



Figure 1. The head of the nocturnal bee *Megalopta genalis*, showing the relatively large compound eyes and ocelli.

Columbines

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What are columbines?

Columbines are compact, herbaceous, flowering plants belonging to the genus *Aquilegia* which have long been of interest to both garden enthusiasts and scientists. Species of *Aquilegia* are found primarily in mountainous regions of Europe, Asia and North America. Their flowers are usually brightly coloured and each petal is modified to have a tubular outgrowth, the nectar spur (Figure 1). Nectar is made at the very tip of the spur, filling it partially. Animals probe the spurs to obtain the nectar, thereby achieving pollination of the flowers. Species of *Aquilegia* can differ dramatically in flower color and shape, yet they can usually be hybridized, which has resulted in both natural hybrid zones and a startling number of horticultural varieties. In the Figure, panels A and B show the species *A. formosa* and *A. pubescens*, respectively, while C shows a naturally occurring hybrid of the two. The ability of very divergent columbines to hybridize also piqued the interest of Darwin, as he discussed the concept of species in letters with J.D. Hooker during the 1850s, and continued to attract scientific interest throughout the twentieth century, resulting in classic studies such as Munz's taxonomic treatment in the 1940s, Verne Grant's analysis of ecological and pollinator isolation in the 1950s and Prazmo's genetic studies of floral differences in the 1960s.

How have columbines evolved?

Aquilegia has about 70 species that vary in their flower color and shape as well as the habitats in which they grow. Despite their geographic, morphological and ecological diversity, very few genetic differences can be found among the species. This has led to columbines becoming a textbook example of a recent adaptive radiation, where speciation has occurred rapidly to produce a variety of species, each

visually, nor whether vision is used to determine directions and distances travelled to the foraging site, there is no reason to rule out the possibility.

Can nocturnal bees really see so well with such tiny eyes?

Remarkably yes. But there is no question that their visual systems are operating near the limit and that only the coarser and slower features of the world can be seen. Light intensity plays a deciding role in whether a particular bee species is able to forage or not — many species of bees that are capable of visual foraging in the early dusk may be forced back to the nest just a short time later before light levels have become unacceptably dim. Those able to forage at the very dimmest light levels (such as *Megalopta genalis* (Figure 1) and the giant Indian carpenter bee *Xylocopa* sp.) typically have visual specialisations that permit nocturnal foraging. Apart from giant ocelli, these bees have very large ommatidial facet lenses and massive photoreceptors, adaptations that maximise light capture and improve vision. Moreover, it is very likely that nocturnal bees (and indeed many other nocturnal insects) are also able to enhance vision at a higher level in the visual system by neurally summing photons in space and time. This strategy, which improves visual sensitivity

at the expense of spatial and temporal resolution, requires the presence of specialised circuits in the optic lobes of the brain. Even though we have considerable evidence for the presence of such circuits, their existence remains to be established with certainty. But the remarkable visual abilities of nocturnal bees — which allow landmark-based homing at light levels where humans are practically blind — are beyond question.

Further reading

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filling different ecological roles. What is unique about columbines, compared to their close relatives that have not gone through an adaptive radiation, is that they have evolved nectar spurs. The colour of the nectar spurs tends to attract different types of pollinator, while their length and shape can affect which animals are able to obtain the nectar. Thus, some species of *Aquilegia* are specialized for bumble-bees while others are specialized for hummingbirds (Figure 1A) and others for hawkmoths (Figure 1B). Once populations have specialized to different pollinators, they are unlikely to mate with one another and, ultimately, speciation occurs. Other groups of flowering plants have independently evolved nectar spurs and most of these groups are also associated with increased species diversification compared to their close relatives.

What can columbines tell us about the genetics of speciation? Speciation is the process by which two populations of a species diverge until they can no longer produce viable offspring. Studies of *Aquilegia* have shown that, even when populations are capable of producing fertile offspring, they largely do not if they are adapted for pollination by different animals. Therefore, shifts in ecological associations occur before changes that make hybridization impossible altogether. This is a general theme that is being found in both plants and animals. The next goal is to identify the types of gene and mutation that allow these differences to evolve. Because species of *Aquilegia* are so similar at the DNA level this should be much easier than for other groups of plants.

Can columbines be used to address other evolutionary questions? Yes, because of its phylogenetic position, *Aquilegia* is well suited for studies of some of the major transitions that have occurred during the evolution of flowering plants. About 75% of all flowering plants are members of a lineage called the eudicots, which are distinct from the monocots (for

example, grasses and orchids), and other basal lineages (for example, Magnolids and water lilies). *Aquilegia* is a member of the very earliest diverging branch of the eudicots and thus can be used in comparative studies to determine when important genomic changes occurred leading to the major diversification of the flowering plants. In addition to providing insight into the eudicot ancestor, *Aquilegia*'s phylogenetic position is roughly intermediate between the highly divergent model systems in the monocot grasses (for example, rice and maize) and core eudicots (for example, *Arabidopsis*, snapdragon). This allows *Aquilegia* to provide an important third data point in genomic studies comparing these groups.

Any other interesting traits?

