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The Evolution and Ecology of Floral Morphology in *Aquilegia* and the Influence of Horticulture on Its Emergence as a Model System

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Abstract

The columbine genus *Aquilegia* has been grown as a garden ornamental for centuries. Because of its unusual floral shape with the sepals being petaloid and colorful and the petals forming slender nectar spurs, many collections were made and species have been available to scientists as well as horticulturalists. Given its widespread natural distribution throughout the northern hemisphere, this availability was essential for early studies establishing that species in the genus were often highly intercompatible and their hybrids could be established. This led to a number of studies showing the relatively simple genetic inheritance of many of the dramatic differences among flowers of various species. More recent work has established the phylogenetic relationships among most of the species and showing that the genus had a burst of diversification after evolving the distinctive nectar spurs. Other studies have shown that this burst of diversification was likely due to adaptation to different pollinators, especially in North America, with changes in flower color, orientation and spur length to match the preferences and tongue lengths of new pollinators. Further studies have dissected the genetic basis of some of these traits. *Aquilegia* has also been noteworthy for its contributions to our understanding of the evolution of floral organ identity and horticultural varieties have, and will continue, to aid in these efforts. *Aquilegia* has now been the subject of substantial effort to develop it as a new model genomic system with the establishment of a high-quality reference sequence, derived from a highly inbred horticultural line as well as other resources such as a functional assay to assess specific gene functions. These resources poise *Aquilegia* to continue to be at the forefront of plant evolutionary and ecological research.

INTRODUCTION

The columbine genus, *Aquilegia*, has long been admired and cultivated due to its striking floral shape and extensive range of floral colors. The common name “columbine” is derived from the Latin *columba* for dove as the inwardly curled nectar spurs being thought to resemble a cluster of doves while *Aquilegia* is derived from *aquila* for eagle with the same nectar spurs being thought to resemble an eagle’s claw. For centuries *Aquilegia* has been cultivated and mutant forms selected as illustrated in herbals and floras from at least the late 1500s. The earliest depiction of *Aquilegia* I have found is from 1592 by Fabio Colonna in his *Phytobasanos* (Fig. 1A), though he labels the drawing as *Isopyrum*. Other depictions include homeotic mutants, clearly selected for their increased numbers of colorful sepals and or petals (Fig. 1B,C). Similar forms are still available in the trade and are prized garden additions. While the beautiful flowers have attracted gardeners and horticulturalists for hundreds of years, *Aquilegia* has also played an important scientific role in our understanding of adaptation, speciation and floral development. Interestingly many of the traits that have attracted horticulturalists turn out to be the same traits of interest to many scientists studying ecology and evolutionary biology. Here I review a number of the findings regarding the evolution of floral traits in *Aquilegia* and their ecological context and the role this genus will likely play in many future studies.

THE BIOGEOGRAPHY AND EVOLUTION OF *AQUILEGIA*

Early studies of *Aquilegia* revealed that most species are intercompatible and that fertile hybrids can be created (Taylor, 1967; Prazmo, 1965b). This finding led to the suggestion that the genus was quite young (Clausen et al., 1945). They pointed out that this young age would be especially interesting as *Aquilegia* could represent a model system for studying the early stage of species diversification. However, others (Stebbins, 1950; Leppik, 1964; Prazmo, 1965b) stated that the widespread distribution of the genus throughout the northern hemisphere suggested that the genus was much older in chronological time (at least mid-Tertiary) and had simply retained the ability to interbreed. The first attempt to reconstruct a phylogenetic tree for the genus using DNA sequences utilized both rDNA and cpDNA (Hodges and Arnold, 1994). Despite sampling taxa from across the geographic range of the genus, they found very little DNA sequence variation. In contrast, closely related genera had 3-45 times this level of variation (Hodges and Arnold, 1994). Thus, these data strongly supported the notion that *Aquilegia* had indeed arisen recently and had simply not had enough time to accumulate sequence differences between species. These findings also suggested that the genus was much more akin genetically to a species flock such as the African Cichlid fishes or Darwin's Finches but was very unusual because *Aquilegia* occurred over a much larger geographic area rather than being isolated on newly formed islands or lakes. Finally, this study suggested that the evolution of nectar spurs likely fostered an increased rate of speciation because *Aquilegia* has approximately 70 species while its sister group, *SemiAquilegia*, which lacks nectar spurs (as do all the other closely related genera in the *Ranunculaceae*), has only one species (*S. adoxoides*). Because these genera must be the same age, *Aquilegia* has diversified to a much greater extent. The difference in the presence of nectar spurs and their intimate association with pollinators suggested that they may act as a "key innovation" promoting diversification. Supporting this finding is the fact that the vast majority of independent origins of nectar spurs are also associated with large increases in species diversity relative to their sister groups that lack spurs (Hodges and Arnold, 1995; Hodges, 1997).

