divisions of the apical initials. Is this an ancestral auxin-mediated mechanism or did it evolve independently? Could mechanics have perhaps been a crude ancestral mechanism that predated auxin-dependent patterning? What about phyllotactic patterns in flowers? Phyllotaxis reaches its greatest diversity in the flower, where it is obviously related to different reproductive strategies. How do regulatory mechanisms of phyllotaxis interact with the extremely well-studied floral organ identity determinants? It is not that we have run out of basic questions to be asked in model plants; instead, there is a world of diversity waiting to be explored.

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# The ABC model of floral development

Vivian Irish

Flowers are organized into concentric whorls of sepals, petals, stamens and carpels, with each of these floral organ types having a unique role in reproduction (Figure 1). Sepals enclose and protect the flower bud, while petals can be large and showy so as to attract pollinators (or people!). Stamens produce pollen grains that contain male gametes, while the carpels contain the ovules that when fertilized will produce the seeds. While the size, shape, number and elaboration of each of these organ types can be quite different, the same general organization of four floral organ types arranged in concentric whorls exists across all flowering plant (angiosperm) species. As I shall explain in this Primer, the 'ABC model' is a simple and satisfying explanation for how this conserved floral architecture is genetically specified.

What is the ABC model?
The ABC model was first explicitly articulated in 1991, in a seminal paper by Enrico Coen and Elliot Meyerowitz. Although homeotic mutations affecting floral organ identity had been known for centuries, it was the systematic analyses of these mutations, and of the phenotypes produced by double and triple mutants, that proved to be critical in developing the ABC model. Building on previous genetic analyses in two different species, *Antirrhinum majus*
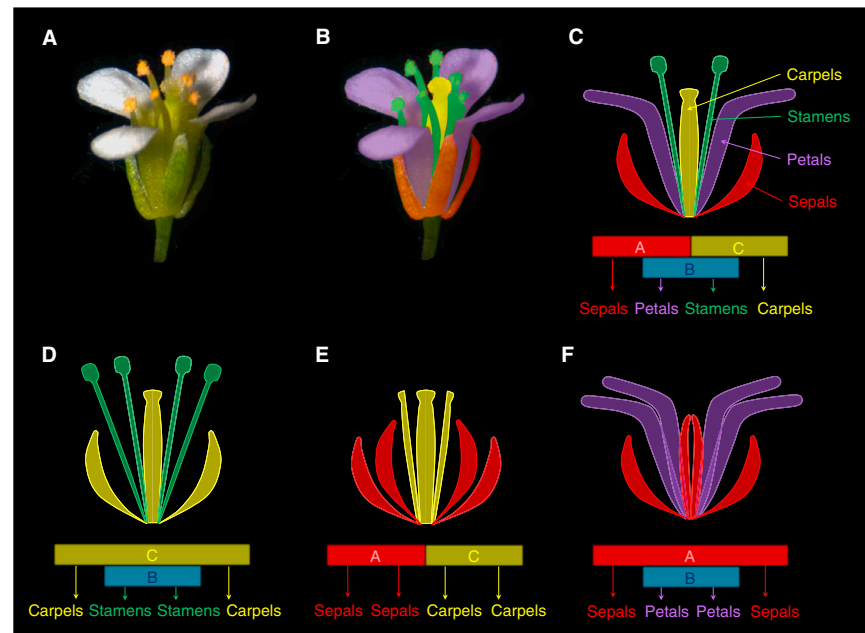
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Figure 1. The ABC model.
Wild type *Arabidopsis* flower (A), color coded in (B) to demarcate the sepals (red), petals (purple), stamens (green) and carpels (yellow). (C) A cartoon version of an *Arabidopsis* flower, with the domains of *ABC* gene function shown below. The function of *A* alone specifies sepal identity (red) in the first whorl, while a combination of *A* (red) + *B* (blue) function specifies petal identity (purple) in the second whorl. The combination of *B* (blue) + *C* (yellow) function specifies stamen identity (green) in the third whorl, while *C* function alone specifies carpels (yellow) in the fourth whorl. (D–F) How the ABC model explains different mutant phenotypes. (D) The elimination of *A* function results in flowers composed of carpels in the first whorl, stamens in the second and third whorls, and carpels in the fourth whorl; the lack of *A* function in the first two whorls results in ectopic expression of *C* function in those regions. (E) A flower lacking *B* function consists of sepals in the first and second whorls and carpels in the third and fourth whorls. (F) A flower lacking *C* function consists of sepals in the first whorl, petals in the second and third whorls, and sepals in the fourth whorl.
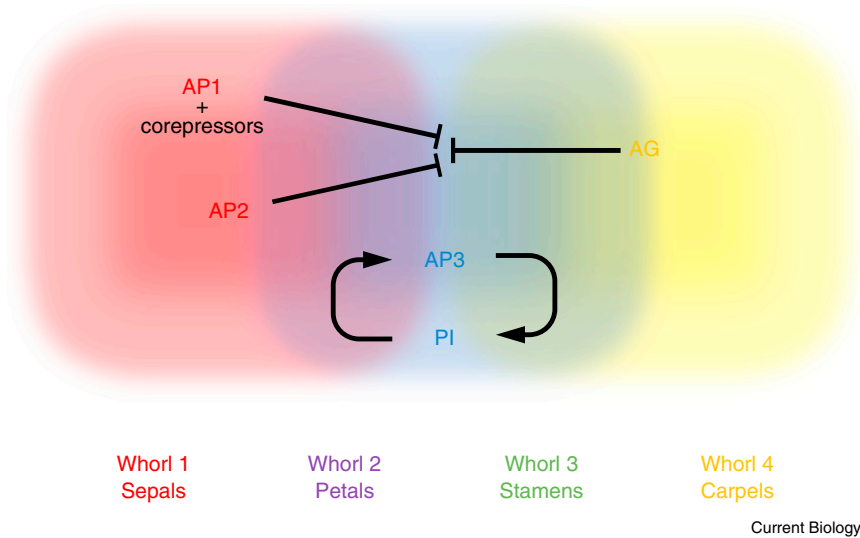
Figure 2. Domains of *ABC* gene function.
The *Arabidopsis ABC* genes function in discrete domains; *AP1* and *AP2* are *A* class genes (red), the products of which act in combination with other factors to countermand the activity of the *AG C* class gene (yellow) in the first and second whorls, while AG inhibits the function of the *A* class genes in the third and fourth whorls. *AP3* and *PI* are *B* class genes (blue) that positively regulate each other's expression in the second and third whorls.

