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My favourite flowering image

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Abstract

When Nick Battey asked me to write an essay on my favourite flowering image, I rummaged through my mind and some old photographs, and while there are several aesthetically striking images (after all, the photographs are of flowers) three candidates stood out. In each of the cases, it was the first photographic evidence of a ‘eureka’ moment, one of those rare moments of revelation that one has in their scientific career.

Key words: AGAMOUS, determinacy, flower development, homeosis.

Based on single mutant phenotypes of the Arabidopsis floral homeotic mutants, we reasoned that loss of all ABC gene activity would result in a flower in which all floral organs develop as leaves. It was late at night when I first observed the ‘ABC’ triple mutant (apetala2-1 apetala3-1 agamous-1) in early 1990, and no one else was present in Elliot Meyerowitz’s laboratory. So I ran down the hallway to show Joe Minor, my roommate during much of graduate school, and who worked on fertilization in sea urchins. While it was the phenotype that we had all expected, the reality of seeing our predictions confirmed represents one of my favourite memories from graduate school, and even excited the interest of those, such as Joe, who were working on very different aspects of biology. In addition, it was an affirmation of JW von Goethe’s statement that ‘alles ist blatt’, made two centuries previously. A photograph of this genotype was eventually published as Fig. 7A of Bowman et al. (1991). The second considered image was taken in January of 1996, and is the first photograph of a crabs claw gymnos double mutant Arabidopsis gynoecium, with ovules developing on the outside of the ovary. It is of significance to me as it presents both a ‘eureka’ moment, where we immediately understood that CRABS CLAW was involved in determining carpel polarity, and has also led, directly or indirectly, to nearly all of the paths my laboratory has taken in the years since. After searching through old publications, the first photograph was never published, but similar images appear in Eshed et al. (1999) and in a previous Flowering Newsletter (Bowman et al., 2001).

Instead, my choice represents the first time I was fortunate enough to have a scientific revelation through observation. The chosen images (Fig. 1) are of developing Arabidopsis agamous-1 flowers and were taken on 15 August 1988. Upon viewing the flowers depicted in the photographs, the developmental basis for the agamous mutant phenotype immediately became obvious: a homeotic conversion of stamens to petals and, surprisingly, what appeared to be a resetting of the remaining floral meristem into a new flower bud, providing an explanation of the indeterminacy of agamous flowers. The remainder of this essay describes the context for how the images were produced and some of thoughts and subsequent studies our initial interpretations spawned.

In 1988, David Smyth joined Elliot Meyerowitz’s laboratory as a sabbatical visitor during the second year of my PhD, and we embarked on a collaboration – one that has continued on and off for more than 20 years. Our aim was to look more closely at Arabidopsis flower development, specifically comparing the floral homeotic mutants with the wild type, since, whenever a new technique is applied to an old problem, novel insights are often obtained. We were inspired by earlier studies in which flower development was observed from organ inception (Breiger, 1935; Natarell and Sink, 1971) and in particular by a then recent paper by Polowick and Sawhney (Polowick and Sawhney, 1986) describing Brassica napus flower development using scanning electron microscopy (SEM). With the beautiful images of B. napus in mind, we fixed Arabidopsis inflorescences using a similar
protocol and discussed with Pat Koen, who was in charge of the electron microscope facility at Caltech in the subbasement of Alles Hall, methods to mount and dissect the fixed flower buds. It was at this point we realized that the sizes of Arabidopsis buds of appropriate developmental stages were significantly smaller than those of Brassica, and hence dissections were a caffeine-free zone. As Elliot’s laboratory also worked on Drosophila at the time, it was suggested that we try dissections with glass needles, such as those that were being used to inject DNA into Drosophila embryos.

There were two stages of dissection: the mounting of young buds removed from critically point dried inflorescences; and their subsequent dissection, which primarily consisted of the removal of sepals to reveal the inner floral whors. The needles had the advantage of being small enough to manoeuvre between the flower buds of the inflorescence, but their sharpness often inflicted serious wounds in the dried material. The mounting of individual buds to the SEM stub was a something of an art, as the adhesive we were using at the time, a silver paste, needed to be almost dry before the bud could be placed into the paste without having the buds become completely entombed by the solvent wicking over all surfaces. Conversely, if the paste was too dry the buds were not affixed well enough, making the subsequent dissection of the sepals impossible: the buds were dislodged by the needle, a little black fleck of tissue would fly away, and at less than 100μm across in total, there was little chance of retrieval. The dissection of the youngest buds was particularly difficult because the magnifying power of our dissecting microscope was not enough to resolve the sepals of stage 5–6 buds clearly. Thus, we obtained, over time and many mangled flower buds, a ‘feeling’ for how to remove the sepals without damaging the internal whors, as we could not be sure that the samples were not damaged until we observed them in the SEM.

David and I worked in parallel, with David focusing on wild-type flowers (Smyth et al., 1990) and myself concentrating on various mutant genotypes, fixing and dissecting. We then descended to the sub-basement together to observe our handiwork. In our first forays into SEM, the facility seemed to demand the respect required of a Roman legionary, as there were very strict rules about what we could or could not do and how it should be done, but over several months, we made the place our own and spent many pleasurable hours looking at and thinking about flower development. One aspect of photography at the time provided both an advantage and a disadvantage. Image capture on the SEM was onto large format film, which required manual processing in a dark room. While this may seem inconvenient by today’s standards, it provided ample time to talk and think about what we had just observed on the microscope, something that is sometimes lacking in the data driven experimental atmosphere of the present.