Though the findings of Hodges and Arnold (1994) suggested that the columbines had arisen recently, it remained unclear exactly what "recently" meant. In the twenty plus years since that publication, DNA sequencing and phylogenetic methods have improved immensely. Fior et al. (2013) sequenced 24 kb of the cpDNA from 84 individuals spanning the geographic distribution of *Aquilegia*. Using this especially large data set, they were able to conduct a chronological dating analysis by calibrating nucleotide changes with fossil data for the family *Ranunculaceae*. These data suggest that columbines started to diversify about 5 million years ago (mya) in Asia. The genus then spread west to Europe and east to North America where in both localities they started to diversify about 3 mya. Part of the European clade then re-invaded Asia (Fior et al., 2013).

The North American clade of *Aquilegia* has been studied in somewhat more detail phylogenetically than the European and Asian clades. Whittall and Hodges (2007) produced a species-level phylogeny using AFLP data for 176 individuals spanning all 25 North American taxa. These data suggest that the initial species invading North America were small, short-spurred and blue-flowered species that were pollinated by bumblebees (e.g., *A. jonesii* (Fig. 2A) and *A. saximontana*). This makes sense as the Asian progenitors of the North American clade were likely also bumblebees pollinated. However, invasion of North America brought *Aquilegia* into contact with a new type of pollinator, hummingbirds. Whittall and Hodges (2007) documented two independent origins of hummingbird pollination with intermediate nectar spur lengths and red flowers (Fig. 2B). From these hummingbird-pollinated species, pollination by hawkmoths has apparently evolved at least five separate times (Fig. 2C). These shifts have led to upright flowers, with long nectar spurs (up to 15 cm in *A. longissima*) and the loss of anthocyanin pigments rendering them either white or yellow depending on the presence or absence of yellow carotenoid pigments. Particularly interesting from this analysis was the finding that petal nectar spurs seem to evolve to ever greater lengths, starting with short spurs,

pollinated by bumblebees, then proceeding to intermediate lengths with hummingbird pollination and culminating with long spurs and hawkmoth pollination (Fig. 2). There was very little evidence that spurs ever evolved to shorter lengths suggesting directionality to evolution in the genus. Furthermore, these shifts in flower morphology and color were concentrated during speciation events rather than occurring gradually within species supporting a punctuated tempo to evolutionary change. Lastly, these data suggested that similar traits had evolved independently multiple times, which allows testing of whether natural selection produces similar phenotypes by selecting the variation in the same genes or pathways.

Studies within populations have also supported the premise that pollinators have played a critical role in species diversification, particularly in the North American clade of *Aquilegia*. Most evolutionary biologists use the evolution of reproductive isolation as the definition of the process that produces new species. Using this definition, for flower morphology and color to affect the speciation process, divergence in these traits must reduce the likelihood of plants mating – mediated through pollinator visitation and/or how they transfer pollen. Studies with *A. formosa*, a hummingbird-pollinated species and *A. pubescens*, a hawkmoth pollinated species addressed this issue. These two species are very closely related (Whittall and Hodges, 2007) with *A. pubescens* being derived from hummingbird pollination. *Aquilegia formosa* has scarlet red and yellow flowers that are pendant with medium length spurs. In contrast, *A. pubescens* has white or pale yellow flowers that are upright with long spurs (Fig. 2C). Thus, evolution has converted red flowers to white, pendant flowers to upright and medium spurred flowers to long-spurred flowers. When flowers of these two species are presented (in a regular array alternating between the species) to hummingbirds and hawkmoths, hummingbirds nearly exclusively visited *A. formosa*, avoiding *A. pubescens* while hawkmoths did the opposite, visiting *A. pubescens* and avoiding *A. formosa* (Fulton and Hodges, 1999). Thus, pollen would not be transferred between these intercompatible species and reproductive isolation would be enforced.