(snapdragon) and *Arabidopsis thaliana*, Coen and Meyerowitz proposed that distinct organ identities are specified by a unique combination of homeotic 'A', 'B' and 'C' gene activities within each whorl (Figure 1). The second tenet of the model was that the A and C functions negatively regulate each other. Together, these two components of the model can explain normal organ identity in each whorl, and at the same time explain a wide variety of homeotic mutant phenotypes (Figure 1).

Because the ABC model was initially based on comparable genetic analyses and mutant phenotypes in snapdragon and *Arabidopsis*, Coen and Meyerowitz also suggested that the model could be widely applied to all flowering plant species. The basic aspects of the ABC model, along with the generalization of the model across flowering plants, have now been largely substantiated by decades of work from labs around the world. This burgeoning area of research has led to the elucidation of the molecular nature of the ABC genes, how they act, and how their functions have been conserved or have diversified.

What are the ABC genes and how do they work?
Unlike the homeotic genes in animals that encode homeodomain proteins,

most plant homeotic ABC genes encode members of the MADS-domain class of transcription factors. In *Arabidopsis*, the A function is provided by the MADS box gene *APETALA1* (*AP1*) together with *APETALA2* (*AP2*), which encodes a member of the EREBP family of transcription factors. The B function is provided by two MADS box genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), while the C function is provided by the MADS box gene *AGAMOUS* (*AG*).

