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The ABC model of flower development: then and now

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Summary

In 1991, we published a paper in *Development* that proposed the ABC model of flower development, an early contribution to the genetic analysis of development in plants. In this, we used a series of homeotic mutants, and double and triple mutants, to establish a predictive model of organ specification in developing flowers. This model has served as the basis for much subsequent work, especially towards understanding seed plant evolution. Here, we discuss several aspects of this story, that could be a much longer one. One surprising conclusion is that materials and methods that might have led to similar work, and to the same model, were available 100 years before our experiments, belying the belief that progress in biology necessarily comes from improvements in methods, rather than in concepts.

Flower development before the ABC model

It is straightforward to review the state of the field of genetic approaches to flower development in the late 1980s, as we published a historical review as a background to our work – like the 1991 paper (Bowman et al., 1991), this was also in *Development* (Meyerowitz et al., 1989). We pointed out that what later would be considered to be mutants with altered flower development – in types, numbers and positions of the floral organs (sepals, petals, stamens and carpels) – were known from ancient Greece, and had been studied in great detail in the 19th century, as had normal flower development, the microscopic study of which was first extensively published by Payer in 1857 (Payer, 1857). Plant genetics was of course well developed at the time, as it had been in progress since the original discoveries of Mendel in 1865. Homeotic flower variants, in which one floral organ type is replaced by another, were specifically recognized and studied in the 19th century (e.g. Masters, 1869) under the term 'metamorphy', while Bateson introduced the concept of homoeosis in 1894. A collection of homeotic mutants of snapdragon existed by the 1930s in the laboratory of Erwin Baur and co-workers (Stubbe, 1966). Thus, both the materials (at least in other species than the one we used) and methods (not scanning electron microscopy, but microscopic and detailed analysis of early flower development) used in our 1991 paper were available more than 100 years earlier, and the exact materials with which cognate experiments could have been carried out existed more than 50 years earlier. The key to our model was the use of double and triple mutants. The Baur laboratory made many mutants, but none (of which we have found a record) that involved more than a single homeotic mutant.

Our paper therefore serves as a counter-example to the notion that advances in biology derive only from advances in methodology. Our 1991 work could have been carried out a century earlier, except that the conceptual framework of biology at that time (especially the notion of a regulatory gene), was such that no one had thought to do it.

The paper

We began with a set of four homeotic mutants of *Arabidopsis* in which fairly normal floral organs were found in floral whorls where they would not be expected in wild-type flowers. The mutants were obtained from the generosity of colleagues, particularly Maarten Koornneef, then at the University of Wageningen (The Netherlands), and had already been described in detail as single and double mutants in the first issue of *The Plant Cell* (Bowman et al., 1989). We concluded that the genes function in overlapping fields that occupy two adjacent floral whorls, and that they 'act in allowing cells to recognize their position in the developing flower'.

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Subsequently, additional mutant alleles were obtained and analyzed, revealing the likely null phenotypes, and triple mutants were generated (Fig. 1; for further commentary, see Bowman, 2010). The new data were the foundation on which we built 'A simple model', now known as the ABC model of floral organ identity. The letters came from the three overlapping fields, named A (APETALA2 gene function, AP2), B (APETALA3 and PISTILLATA, AP3/PI) and C (AGAMOUS, AG). AP2 was proposed to function in whorl 1 to define sepals, and AG in whorl 4 to control carpel identity. The overlaps explained the combinatorial roles of AP2 and AP3/PI in whorl 2 that normally defined petals, and AP3/PI and AG in whorl 3 that specified stamens. A key new component, without which the model does not work and that distinguishes this model from others, was the proposal that genes acting in the A and C fields, *AP2* and *AG*, were mutually antagonistic, something shown at the molecular level beginning very shortly afterwards (Drews et al., 1991). It was

A *Development* classic

The year 2012 marks 25 years since the journal *Development* was relaunched from its predecessor, the *Journal of Embryology and Experimental Morphology* (*JEEM*). In 2008, we fully digitised our *Development* and *JEEM* archives, and made them freely available online. At the same time, we took the opportunity to revisit some of the classic papers published in *JEEM*, in a series of commentaries (see Alfred and Smith, 2008). Now, to mark a quarter century of *Development*, we have been looking through our archives at some of the most influential papers published in *Development*'s pages. In this series of Spotlight articles, we have asked the authors of those articles to tell us the back-story behind their work and how the paper has influenced the development of their field. Look out for more of these Spotlight papers in the next few issues.