To understand which of these floral features affected pollinator behavior, Fulton and Hodges (1999) examined how hawkmoths responded to changes in specific traits. They first examined flower orientation by manipulating half of the flowers in an array of *A. pubescens* flowers to have a pendent orientation (like *A. formosa*) and the other half the normal upright orientation. In this case, hawkmoths avoided the pendent flowers and primarily visited the upright ones. Thus flower orientation has a very large effect on reproductive isolation. They then shortened the spurs of half of the flowers in the array by clipping off the spur to the length of *A. formosa* spurs and tying them off to retain the nectar that had accumulated. In this case hawkmoths showed no preference for short versus long-spurred flowers. However, the short-spurred flowers caused the hawkmoths to not probe as deeply into the flowers and their large bodies had little contact with the flower's reproductive organs. To quantify this effect, Fulton and Hodges (1999) counted how much pollen remained in the flowers after hawkmoth visitation and found that indeed, short-spurred flowers had significantly great pollen remaining in their anthers compared to the normal long-spurred flowers. So, petal spur-length does not affect hawkmoth visitation but profoundly affects whether pollen will be transferred between species. To determine how changes in a single trait might affect hawkmoth visitation to the normally hummingbird pollinated *A. formosa* a third experiment was conducted. Because hawkmoths had preferred upright flowers in the first experiment described above, *A. formosa* flowers were presented with their normal pendent orientation or in an upright orientation along with normal upright *A. pubescens* flowers. Hawkmoths again strongly preferred *A. pubescens* flowers but visited upright *A. formosa* flowers significantly more than the pendent *A. formosa* flowers (Hodges et al., 2002). The large difference in visitation between upright *A. formosa* and the upright *A. pubescens* was attributed to the stark difference in flower color (though floral scent differences could also contribute to this difference).

Reproductive isolation between *Aquilegia formosa* and *A. pubescens* has also been addressed at the population genetic level. These two species overlap in their distributions with *A. formosa* being very widespread occurring in the western North America from Alaska to southern California. In contrast, *A. pubescens* only occurs in the southern Sierra Nevada Mountains of California. In the southern Sierra Nevada the two species are largely separated by altitude with *A. formosa* occurring in shady environments along streams up to tree line (~3000 m) while *A. pubescens* occurs in open habitat with rocky scree above tree line. At tree line the two species often come into close proximity and local natural hybrid zones occur with a broad range of floral phenotypes. Classically, such hybrid zones have been interpreted as allowing a great deal of gene exchange between species (Abbott et al., 2013) and thus a lower degree of reproductive isolation, especially for those parts of the genome not involved with traits that do affect mating patterns. In a recent study, Noutsos et al. (2014) used 79 SNP markers and genotyped nearly 1000 individuals of both species and hybrid zones in populations spanning the geographic overlap between the species. Surprisingly they found that estimates of gene exchange, on the same spatial scale, differed remarkably from within species to between species comparisons. Within species, estimates of gene exchange were high among populations up to 30 km apart in *A. formosa* and up to 100 km apart in *A. pubescens*. In contrast estimates of gene exchange between the species was not significant and at most occurred on the scale of just a few km. Once again, these data suggested that the floral morphology is likely strongly reducing gene exchange between these intercompatible species.

Thus it appears that natural selection and horticulturalists have acted in similar ways in manipulating *Aquilegia* flowers. Certainly floral color is of paramount importance to breeders as is flower shape. In addition, the orientation of flowers alters the floral display sought by gardeners. As we have seen above, natural selection has converged on the same outcome multiple times. Hawkmoth pollination with upright, pale colored and long-spurred flowers having evolved independently multiple times as have the bright red, pendent and medium spurred flowers of species visited by hummingbirds.

THE GENETICS OF FLORAL TRAITS

As noted above, floral traits associated with specific pollinators include nectar spur length, flower orientation and flower color. *Aquilegia* has been long been used for genetic studies and even Mendel did crosses with *Aquilegia*. In the 1960s, Prazmo conducted now classic studies that showed that the lack of a nectar spur in *A. ecalcarata* segregated as one or two recessive loci, depending on which species it was crossed with (Prazmo, 1960, 1961, 1965a). She also found evidence for single loci controlling flower color, curvature of spurs and flower orientation (Prazmo, 1965a). Taylor (1967) also found similar evidence for single loci controlling these traits. In contrast, both of these authors found that the length of the nectar spur was polygenic. Below I summarize the more recent work aimed at finding the genes causing variation in floral traits of *Aquilegia*.

Flower Color

As we have seen above, flower color is strongly associated with different broad classes of pollinators. In Europe and Asia, most species produce blue or purple anthocyanin pigments and are pollinated by bees and bumblebees. There are some exceptions such as the yellow *Aquilegia aurea* in eastern Europe and the white *A. fragrans* from the Himalaya. Anthocyanins are produced through a highly conserved metabolic pathway the anthocyanin biosynthetic pathway (ABP) involving six main enzymes chalcone synthase (CHS), chalcone flavone isomerase (CHI), flavanone-3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP flavonoid glucosyltransferase (UFGT). Pelargonidins, which produce the strongly red colored flowers are the result of these six main enzymes. Cyanidins, which can have a range of colors from red to blue, require an additional enzyme flavonoid 3'-hydroxylase (F3'H). Delphinidins, which produce deep blue and purple colors, require either the addition of flavonoid 3'5'-hydroxylase (F3'5'H) or both F3'H and F3'5'H.

