Journal of Experimental Botany, Vol. 71, No. 22 pp. e1–e3, 2020
doi:10.1093/jxb/erz035 Advance Access Publication May 12, 2020

FEATURED IMAGE

My favourite flowering image: an *Aquilegia* flower

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Editor: Frank Wellmer, Trinity College Dublin, Ireland

When I started my faculty position, I had a lot of ideas about new research projects, like most naïve assistant professors. In particular, I was interested in working on the evolution of petal identity, and the eudicot family Ranunculaceae (the buttercups) seemed especially promising. For over a century, botanists had suggested that the often bizarre petals of this family had evolved many times independently from outer whorls of stamens (Prantl, 1888). I wanted to know whether there were any molecular signatures of these hypothesized parallel events. My original target genus was actually *Ranunculus* itself. I had already done some cloning and expression studies and knew that there were homeotic mutants available in the horticultural trade. Unfortunately, I quickly discovered that the genus also has a relatively large genome, wild variation in ploidy, a poorly resolved phylogeny, and serious seed dormancy. Not your best candidate for a new model system, even in the world of evo-devo. My next stop was the Kew C Value Database. A quick search revealed that the genus *Aquilegia* had the smallest genome in the family, a little over 300 Mbp (Ingle *et al.*, 1975), plus readily available homeotic mutants, a manageable number of interfertile species (~70; Munz, 1946), and a long history as a model for speciation processes (Hodges and Arnold, 1994). In one of those strokes of luck that sometimes put you on a whole different path, I had recently been introduced to Scott A. Hodges from the University of California, Santa Barbara, an international expert on *Aquilegia* evolution, and he was just in the process of putting together a collaborative initiative with Justin Borevitz (Australian National University) and Magnus Nordborg (Gregor Mendel Institute, Austrian Academy of Sciences) to develop genetic and genomic resources for the genus. Fifteen years later, we now have a fully sequenced reference genome along with more than a dozen re-sequenced species genomes (Filiault *et al.*, 2018), tractable RNAi-based tools for studying gene function (Gould and Kramer, 2007; Sharma and Kramer, 2013*b*), and a range of established molecular protocols. It is also a heck of a lot prettier than Arabidopsis (sorry, I have to be honest).

Aquilegia flower. Image courtesy of Ya Min, Kramer Lab, Harvard University.

So what have we learned from *Aquilegia* in terms of my original interest? There are many instances across the angiosperms where we believe that novel floral organ identities have arisen, in some cases from pre-existing floral organs and, in other instances, completely *de novo* (Endress, 1994). Often, these hypotheses relate to relatively ancient evolutionary events or focus on taxa that are not genetically tractable. The buttercup family offers an interesting example in which sterile, nectiferous petals are thought to have been derived from outer stamens several times independently across the family (Worsdell, 1903; Kosuge, 1994). Our comparative studies started by identifying homologs of the floral organ identity genes, with a focus on the B class genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) that control petal and stamen identity (Bowman *et al.*, 1989). This revealed that there are many paralogs of these MADS

box genes expressed in most sampled genera (Kramer *et al.*, 2003). In particular, there are three relatively ancient lineages of *AP3* paralogs, which we termed *AP3-I*, *AP3-II*, and *AP3-III*. The first two of these lineages have quite variable floral gene expression, but the third appears to have experienced an early subfunctionalization event that produced highly conserved petal-specific expression (Rasmussen *et al.*, 2009). This narrow expression domain is found among *AP3-III* orthologs across both the Ranunculaceae and Berberidaceae, with some potential instances in other ranunculid families. Further, functional studies in both *Aquilegia* and another Ranunculaceae genus, *Nigella*, confirm that these genes are specifically required for petal identity (Sharma *et al.*, 2011; Goncalves *et al.*, 2013; Zhang *et al.*, 2013). Finally, studies of closely related genera that either have or lack petals have found that while the *AP3-III* ortholog is expressed in the flowers bearing petals, it has been silenced in those lacking petals, and often shows evidence of pseudogenization (Zhang *et al.*, 2013). As a complement to this molecular evidence, an unbiased reconstruction of the presence/absence of second whorl sterile organs in the modern Ranunculaceae phylogeny suggests that petals were, in fact, present in the last common ancestor of the family and have been lost many times independently (Zhang *et al.*, 2013). So the upshot of this study is actually that Ranunculaceae petals are not independently derived. They appear to be homologous in a phylogenetic sense and also from the perspective of process homology by sharing a common identity program that has simply been turned off repeatedly. The alternative interpretation would require that in many separate instances, the same specific *AP3* paralog (out of three) underwent petal-specific subfunctionalization in the context of the evolution of those organs but then, for some mysterious reason, the expression of this same paralog was lost in the apetalous ancestor. Apologies to Prantl (1888), but this scenario is both unparsimonious and inconsistent with the observed data.