The combinatorial action of the ABC proteins results in part from expression of their genes in discrete domains of the developing flower. While the initial domains of expression of these genes can be broad, their expression and function quickly become refined in response to a series of cross regulatory interactions (Figure 2). For instance, in *Arabidopsis*, *AP1* is initially expressed throughout the young flower bud, but *AP1* transcripts are rapidly constrained to the first and second whorls through the negative regulatory effects of AG protein in the third and fourth whorls. How *AG* expression is limited to the third and fourth whorls is still not entirely understood, but appears to be due in part to the action of AP2 protein.

Although *AP2* transcripts are ubiquitous throughout the developing

flower, AP2 function is limited to the first and second whorls. This restriction of AP2 function occurs somewhat differently in different flowering plant lineages, and is often dependent on post-transcriptional control mechanisms. The delimitation of AG activity to the third and fourth whorls also depends on the action of AP1 in conjunction with several co-repressor proteins that repress *AG* expression in the first two whorls. The B-class genes *AP3* and *PI* are also initially expressed in broader domains; positive cross-regulatory interactions where these domains overlap serve to rapidly refine their expression to the second and third whorls during flower development.

In whorls with expression of multiple ABC genes, their protein products physically interact to drive the processes of specifying organ identity. For instance, specification of stamen identity appears to depend on the physical interaction of AG, AP3 and PI proteins along with other MADS box proteins to regulate transcription of stamen-specific genes. These protein complexes in turn associate with a number of other cofactors to stabilize protein–DNA interactions, to facilitate cooperative binding at specific promoter regions, and to promote transcriptional activation or repression. The ABC proteins also have been shown in some cases to recruit histone modification enzymes, indicating a role for chromatin remodeling in the control of organ identity specification.

What do the ABC genes regulate?
One could imagine that the activity of the ABC genes in specific whorls could be sufficient to set in motion an ABC-independent cascade of events that would lead to organ differentiation. It is clear, though, that the ABC gene activities are necessary throughout floral development to maintain organ identity. For instance, turning off B gene activity late in floral development results in a patch of sepal tissue in an otherwise normal petal; in other words, B gene function is necessary late in development to maintain appropriate organ type-specific differentiation. This in turn suggests that over the course of flower development, the ABC proteins are responsible for turning on and

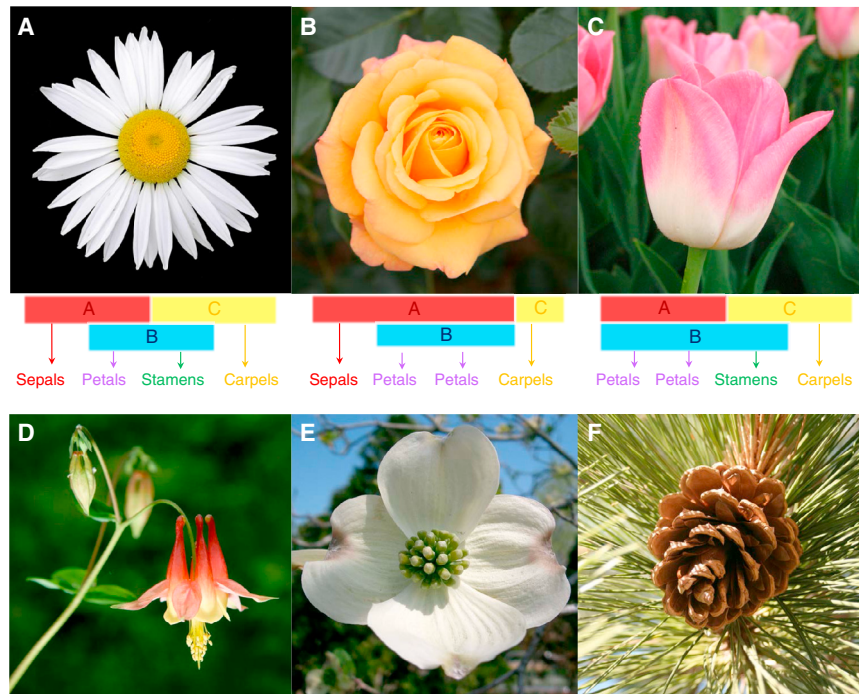turning off the expression of different panoplies of genes.