These latter two enzymes add 1 or 2 hydroxyl groups to the β -ring of the product of F3H (Grotewold, 2006; Rausher, 2008). Across the angiosperms, the expression of these enzymatic genes are controlled by a group of transcription factors including a R2R3 Myb, a bHLH and a WD40 though which specific genes are co-controlled varies from taxa to taxa (Grotewold, 2006).

The transition from hummingbird to hawkmoth pollination involves the loss of anthocyanin pigmentation resulting in white or yellow flowers depending on the presence of yellow carotenoid pigments. As this transition has occurred multiple times, it is possible to determine if this evolutionary transition occurred in the same manner at the molecular level during each transition. A number of authors have predicted that evolution will favor molecular mechanisms that result in the fewest pleiotropic effects and thus that changes in transcription factors will be favored during evolution (Clegg and Durbin, 2000). Such a scenario envisions that the enzymatic genes thus remain functional and can perform their functions in other tissues and at other developmental stages, which could be an evolutionary advantage. This is in contrast to many other possible mechanisms that could cause the loss of floral pigmentation such as having any of the enzymatic genes lose their enzymatic capabilities. Another possible mechanism is changing the flux of side branches of the broader flavonoid pathway and removing the flux of metabolites feeding into the ABP (Hodges and Derieg, 2009).

Aquilegia species with white or yellow flowers appear to have largely been derived through changes in transregulators as predicted by the minimal-pleiotropy hypothesis. In a set of experiments that determined the expression level of the core ABP enzymatic genes, Whittall et al. (2006) found that most transitions to loss of anthocyanin production involved down-regulation of multiple ABP genes, especially DFR and ANS. This finding, coupled with multiple crossing studies that have found strong evidence that a single genomic region is involved with anthocyanin loss in *Aquilegia* (Prazmo, 1961, 1965a) strongly suggest that mutations to a common transregulator are responsible. More recently we have identified a very large array of candidate genes involved in the broader anthocyanin pathway (Hodges and Derieg, 2009), which will be studied to understand shifts in color among anthocyanin producing species. For example the transition from blue to red flowers likely involves the loss of F3'5'H activity (Rausher, 2008) but more subtle changes in color are likely to be more complicated.

Floral Orientation and Spur Length

As discussed above, the orientation of flowers can be critical for attracting pollinators such as hawkmoths and nectar spur length affects how floral visitors are positioned with respect to the reproductive organs of a flower and thus how well they will act as pollen vectors. Prazmo (1960) crossed *A. ecalcarata*, which has pendent flowers with *A. chrysantha*, which has upright flowers. She found that the F₁ plants had pendent flowers and the F₂ segregated 209 pendent to 66 upright suggesting a single locus with the pendent allele dominant. In a cross between *A. formosa* (pendent) and *A. pubescens* (upright), Hodges et al. (2002) found a similar pattern with about 75% of the F₂ having flowers held at an angle below horizontal and 25% above. However, there was a great deal of variability within each of these classes with some plants, for example, having flowers fully pendent and others just slightly below the horizontal position. Thus, it appears that there is likely a major locus controlling differences in flower orientation between *A. formosa* and *A. pubescens* but that there are also other modifier loci of smaller effect as well. In her crosses, Prazmo (Prazmo, 1960, 1961) found spur length in F₂ populations to vary continuously and concluded that this trait was controlled by many loci. Our results are similar (Hodges et al., 2002).

Interestingly, we found that flower color, orientation and spur length were highly correlated with one another in the F₂ population of *A. formosa* and *A. pubescens* (Hodges et al., 2002) suggesting that genes underlying these traits were linked or caused by pleiotropy. In a separate F₂ population QTL for these traits were often co-localized substantiating these conclusions (Hodges et al., 2002). Thus many of the floral traits

differentiating species may be due to few genes clustered together or with pleiotropic effects on multiple traits important for reproductive isolation.