Aquilegia has a number of morphological and physiological traits that are of considerable interest. For example, the compound leaves of *Aquilegia* provide a useful model for testing hypotheses that have largely been developed in core eudicot systems such as tomato. *Aquilegia*'s flowers are also of particular utility and their features may help us to understand how novel morphological traits evolve. Often, major morphological novelties differentiate groups that have diverged long ago, which makes understanding their origin difficult. *Aquilegia* has two novel floral features that have evolved recently: the nectar spur and an entirely unique floral organ, the staminodium, which surrounds developing fruit. Developmental and genetic studies of these structures will help us understand how novelties evolve. In terms of physiology, there are species of *Aquilegia* that have adapted to very different environments and exhibit traits such as tolerance for high salt, high altitude or drought conditions. The ability to make inter-species hybrids in *Aquilegia* will allow the genetic basis for these adaptations to be determined.

What do we know about *Aquilegia*'s genome and genetics?

Aquilegia has by far the smallest genome in its family (~400 Mb) and

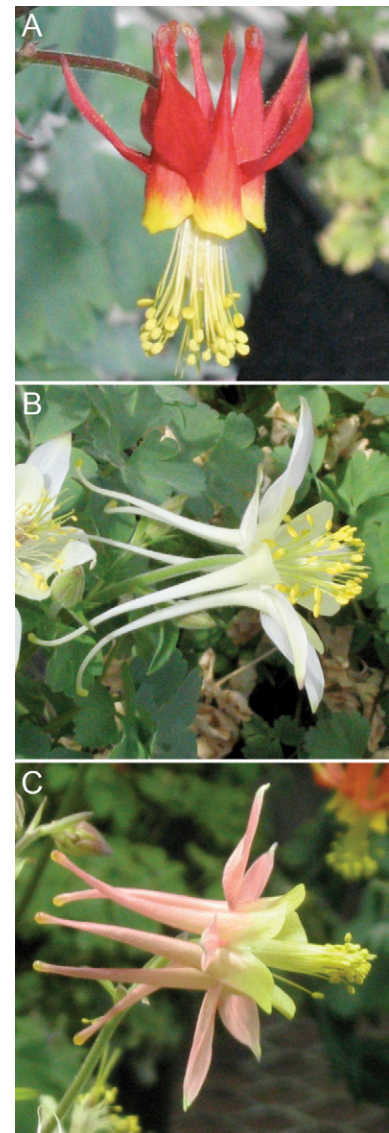


Figure 1. Variable flowers of *Aquilegia*. (A) *A. formosa* flower. (B) *A. pubescens* flower. (C) Flower from *A. formosa* x *A. pubescens* naturally occurring hybrid.

the species are religiously diploid. The application of a wide array of approaches is helping us to learn more about *Aquilegia*'s genome, including the construction of a detailed genetic map and defining DNA markers that can be used in most species. There is also a large database of expressed sequence tags (ESTs, see <http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=aquilegia>) and a physical map of the genome. Perhaps most exciting, columbine is slated for whole-genome sequencing by the Joint Genome Institute (see <http://www.jgi.doe.gov/sequencing/why/CSP2007/aquilegia.html>).

Two aspects of the genetics of floral traits in *Aquilegia* have been studied: flower color and flower development. A recent comparative study showed that species that did not produce floral anthocyanins (the red and blue pigments in most flowers) also had significantly lower expression levels for multiple genes needed to produce the pigments. However, crossing studies suggest that a single gene is usually responsible for losses of these pigments. Taken together, this suggests that changes in flower color are usually due to mutations in transcription factors that control the expression of multiple steps in the biochemical pathway rather than changes in the amino acid sequences of enzymes. Other studies have focused on the genetic basis for *Aquilegia*'s novel floral organs, especially the staminodia. This work has found that ancient duplication events in the genes that normally produce stamen identity may have facilitated the evolution of the novel staminodium identity program. Additional tools for functional genetics, including RNAi and transgenics, are currently being optimized in *Aquilegia* and will greatly facilitate the development of these lines of research.

Where can I find out more?

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Fabled oaks hope

Britain is not known for forests: in fact it has one of the lowest levels of tree cover in Europe. But it does provide home to some of the oldest trees on the continent. In Sherwood Forest, in the English midlands, oak trees survive that are believed to be more than 1,000 years old. Such venerable trees are made more famous by their association with the legend of Robin Hood, the outlaw who lived amongst them while fighting with the British authorities at the time of the Crusades.

But Sherwood Forest now comprises just fragments of its former extent and the ancient oaks are finally crumbling. So a new project aims to create corridors between these ancient fragments and replant with oak trees to try to restore the forest to something of its former size before all the ancient oaks have all vanished. Researchers calculate that within 50 years Europe's oldest trees will have gone. A clue to the age of these trees can be gleaned from the fact that oak trees were cleared from the forest in the great rush to build warships against the Spanish

Armada, but these trees were already considered too old to provide useful timber, and so were left standing.

Given their parlous present state, a rescue plan has been drawn up by conservationists which includes a £59 million bid to the country's lottery fund this winter with the aim of planting 250,000 new oaks to link the surviving fragments and create new stretches of equally important grazed open heath.

The restoration of the forested regions remains a paramount goal for conservationists. Ancient maps studied by archaeologist, Ursula Spence, show that at the time when Robin Hood was believed to be living in the forest, should he have fled he would have been able to ride under cover to Sheffield 80 kilometres away. Now he would barely manage five kilometres.

But beyond the Robin Hood stories, the minimal disturbance amongst the remaining fragments has also created a wildlife refuge. Several beetles are found here and nowhere else in Britain and many other rare species are home here.

Conservationists are hoping the lottery can prove a winner in helping this site.

Nigel Williams



Ancient: Researchers are keen to restore Sherwood Forest as the ancient oaks, reaching the end of their life, provide a key habitat. (Photo: © Ian Evans/Alamy.)