Surprisingly little is known of the genes and processes regulated by the ABC gene products. Systematic searches for targets of ABC gene activity have relied on genome-wide surveys to identify DNA sequences bound by ABC proteins. Thousands of such sites have been identified in the *Arabidopsis* genome, but comparatively few of these sites have been independently validated. Identification of transcripts whose levels are altered in the presence or absence of the ABC proteins has also yielded a number of potential candidate target genes. These targets include genes encoding other transcription factors, genes required for hormone synthesis or response, and genes necessary to modulate cell growth, cell division or cell-type differentiation.

Despite these riches, few guiding principles have emerged as to how the ABC proteins actually specify the different organ types. We do know, for instance, that the B proteins control cell division genes that act to shape the petal; that petal epidermal cells are sculpted in part by genes that are turned on in response to B gene activity; and that *Arabidopsis* petals are white because B protein function results in the downregulation of photosynthetic gene expression. To what extent, though, are the *Arabidopsis* ABC proteins regulating similar or different sets of target genes as compared, for instance, to those of snapdragon? We are still far from understanding the intricacies of these regulatory interactions, the extent to which they are conserved, and the many players involved.

What about daisies, roses or tulips?
Much of the recent experimental work has focused on *Arabidopsis* and its unprepossessing small flowers. Many flowers are quite dramatic, though, with eye-catching colors and elaborate shapes. To what extent does the ABC model apply when considering the exquisite variation seen in the flowers of the other 350,000 flowering plant species?

Daisies, and their close relatives the dandelions and sunflowers, have a compact head that is actually

Figure 3. Variations on the ABC theme.
(A) Ox-eye Daisy (*Leucanthemum vulgare)* showing the marginal ray flowers and the central disc flowers; despite the different morphologies of each flower type, the organization of each can be explained by the ABC model (illustrated below). (B) Rose (*Rosa* spp.) with multiple whorls of petals that correspond to an expansion of A + B gene activities (below). (C) Tulip (*Tulipa gesneriana*) with sepal-like organs in the first and second whorls; this can be explained by a shift in the domain of B gene function (below). (D) Columbine (*Aquilegia formosa*) flowers contain stamenodia, a novel organ type situated between the stamens and the carpels. (E) Flowering dogwood (*Cornus florida*) possesses small greenish flowers surrounded by four large, showy petaloid bracts. (F) A female pine cone (*Pinus strobus*). (All images in Figure 3 from Wikimedia commons.)

composed of many small flowers (Figure 3). The entire head gives the impression of one large flower, the better to attract insect pollinators. The outermost marginal flowers, or 'ray' flowers, are asymmetrically shaped, with one extremely enlarged petal and non-functional stamens that do not develop beyond a rudimentary stage. The inner 'disc' flowers have considerably reduced petals, but do possess functional stamens and carpels. Despite these morphological variations, ABC genes have been identified and shown to function within each tiny daisy flower in a very similar manner to their *Arabidopsis* counterparts. The differences in how the ABC program plays out in ray versus disc flowers appears to depend on the interaction of the ABC proteins with other MADS box proteins whose expression is graded across the flower

head, fine tuning the output of these developmental pathways in each flower type.

Cultivated roses have many extra whorls of petals while tulips lack obvious sepals (Figure 3). Both of these examples can be explained by simple shifts in the patterns of expression of ABC genes. Roses have been cultivated for millennia, and 'double flowered' forms with many extra petals were selected for independently in Europe and China. Both European and Chinese double flowered rose cultivars display a significant contraction in the domain of C gene expression, allowing for a much larger domain of A+B expression and concomitant extra petals. Tulips, on the other hand, display an outward shift in the expression of the B class genes, resulting in petal-like organs developing in the first whorl. The dramatic forms

displayed by many cultivated garden flowers are no doubt due to the horticultural selection of alterations in the underlying ABC program.