Flower Development

A great deal of study, primarily from the laboratory of Elena Kramer, has centered on elucidating the genetic programs underlying the development of floral organs in *Aquilegia*. These studies have recently been reviewed (Sharma et al., 2014) and thus I will only briefly touch on them here. The *Aquilegia* flower presents a number of novel features making it particularly interesting for studies of developmental evolution including two petaloid organs (the sepals and petals), the highly modified petal with a tubular nectar spur and an entirely distinct fifth floral whorl, the staminodium (Fig. 3). Studies have centered on determining the degree of conservation for the ABC model of floral organ identity, which suggests that the interaction of three classes of genes establishes identity. In *Arabidopsis* it has been determined that the A class alone determines sepals, A and B, petals; B and C, stamens; and C alone, carpels (Coen and Meyerowitz, 1991). There are two B class genes in *Arabidopsis*, *PISTILLATA* (*PI*) and *APETALA3* (*AP3*). In the Ranunculid order there have been two duplications of *AP3* resulting in three paralogous lineages and in *Aquilegia* there has been one additional recent duplication such that it possesses four *AP3*-like genes, *AqAP3-1*, *AqAP3-2*, *AqAP3-3*, and *AqAP3-3b* (Kramer et al., 2003, 2007). Some of these genes have apparently become subfunctionalized or neofunctionalized as, for instance, *AP3-3* specifies petal identity (Sharma et al., 2011) and *AP3-1* specifies staminodia (Sharma and Kramer, 2013).

Horticultural varieties have played a role in studies of *Aquilegia* flower development and likely will continue to do so in the future. This is because a number of floral homeotic mutants have been selected and are available in the trade. For instance *stellata* and *clematiflora* are names for mutants that have their second whorl transformed from petals into a second set of sepals. Substantiating the role of *AP3-3* in specifying petal identity, its expression is completely absent in *clematiflora* (Kramer et al., 2007). The *stellata* form not only lacks petals but has numerous whorls of sepals suggesting that not only petals have been transformed but stamens as well (Fig. 1B,C). This suggests a reduction in the extent of C-class gene expression. Similarly, there are numerous cultivated forms of *A. vulgaris* where the number of petal whorls has expanded (Fig. 1C). Thus these varieties could be used to study how the boundary of C-class gene expression is controlled.

Genomics

A number of groups have been working to develop genetic and genomic resources for *Aquilegia*. Interest in acquiring these tools comes for a variety of reasons including *Aquilegia* being a member of the basal eudicot lineage, the *Ranunculales*, which allows very deep phylogenetic comparisons with the commonly used model systems *Arabidopsis* and rice. In addition, as noted here, the species of *Aquilegia* display a great diversity in floral form and ecology and the species are largely intercompatible so genetic dissection of these traits is possible. Finally, the genome of *Aquilegia* is quite small for an angiosperm at about 300 Mb. For all these reasons, the Joint Genome Institute (JGI) undertook the sequencing of *Aquilegia*. Again, a horticultural line has played a very significant role in this effort. Ideally, highly inbred lines are used in a sequencing project as the reduction in variation between homologous chromosomes makes assembly of the genome much easier. Goldsmith Seeds had produced such an inbred line (*A. × coerulea* ‘Goldsmith’) and donated it to our sequencing effort. JGI has now produced a high-quality Sanger-sequenced genome of this line and provided detailed gene annotation derived from deep sequencing expressed sequence tags and RNAseq (<http://phytozome.jgi.doe.gov>). In addition, 13 species from throughout the genus have been resequenced as well as the sister genus *SemiAquilegia*. Two BAC libraries are available (Fang et al.,

2010) and a virus induced gene silencing (VIGS) protocol has been developed allowing the selective knockdown of genes (Gould and Kramer, 2007).

CONCLUSIONS

Horticultural interest in *Aquilegia* has played a major role in allowing columbines to be developed into a genetic model system. The early experiments documenting the intercompatibility among species and the genetic basis of many traits would not have been possible without the interest in and trade of seed from across the globe. These studies established the unique opportunities offered with this genus for studying plant diversification and the genetics of adaptation and speciation. Furthermore, collection and propagation of floral mutants has, and is, providing material for understanding the genetic basis of floral development and its evolution. And, of particular importance for many future studies is the use of a horticultural inbred line for the high-quality reference genome for *Aquilegia*, *A. × coerulea* 'Goldsmith'. This resource and others noted here position *Aquilegia* as a major emerging model system for understanding plant evolution and development.