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Fig. 1. The *Arabidopsis* research group at Caltech in late 1988. From left to right, Hong Ma (post-doc), Tony Bleecker (post-doc), Yi Hu (technician), John Bowman (PhD student), Usha Vijayraghavan (post-doc), Marty Yanofsky (post-doc), Elliot Meyerowitz (group leader) and David Smyth (sabbatical visitor) (absent: Sherrie Kempin, research assistant). At this time, John Bowman and David Smyth were focusing on the developmental genetics of ABC genes, and Marty Yanofsky and Hong Ma were characterizing the C function gene *AGAMOUS*.

aesthetically satisfying to be able to account for the eight floral phenotypes in terms of the model, and its attraction lay in its simplicity and predictive power. Even so, it only explained organ identity, and we listed a series of 'unexplained complications' associated with mutant phenotypes, including mosaic organs, secondary flowers, floral indeterminacy and missing organs.

We chose to send the manuscript to *Development* rather than to a plant-oriented journal on the encouragement of editor Keith Roberts, who seemed to share our holistic view that developmental principles are shared across kingdoms. And, color illustrations were free and there were then no page limits. The two reviewers were positive, with one saying 'This is a stimulating paper which should have a large impact on the field. Interest rating 9.' The other reviewer thought it 'potentially an excellent paper', but 'the writing style could be improved' and wanted it shortened by half. However, the first reviewer, and the editor, disagreed, and its 20 pages stood. We suggested a 'fruit salad' cover photo of the wild-type and all mutant phenotypes that was also accepted (Fig. 2).

Its impact

The ABC model was popularized in a review in *Nature* published later in 1991 by the senior author and Enrico Coen, whose group had been making parallel new findings of similar homeotic mutants in snapdragon (*Antirrhinum majus*) (Coen and Meyerowitz, 1991). In this review, functions were distinguished from regions (or fields) by using a, b and c for functions and A, B and C for regions, although it is now customary to capitalize the functions. The importance of comparative findings in *Antirrhinum* was highlighted by the earlier contribution of Zsuszanna Schwarz-Sommer and colleagues, who independently generated a floral organ identity model that proposed the equivalent of B and C functions, but lacked A function and was not tested using multiple mutants (Schwarz-Sommer et al., 1990).

The ABC model is still widely used as a framework for understanding floral development today (Krizek and Fletcher, 2005; Causier et al., 2010). The impact of the *Development* paper is reflected in its continued high citation rate (it still gathers over 30 citations a year according to the Web of Knowledge, http://wokinfo.com). Significant advances since 1991 include findings that: (1) all genes, except *AP2*, encode MADS transcription factors (e.g. Weigel and Meyerowitz, 1994); (2) another A function gene, *APETALA1*, exists in *Arabidopsis* (Bowman et al., 1993; Gustafson-Brown et al., 1994); (3) four other MADS genes (*SEPALLATA*s) are involved in establishing the floral nature of flower organs in *Arabidopsis* (Pelaz et al., 2000) (often called E function, although using our terminology these would be meristem identity, not organ identity, genes); (4) SEP proteins likely act in multimeric combination with A, B and C function MADS proteins (the quartet model) (e.g. Melzer and Theissen 2009); (5) AP2 transcripts are regulated post-transcriptionally by microRNAs (Aukerman and Sakai, 2003; Chen, 2004); and (6) AP2 is a direct negative regulator of AG expression, a very recent finding (Dinh et al., 2012).

The ABC model is still widely used as a framework for understanding floral development today

Perhaps owing to its relative simplicity, and to the ubiquity of the appreciation of flowers in human society, the ABC model was rapidly introduced into university textbooks, not only those focused on developmental biology (e.g. Wolpert and Tickle, 2011), but also to first year general biology textbooks (e.g. Campbell et al., 1999; Freeman, 2008), cell biology texts (e.g. Alberts et al., 1994) and those focused on genetics (e.g. Griffiths et al., 1993; Sanders and Bowman, 2012). The ABC model is even being taught to high school students in some locales (Fig. 3).

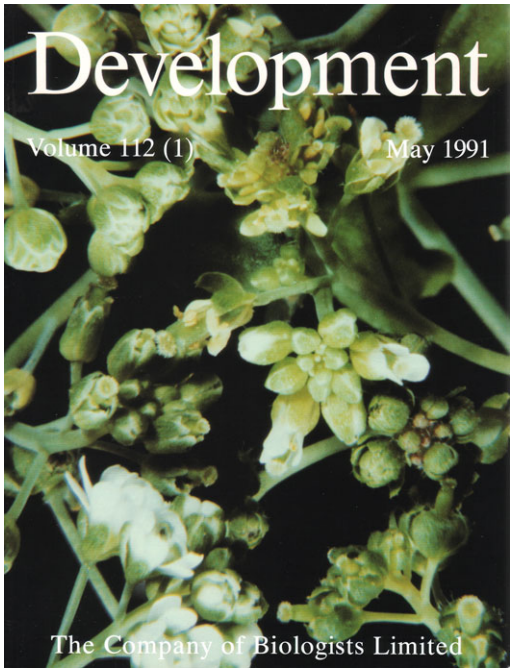

Fig. 2. The cover of the issue of *Development* in which the ABC paper appeared. The photograph depicts eight different genotypes of *Arabidopsis* flowers. Wild-type flowers are shown with three different single floral homeotic mutants (*ap3-1*, *ap2-1* and *ag-1*), and the three double mutant and the triple mutant combinations of these alleles.