There are many further variations to the basic ABC model. In many lineages, gene duplications have led to multiple copies of the ABC genes. In turn, these gene duplicates can evolve so as to parse the original function or to acquire new roles. There also are instances in which the original gene copy has been lost and a derived duplicate version has taken on the role of specifying organ identity. An additional consequence of ABC gene evolution is that the changes in the resulting ABC proteins can diversify their interaction partners, resulting in new or modified protein complexes with altered affinities for target gene binding sites.

In some cases, the evolution of new functional roles for ABC gene duplicates may well explain morphological novelties observed in some species. For instance, a duplicated B gene is responsible for the development of a novel floral organ type, the stamenodium, in columbines (Figure 3). ABC gene functions can even be heterotopic; expression of B class genes imparts petalloid characteristics to the petal-like bracts of dogwoods (Figure 3). What is remarkable about the ABC model, though, is its robustness; although the ABC gene network is constantly evolving, the overall logic of the network has been retained across the vast majority of flowering plants.

What about pine trees and other non-flowering plants?

The angiosperms and the gymnosperms together comprise the seed plants. Gymnosperms, including evergreens, cycads, and gingkos, produce cones, not flowers (Figure 3). While flowers generally produce stamens and carpels on the same axis, cones are either male and produce pollen, or female and contain ovules. Gymnosperms ('naked seed') are so named because they lack the carpels typical of flowering plants. Other land plant lineages include the non-vascular plants, such as mosses, and the seedless vascular plants, such as ferns and lycophytes. To what extent does the ABC model apply to these non-flowering plant lineages?

Gymnosperms possess copies of B and C class genes. The gymnosperm C genes are expressed in male and female cones, while B gene expression is limited to male cones. Thus, specification of male and female identity appears to be conserved across angiosperms and gymnosperms. By contrast, analyses of the genomes of the moss *Physcomytrella patens*, or the lycophyte *Selaginella moellendorffii*, indicated that these species lack orthologs of any of the ABC genes. It thus appears that the ABC gene network arose coincident with the angiosperms. The angiosperms are easily the most speciose group within the plant kingdom; the foundations for their considerable success could well be due to the evolution of this robust gene network.

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Primer
Succulent plants

Howard Griffiths and Jamie Males*

The peculiar morphologies of succulent plants have been variously considered as grotesque monstrosities and exotic curiosities, but succulents have always been perceived as unique. The succulent syndrome is considered to be one of the most remarkable examples of convergent evolution across the plant kingdom. Common to all succulents is the presence of large cells for water storage. However, cellular succulence can occur in any vegetative plant organ, with the level of succulence in roots, stems, and leaves being subject to a certain degree of evolutionary coordination. Furthermore, cellular succulence scales up to morphological succulence according to various anatomical schemes that confer contrasting functional characteristics. This means that succulence is associated with a broad range of ecophysiological strategies and occurs in plants that have evolved in many different environments.

The term 'succulence' itself is generally agreed to refer to the storage of a significant amount of withdrawable water in living cells. Since it is dependent on variation in quantitative parameters, such as cell volume, cell packing, and tissue thicknesses, succulence is better considered as a continuous rather than a binary trait. Running parallel to this spectrum of succulence is a corresponding and underappreciated spectrum of ecological strategies based on contrasting water-use characteristics. Nevertheless, categorisation is sometimes necessary, and approximately 3–5% of all flowering plants are commonly described as succulents — a small but important proportion. Although some succulent lineages have undergone much greater net species diversification than others, there is no strong bias in the phylogenetic distribution of origins of succulence, suggesting that it is a relatively 'evolutionarily accessible' syndrome.

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