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Literature Cited

- Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J.W., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Most, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Vainola, R., Wolf, J.B.W. and Zinner, D. 2013. Hybridization and speciation. *J. Evol. Biol.* 26:229-246.
- Clausen, J., Keck, D.D. and Hiesey, W.M. 1945. *Studies on the Nature of Species II*, Carnegie Inst. Wash. Publ. 564, Carnegie Inst., Washington, DC.
- Clegg, M.T. and Durbin, M.L. 2000. Flower color variation: a model for the experimental study of evolution. *Proc. Natl. Acad. Sci. USA* 97(13):7016-23.
- Coen, E.S. and Meyerowitz, E.M. 1991. The war of the whorls - genetic interactions controlling flower development. *Nature* 353:31-37.
- Fang, G.C., Blackmon, B.P., Henry, D.C., Staton, M.E., Sasaki, C.A., Hodges, S.A., Tomkins, J.P. and Luo, H. 2010. Genomic tools development for *Aquilegia*: construction of a BAC-based physical map. *BMC Genomics* 11.
- Fior, S., Li, M.G., Oxelman, B., Viola, R., Hodges, S.A., Ometto, L. and Varotto, C. 2013. Spatiotemporal reconstruction of the *Aquilegia* rapid radiation through next-generation sequencing of rapidly evolving cpDNA regions. *New Phytol.* 198:579-592.
- Fulton, M. and Hodges, S.A. 1999. Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proc. Royal Soc. London Ser. B-Biol. Sci.* 266:2247-2252.
- Gould, B. and Kramer, E.M. 2007. Virus-induced gene silencing as a tool for functional analyses in the emerging model plant *Aquilegia* (columbine, *Ranunculaceae*). *Plant Meth.* 3.
- Grotewold, E. 2006. The genetics and biochemistry of floral pigments. *Ann. Rev. Plant Biol.* 57:761-780.
- Hodges, S.A. 1997. A rapid adaptive radiation via a key innovation in *Aquilegia*. In: T. Givnish and S. Kenneth (ed.), *Molecular Evolution and Adaptive Rad.* Cambridge University Press, Cambridge.
- Hodges, S.A. and Arnold, M.L. 1994. Columbines - a geographically widespread species flock. *Proc. Natl. Acad. Sci. USA* 91:5129-5132.

- Hodges, S.A. and Arnold, M.L. 1995. Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. Royal Soc. London Series B-Biol. Sci.* 262:343-348.
- Hodges, S.A. and Derieg, N.J. 2009. Adaptive radiations: from field to genomic studies. *Proc. Natl. Acad. Sci. USA* 106:9947-9954.
- Hodges, S.A., Whittall, J.B., Fulton, M. and Yang, J.Y. 2002. Genetics of floral traits influencing reproductive isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Amer. Naturalis.* 159:S51-S60.
- Kramer, E.M., Di Stilio, V.S. and Schluter, P.M. 2003. Complex patterns of gene duplication in the APETALA3 and PISTILLATA lineages of the *Ranunculaceae*. *Intl. J. Plant Sci.* 164:1-11.
- Kramer, E.M., Holappa, L., Gould, B., Jaramillo, M.A., Setnikov, D. and Santiago, P.M. 2007. Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot *Aquilegia*. *Plant Cell.* 19:750-766.
- Leppik, E.E. 1964. Floral evolution in the *Ranunculaceae*. *Iowa State J. Sci.* 39:1-101.
- Noutsos, C., Borevitz, J.O. and Hodges, S.A. 2014. Gene flow between nascent species: geographic, genotypic and phenotypic differentiation within and between *Aquilegia formosa* and *A. pubescens*. *Mol. Ecol.* 23:5589-5598.
- Prazmo, W. 1960. Cytogenetic studies on the genus *Aquilegia* I. Crosses between *Aquilegia vulgaris* L. and *Aquilegia ecalcarata* Maxim. *Acta Soc. Bot. Pol.* 29:423-442.
- Prazmo, W. 1961. Cytogenetic studies on the genus *Aquilegia* II. Crosses between *Aquilegia ecalcarata* Maxim. and *Aquilegia chrysantha* Gray. *Acta Soc. Bot. Pol.* 30:57-77.
- Prazmo, W. 1965a. Cytogenetic studies on the genus *Aquilegia* III. Inheritance of the traits distinguishing different complexes in the genus *Aquilegia*. *Acta Soc. Bot. Pol.* 34:403-437.
- Prazmo, W. 1965b. Cytogenetic studies on the genus *Aquilegia* IV. Fertility relationships among the *Aquilegia* species. *Acta Soc. Bot. Pol.* 34:667-685.
- Rausher, M.D. 2008. Evolutionary transitions in floral color. *Intl. J. Plant Sci.* 169:7-21.
- Sharma, B., Guo, C., Kong, H. and Kramer, E.M. 2011. Petal-specific subfunctionalization of an APETALA3 paralog in the *Ranunculales* and its implications for petal evolution. *New Phytol.* 191:870-83.
- Sharma, B. and Kramer, E.M. 2013. Sub- and neofunctionalization of APETALA3 paralogs have contributed to the evolution of novel floral organ identity in *Aquilegia* (columbine, *Ranunculaceae*). *New Phytol.* 197:949-957.
- Sharma, B., Yant, L., Hodges, S.A., and Kramer, E.M. 2014. Understanding the development and evolution of novel floral form in *Aquilegia*. *Curr. Opin. Plant Biol.* 17:22-27.
- Stebbins, G.L. 1950. *Variation and Evolution in Plants*, Columbia Univ. Press, New York.
- Taylor, R.J. 1967. Interspecific hybridization and its evolutionary significance in genus *Aquilegia*. *Brittonia* 19:374-390.
- Whittall, J.B. and Hodges, S.A. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* 447:706-U12.
- Whittall, J.B., Voelckel, C., Kliebenstein, D.J. and Hodges, S.A. 2006. Convergence, constraint and the role of gene expression during adaptive radiation: floral anthocyanins in *Aquilegia*. *Mol. Ecol.* 15:4645-4657.