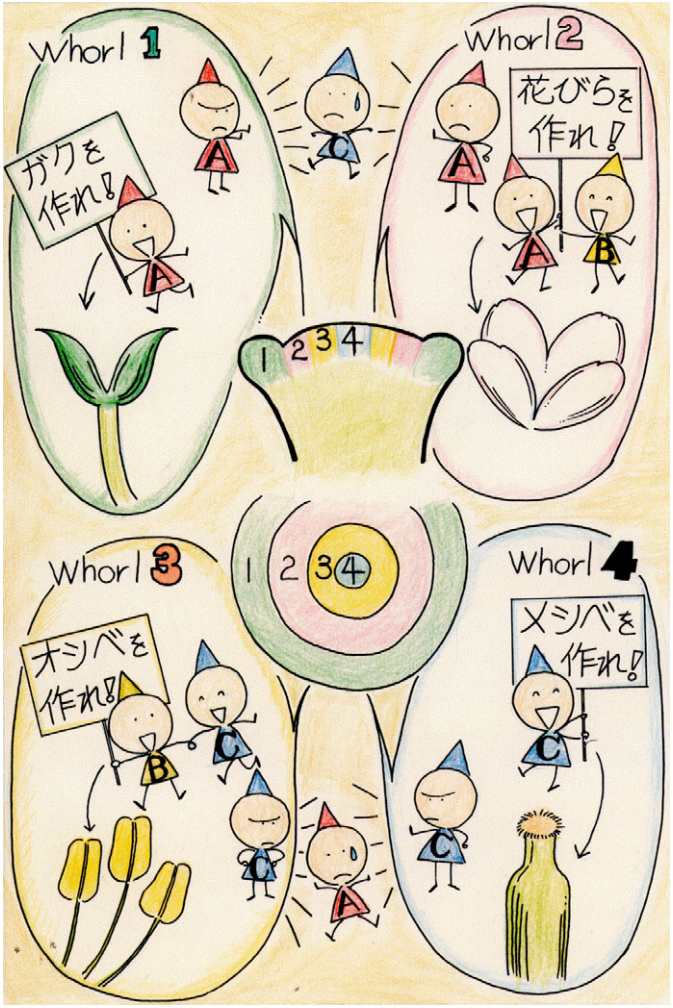

Fig. 3. Cartoon of the ABC model drawn by Ryoko Hirano and which Hiroyuki Hirano used to teach summer school for secondary school students in Japan. Note the colorful inclusion of the critical A-C mutual inhibition. Image courtesy of Hiroyuki and Ryoko Hirano (University of Tokyo).

The ABC model and flower evolution

Given the conservation of floral organ position across angiosperms, it was a natural question as ask whether the ABC model could be applied to all flowering plants – and not only to *Arabidopsis* and snapdragon, where it was developed. Its integration with new findings that were, at that time, defining the course of angiosperm evolution was catalyzed by two Keystone Symposia on evolution and plant development (early evo-devo) held at Taos (New Mexico) in 1993 and 1997. Two early tests of its general applicability were observations that showed the occurrence of B-class mutants in monocots (Ambrose et al., 2000; van Tunen et al., 1993). Such observations formed the foundation for continuing studies into the diversity of flower architecture, from orchids where the complex perianth is patterned by differential expression of multiple B-class gene paralogs (Mondragon-Palomino and Theissen, 2011), to the 'inside-out' flowers of *Lacandonia schismatica*, where central stamens are surrounded by carpels (Alvarez-Buylla et al., 2010).

The ABC model has also illuminated the evolutionary appearance of flowers, a puzzle that has occupied botanists since Charles Darwin. Both B- and C-class orthologs are found in gymnosperms, with C-class genes expressed in both male and female reproductive structures and B-class expression in male reproductive tissues (Mouradov et al., 1999; Shindo et al., 1999; Sundstrom et al., 1999; Tandre et al., 1998). Thus, the specification of stamens and carpels seems to have its origin in the common ancestor of seed plants, and the mystery of flower evolution lies in 'tinkering' (Jacob, 1977) with pre-existing genetic machinery. None of the ABC-type MADS box genes appears to exist outside angiosperms and gymnosperms, suggesting that their origin lies in extensive gene duplications of an ancestral MADS box gene in the lineage leading to seed plants (Floyd and Bowman, 2007).

In summary, althought the relatively simple ABC model we proposed in 1991 has grown in complexity over the past 21 years – particularly with increasing knowledge of its molecular mechanism – it still provides a basis for our understanding of flower development and morphology in model systems, and across evolution.

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