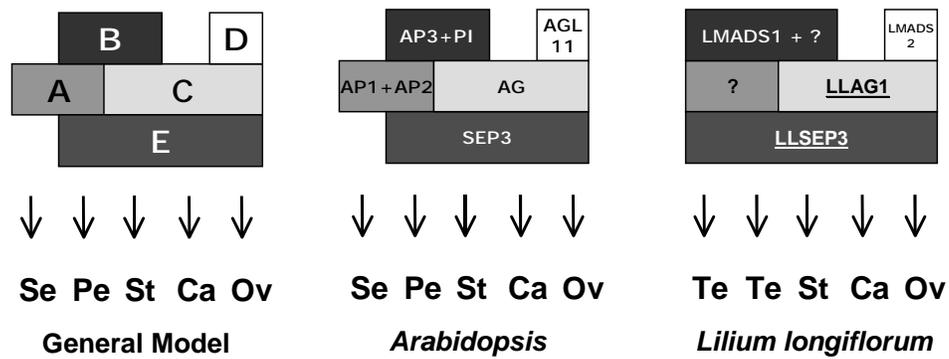


Fig. 1. The ABCDE model for flower development. The current model states that five genetic functions act in an overlapping fashion in order to trigger the development of floral organs. Genes of each function were found in the model species *Arabidopsis*. In *Lilium longiflorum*, some of the ABCDE functions have been already characterised. We are functionally characterising the C and E genetic functions in *L. longiflorum*. Se, sepals; Pe, petals; St, stamens; Ca, carpel; Ov, ovules; Te, tepals

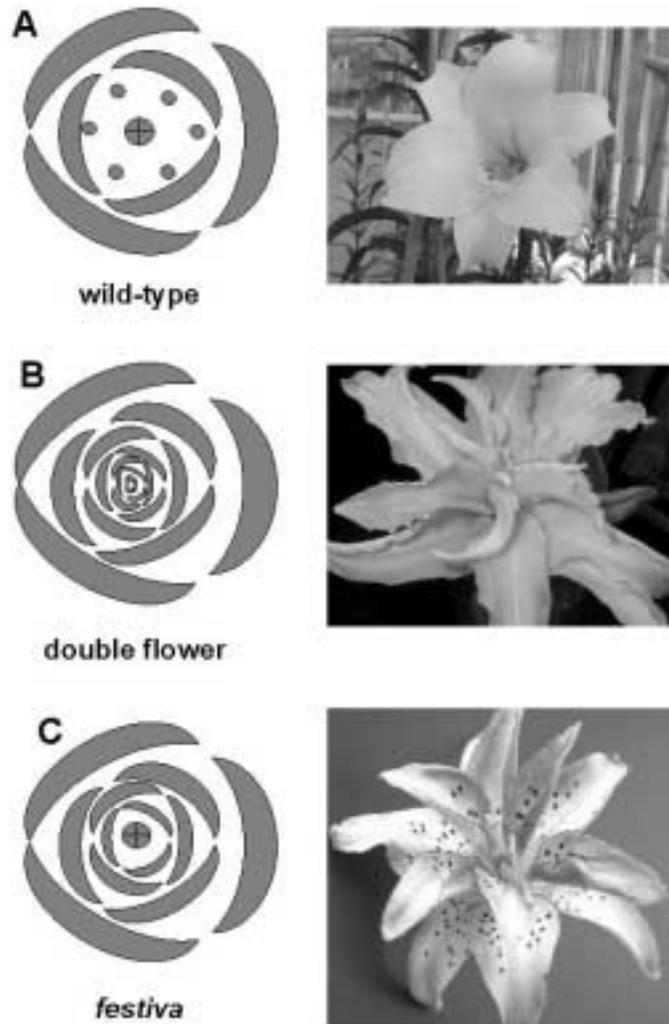


Fig. 2. Diagram representation and picture of *Lilium* floral phenotypes. (A) Wild-type flower, showing tepals in the two outermost whorls, six stamens, and a carpel in the innermost whorl. (B) Double flower, an *agamous*-like phenotype, showing a indeterminate flower with homeotic mutations of stamens into tepals and the carpel into a new flower. (C) *festiva* phenotype, with homeotic change of stamens into tepals. It possesses a normal carpel.