Figures

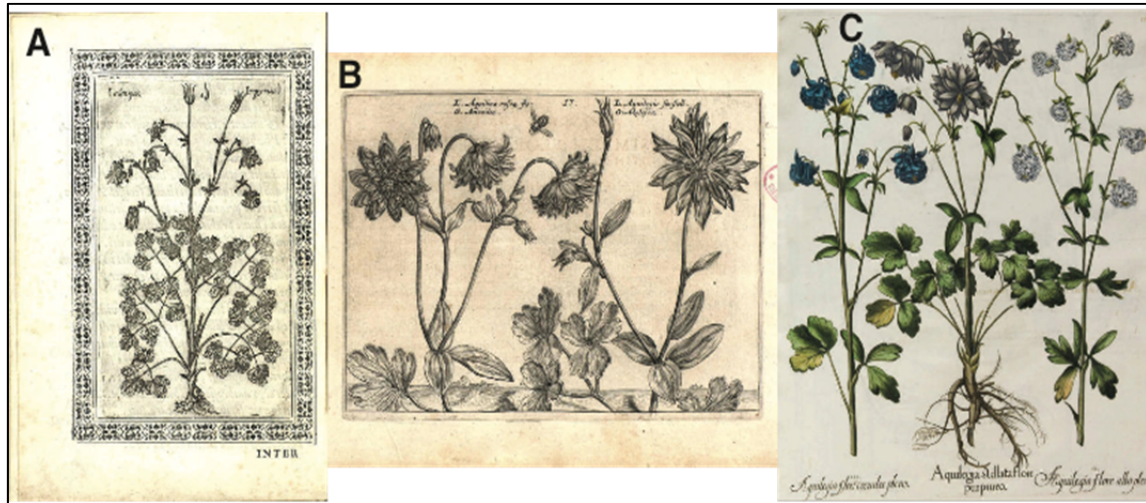


Fig. 1. Examples of early depictions of *Aquilegia*. A. from Fabio Calonna, *Phytobasanos, sive plantarum aliquot historia*, p.1, t. [1] (1592), B. Crispijn van de Passe, *Hortus floridus*, fascicle 1. Vernalis, t. 37, fig. 2 (1614) and C. Basilius Bessler, *Hortus Eystettensis*, vol. 2: *Secundus ordo collectarum plantarum aestivalium*, t. 176, fig. II (1620). Note the stellata and multi-petal forms depicted in B and C.