Although this might have been a disappointment, since what I really wanted to study was the evolution of novel organ identity pathways, it turned out that *Aquilegia* still offered an opportunity to do that. What you cannot see in this picture is that *Aquilegia* has a fifth type of floral organ. In addition to the petaloid sepals, the true petals in the second whorl, the many whorls of stamens, and the inner whorl of unfused carpels, these flowers have a continuous whorl of sterile organs called staminodes positioned between the stamens and the carpels (Kramer *et al.*, 2007). These staminodes are quite recently derived, apparently arising in the last common ancestor of the sister genera *Aquilegia*, *Semiaquilegia*, and *Urophysa*, which has been dated to ~20 million years ago (mya; Bastida *et al.*, 2010). Given that the ABC model does not immediately provide an obvious mechanism for determining the identity of a fifth floral organ, it was natural to ask how staminode identity was controlled. The answer to this question turned out to be to go back to our previously discovered paralogs of *AP3*. Although these three gene lineages are much older than the evolution of the *Aquilegia* staminodes, two of the paralogs, termed *AqAP3-1* and *AqAP3-2*, have undergone a complex pattern of sub- and neofunctionalization (Kramer *et al.*, 2007; Sharma and Kramer, 2013*a*). The two genes are initially expressed in all the stamen and staminode primordia, but, at about the time of carpel initiation, their expression patterns shift such that *AqAP3-2* persists in stamens while *AqAP3-1* becomes concentrated in the novel staminodes. Functional tests confirm that *AqAP3-2* is required for the development of fertile stamens and *AqAP3-1* is specifically necessary for staminode identity (Sharma and Kramer, 2013*a*). The next steps in this study will be to understand how the distinct expression patterns of these paralogs is achieved from a regulatory perspective and how their downstream developmental pathways diverge (and, hopefully, what their actual ecological function is).

Lastly, I will return to the petals, not for their identity but for their wonderfully convoluted final morphology. As I mentioned above, most petals in the Ranunculaceae bear nectaries that provide pollinator rewards, hence their original designation as Honigblätter—honey leaves (Prantl, 1888). These nectiferous petals have evolved diverse morphologies, sometimes being reduced to nothing more than stalked nectaries and at other times wildly elaborated with hairs, pockets, and forks (Rasmussen *et al.*, 2009). Nectar spurs have evolved twice independently in the family, once in *Delphinium* and once in *Aquilegia* (Hodges and Arnold, 1994; Jabbour and Renner, 2012). *Aquilegia* is of particular interest because it has experienced a recent radiation (<10 mya; Fior *et al.*, 2013) that is associated with diversification of spur morphology, especially among the North American clade in which pollinator switches have been common (Hodges and Arnold, 1995; Whittall and Hodges, 2007). As an added benefit, *Aquilegia* species are widely interfertile (Prazmo, 1960), allowing genetic mapping of these morphological differences. Much of our work now focuses on understanding the genetic basis for the original evolution of the *Aquilegia* nectar spur as well as the kinds of mutations that are associated with changes in shape, particularly length and curvature. This effort started with a careful study of spur development, which established that the formation of the spur occurs in two distinct phases: first, a period of localized cell divisions surrounding the nascent nectary that produces the initial spur cup; and, secondly, a longer phase of anisotropic cell elongation that generates most of the spur length (Puzey *et al.*, 2012). This initial study also found that variation in the length of the cell elongation phase appears to be responsible for many of the differences in spur length that are seen between species. Subsequent transcriptomic studies have ruled out a role for the KNOX genes, which participate in generating lateral organ complexity in many other instances (Koenig and Sinha, 2010), and instead implicated localized control of cell division at later developmental stages (Yant *et al.*, 2015). An unexpected outcome of the transcriptomic study was the discovery that homologs of the auxin homeostasis and carpel development gene *STYLISH* have been recruited to control nectary development in *Aquilegia*, and possibly across the family (Min and Kramer, 2019). Now our focus is on merging these traditional developmental genetic approaches with comparative genomics and quantitative genetics to fully flesh out our understanding of the spur developmental program and how genetic variation has produced ecologically relevant morphological diversity.

Acknowledgements

I want to thank Scott Hodges, who taught me everything I know about *Aquilegia* evolution and ecology, and has been a wonderful collaborator over many years. It has also been a pleasure working with Justin Borevitz and Magnus Nordborg on the long process of characterizing the *Aquilegia* genome. Thanks to all the former and current members of the Kramer lab who have advanced our understanding of this beautiful flower, and the National Science Foundantion and Harvard University for funding.

Keywords: *Aquilegia*, evo-devo, floral evolution, gene duplication, nectar spurs.

References

Bastida JM, Alcantara JM, Rey PJ, Vargas P, Herrera CM. 2010. Extended phylogeny of *Aquilegia*: the biogeographical and ecological patterns of two simultaneous but contrasting radiations. Plant Systematics and Evolution **284,** 171–185.

Bowman JL, Smyth DR, Meyerowitz EM. 1989. Genes directing flower development in Arabidopsis. The Plant Cell **1,** 37–52.

Endress PK. 1994. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge University Press.