Double flowers, of which the agamous mutant is an example, have been known for millennia as Theophrastus described double roses in his Enquiry into plants written prior to 286 BC (see Meyerowitz et al., 1989, for a review). Double flowers can have several different developmental origins, due to a combination of meristic and homeotic defects (Reynolds and Tampion, 1983). For Arabidopsis agamous mutants, based on organ counts, the developmental basis was assumed to be homeotic, whereby the organs occupying the third floral whorl develop as petals rather than stamens, and the cells that would normally give rise to the fourth whorl carpels somehow reiterate the developmental processes that has occurred in the outer three whors. The images clearly showed, as expected, the organ primordia that would normally develop into stamens arise in the correct number and position, but in the agamous mutant, differentiate inappropriately into petals. More revealing was the fate of what would normally be the fourth whorl, where development closely resembles flower development at stage 3, as if the remaining floral meristem forgot its identity and started again from the beginning. Thus, while some of our SEM results were confirmatory, new insights were gained. This has been a general theme whenever new technologies have been applied to observe development. In the case of flower development, the next big leap occurred when the floral homeotic genes were cloned and gene expression patterns could be observed directly, demonstrating that the concentric rings of ABC gene expression are established prior to organ primordia emergence (Sommer et al., 1990; Yanofsky et al., 1990; Drews et al., 1991; Jack et al., 1992). More recently techniques for live imaging during development have provided insights into the spatial and temporal dynamics of gene expression (Heisler et al., 2005).

The first morphological difference between wild-type and agamous third whorl organs is their slower growth in agamous mutants, which we considered a reflection of their identity as petals whose development lags behind that of the other floral organs in wild-type flowers. In this sense the third whorl organs of agamous flowers are slightly different from homeotically transformed organs in other mutants.

**Fig. 1.** SEM images of developing agamous-1 flowers. At stage 6 (left), four second (2) and six third (3) whorl organs have initiated in the correct positions [some are hidden underneath the single remaining first (1) whorl lateral sepal]. In wild-type flowers the second whorl organs differentiate into petals, the third whorl organs are stamens, and the cells interior to the third whorl develop into the fourth whorl carpels. By stage 7 in agamous mutants (right) it is clear that the third whorl organs are growing more slowly than in the wild type, and the fourth whorl is behaving as if it constitutes another flower meristem, with four sepals initiated at its periphery. Bar=10 μm.
For example, at the same stage of development, the second whorl organs of pistillata and apetalala3 mutants are diminutive just as are wild-type petals, despite their identity as sepals in the mutants (Bowman et al., 1989). Another exception is in mutants where adjacent organs within whorls or in adjacent whorls are carpels, where all organ primordia tend to fuse, sometimes prior to the formation of distinct organ primordia leading to the hypothesis that identity in the flower meristem is being specified prior to organ primordium initiation. This was later confirmed by gene expression studies. However, experiments with conditional alleles indicated that organ identity is not necessarily irreversibly determined until after the primordia are initiated (Bowman et al., 1989; Schwarz-Sommer et al., 1992). These experiments instilled in me the importance of identifying the earliest stage at which a mutant phenotype can be observed, often leading our present experiments into embryogenesis.

The development of the fourth whorl of agamous mutants as another flower meristem brought up many questions, some of which are still not answered in detail. For example, how does the identity of the flower meristem become reset? With the realization that AGAMOUS represses APETALA1, a flower meristem identity gene, one hypothesis is that gene expression of the cells in the fourth whorl of agamous mutants at stage 6 may be similar to that of stage 2 wild-type flowers (as long as the B class genes are regulated appropriately) (Irish and Sussex, 1990; Bowman et al., 1993; Gustafsonbrown et al., 1994; Mandel and Yanofsky, 1995). AGAMOUS also negatively regulates WUSCHEL, which is required to maintain shoot and flower meristems (Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001), so agamous flowers can develop indeterminately. Thus, agamous flowers produce normal first and second whorls, a homeotypically transformed third whorl, and then the process reiterates indefinitely. However, indeterminacy can take many forms. In an instance of science returning to the past, in the same mutagenesis in the crabs claw mutant background that produced the revelation that CRABS CLAW was a polarity gene, numerous mutants were identified that exhibited indeterminate flowers (Prunet et al., 2008). In these mutant combinations, all four floral organs can be present, with a reiteration of stamens and carpels (or occasionally petals, stamens, and carpels) inside the fourth whorl carpels indicating that the remaining flower meristem can be reset to any previous stage of development. This plasticity in determinacy may explain the many different types of double flowers observed in horticulture, few of which actually resemble agamous mutants. For example, most rose varieties have both stamens and carpels interior to the extra whorls of petals.

The reiterations of ‘Russian doll’ flowers in agamous mutants are often not perfect renditions of the first three whorls. One of the most obvious deviations is that the organs that are ‘sepals’, for example the 4th, 7th, 10th etc whorls, are usually mosaics, with petal identity marginally and sepal identity centrally located (Bowman et al., 1989). Mosaic organs are not specific to agamous mutants, but are found in nearly all the floral homeotic genotypes. The existence of such organs indicates that the formation of organ primordia and their floral organ identity specification can be separated (Bowman et al., 1991). Since organ initiation is directed by auxin biology, knowledge of which has been the revolution in plant development for the past decade (Benkova et al., 2009), a challenge is to determine how the concentric rings of gene expression that determine floral organ identity are precisely co-ordinated with the initiation of floral organ primordia in wild-type flowers. The images of agamous-I represent a visualization of developmental biology that has occupied several laboratories of plant biologists for the past two decades and will continue to do so in the future.

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References