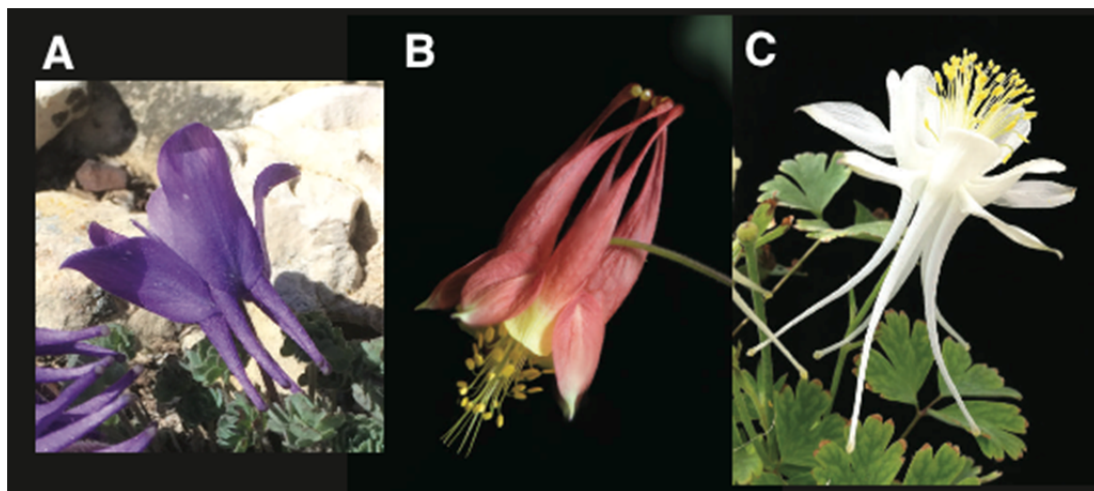


Fig. 2. Examples of the floral morphologies associated with different pollinators in the North American clade of *Aquilegia*. A. *A. jonesii*, a small cushion plant occurring in alpine habitats with short-spurred, upright blue flowers visited by bumblebees. B. *A. canadensis*, a pendent red and yellow flower with medium length spurs pollinated by hummingbirds. C. *A. pubescens*, an upright white-flowered species with long spurs visited by hawkmoths.

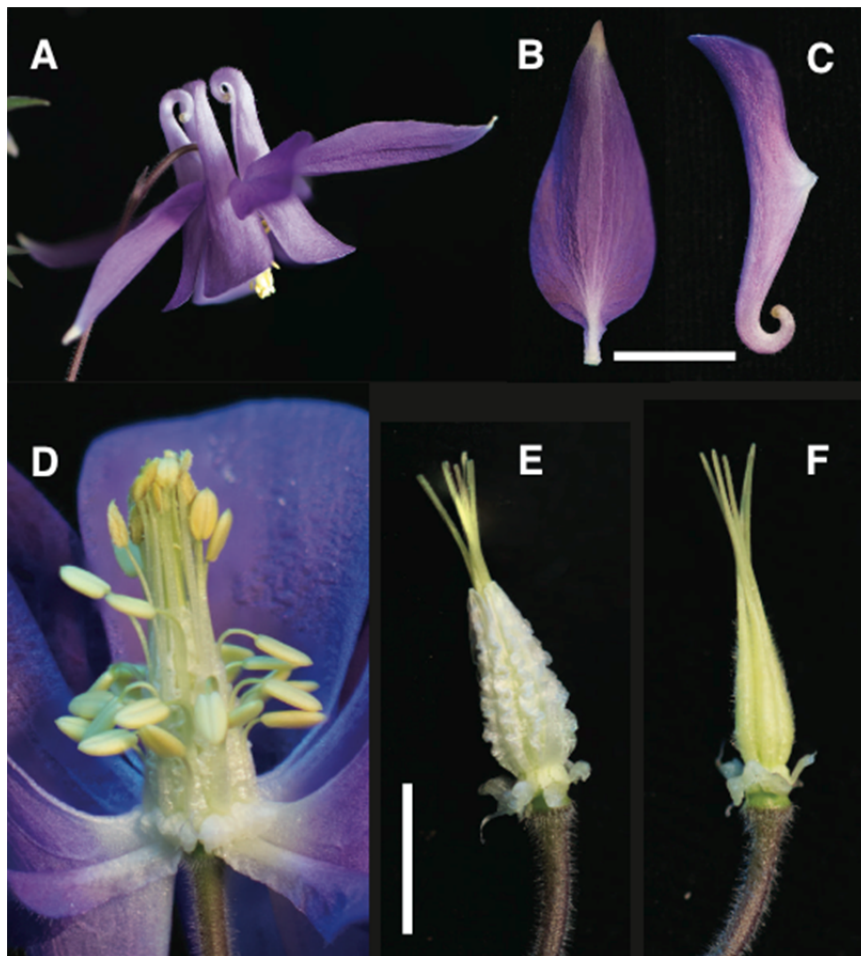


Fig. 3. An *Aquilegia* flower and its separate floral organs. A. whole flower. B. a single sepal, which is colorful and petaloid. C. a single petal with an inwardly curled spur. D. after removal of one sepal and two petals, the whorls of stamens are visible. E. after removal of the stamens, the staminodia surrounding the carpels are visible. F. after removal of the staminodia, the separate carpels are visible. The scale bar for B and C is 1 cm and for D-F is 0.5 cm.