Filiault DL, Ballerini ES, Mandáková T, *et al*. 2018. The *Aquilegia* genome provides insight into adaptive radiation and reveals an extraordinarily polymorphic chromosome with a unique history. eLife doi: 10.7554/eLife.36426.

Fior S, Li M, Oxelman B, Viola R, Hodges SA, Ometto L, Varotto C. 2013. Spatiotemporal reconstruction of the *Aquilegia* rapid radiation through next-generation sequencing of rapidly evolving cpDNA regions. New Phytologist **198,** 579–592.

Gonçalves B, Nougué O, Jabbour F, Ridel C, Morin H, Laufs P, Manicacci D, Damerval C. 2013. An APETALA3 homolog controls both petal identity and floral meristem patterning in *Nigella damascena* L. (Ranunculaceae). The Plant Journal **76,** 223–235.

Gould B, Kramer EM. 2007. Virus-induced gene silencing as a tool for functional analyses in the emerging model plant *Aquilegia* (columbine, Ranunculaceae). Plant Methods **3,** 6.

Hodges SA, Arnold ML. 1994. Columbines: a geographically widespread species flock. Proceedings of the National Academy of Sciences, USA **91,** 5129–5132.

Hodges SA, Arnold ML. 1995. Spurring plant diversification: are floral nectar spurs a key innovation? Proceedings of the Royal Society B: Biological Sciences **262,** 343–348.

Ingle J, Timmis JN, Sinclair J. 1975. The relationship between satellite deoxyribonucleic acid, ribosomal ribonucleic acid gene redundancy, and genome size in plants. Plant Physiology **55,** 496–501.

Jabbour F, Renner SS. 2012. Spurs in a spur: perianth evolution in the *Delphinieae* (Ranunculaceae). International Journal of Plant Sciences **173,** 1036–1054.

Koenig D, Sinha N. 2010. Evolution of leaf shape: a pattern emerges. Current Topics in Developmental Biology **91,** 169–183.

Kosuge K. 1994. Petal evolution in Ranunculaceae. Plant Systematics and Evolution **8,** 185–191.

Kramer EM, Di Stilio VS, Schluter P. 2003. Complex patterns of gene duplication in the APETALA3 and PISTILLATA lineages of the Ranunculaceae. International Journal of Plant Science **164,** 1–11.

Kramer EM, Holappa L, Gould B, Jaramillo MA, Setnikov D, Santiago PM. 2007. Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot *Aquilegia*. The Plant Cell **19,** 750–766.

Min Y, Bunn JI, Kramer EM. 2019. Homologs of the STYLISH gene family control nectary development in *Aquilegia*. New Phytologist **221,** 1090–1100.

Munz PA. 1946. *Aquilegia*: the cultivated and wild columbines. Gentes Herbarium **7,** 1–150.

Prantl K. 1888. Beitrage zur Morphologie und Systematik der Ranunculacean. Botanische Jahrbücher Systematik, Pflanzengeschichte und Pflanzengeographie **9,** 225–273.

Prazmo W. 1960. Genetic studies on the genus *Aquilegia* L. I. Crosses between *Aquilegia vulgaris* L. and *Aquilegia ecalcarata* Maxim. Acta Societatis Botanicorum Poloniae **29,** 57–77.

Puzey JR, Gerbode S, Hodges SA, Mahadevan L, Kramer EM. 2012. Evolution of spur-length diversity in *Aquilegia* petals is achieved solely through cell-shape anisotropy. Proceedings of the Royal Society B: Biological Sciences **279,** 1640–1645.

Rasmussen DA, Kramer EM, Zimmer EA. 2009. One size fits all? Molecular evidence for a commonly inherited petal identity program in Ranunculales. American Journal of Botany **96,** 96–109.

Sharma B, Guo C, Kong H, Kramer EM. 2011. Petal-specific subfunctionalization of an APETALA3 paralog in the Ranunculales and its implications for petal evolution. New Phytologist **191,** 870–883.

Sharma B, Kramer E. 2013a. Sub- and neo-functionalization of APETALA3 paralogs have contributed to the evolution of novel floral organ identity in *Aquilegia* (columbine, Ranunculaceae). New Phytologist **197,** 949–957.

Sharma B, Kramer EM, **eds.** 2013b. Virus induced gene silencing in the rapid cycling Aquilegia coerulea 'Origami'. New York: Springer.

Whittall JB, Hodges SA. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. Nature **447,** 706–709.

Worsdell WC. 1903. The origin of the perianth of flowers, with special reference to the Ranunculaceae. New Phytologist **2,** 42–48.

Yant L, Collani S, Puzey JR, Levy C, Kramer EM. 2015. Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur. Proceedings of the Royal Society B: Biological Sciences **282,** 20142778.

Zhang R, Guo C, Zhang W, *et al*. 2013. Disruption of the petal identity gene APETALA3-3 is highly correlated with loss of petals within the buttercup family (Ranunculaceae). Proceedings of the National Academy of Sciences, USA **110,** 5074–5079.