MINIREVIEW



A PORTRAIT OF THE ANCESTRAL FLOWER

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By comparing the modern day representatives of the earliest diverging lineages of flowering plants, we may derive an anatomical, ecological and molecular description of the common ancestor of the flowering plants that lived around 150 million years ago. By extending these comparisons to the remaining seed plants, the gymnosperms, we may furthermore be able to identify the molecular events that contributed to the evolution of the first flowering plants. Large scale EST sequencing projects and other research efforts are currently poised to extend our ideas of flower development, derived from model systems, to explain how the flower first evolved.

Different ways to paint a picture

From Vincent van Gogh to the Reverend Keble Martin (21), painting pictures of flowers has for long been a preoccupation of both famous artists and celebrated botanical illustrators. All flowering plants are undoubtedly descended from a common ancestor, and it would be interesting to know what the ancestral flower looked like. In the absence of living material, however, we must find alternative ways to paint its portrait. One such method has been available for many years; it is to study the fossil evidence. Unfortunately, the fossil record of soft plant tissues such as flowers is far from complete. For this reason, no known fossil can be said with confidence to bear a strong likeness to the ancestor of the flowering plants. A second method of study has been available for just 5 years; it is to compare living groups known to have diverged at very early stages in the evolution of the flowering plants. Characteristics shared by

such early-diverging groups must either have resulted from parallel evolution, or have been present in their common ancestor. Living plants can be studied by any chosen method, whereas fossils mainly provide structural information. The comparative analysis of living groups may, therefore, be additionally informative on the physiological, molecular and ecological features of the ancestor of the flowering plants.

Discovering the family tree

Comparative methods to study early angiosperm evolution were made possible around 5 years ago by a series of independent molecular phylogenetic studies that arrived at very similar conclusions, providing a robust hypothesis for the evolutionary relationships between the major flowering plant groups, reviewed by Kuzoff and Gasser (20). According to these studies, the 3 most basal orders of angiosperms, whose lineages diverged before those of all other angiosperm groups, are Amborellales, Nymphaeales and Austrobaileyales, collectively referred to as the

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Figure 1. A molecular phylogeny of the seed plants. The extant gymnosperms and angiosperms form 2 monophyletic sister groups. Within the angiosperms, a group of dicot lineages termed the ANITA grade are the first to diverge from the remaining lineage. The monocots form a monophyletic group that evolved from a dicot ancestor, well after the common ancestor of all angiosperms. This tree is based on those given by Kuzoff and Gasser (20) and Qiu *et al.* (23). Approximate species numbers, in parentheses, are taken from Pryer *et al.* (22) and estimated divergence dates from Davies *et al.* (7).

ANITA grade (Fig. 1). Amborellales is represented by a single extant species, Amborella trichopoda, which is a rather straggly tree found only in the under-storey of humid tropical forests in New Caledonia. Nymphaeales is a widely distributed order of aquatic plants, containing the families Nymphaeaceae and Cabombaceae. Austrobaileyales contains the families Austrobaileyaceae, Illiciaceae, Schisandraceae and Trimeniaceae, representing a mixture of endemic and more widely distributed groups. Some slight disagreement in the literature surrounds the order of divergence of Amborellales and Nymphaeales. Most molecular phylogenetic studies support Amborellales alone in the most basal position of the angiosperm tree (7, 20, 37), while others support a first-diverging clade containing both Amborella and Nymphaeales (23). Contradictory conclusions from a further research group (16, 17) have disputed the basal position of the ANITA grade. However, recent reanalyses have demonstrated that the very limited taxon sampling of these latter studies may have caused a distortion of the resulting phylogenetic trees (25, 26). It seems, therefore, that we may continue to regard the ANITA grade as basal to the flowering plants.

The low numbers of extant species (around 180) and higher taxa (14 genera in 7 families) that make up the ANITA grade suggest these groups to have undergone limited speciation events over the long course of their separate evolutionary history. There is, therefore, good reason to suppose that ANITA taxa may possess many characteristics that are pleisiomorphic to the angiosperms: ones that were present in the common ancestor of the living flowering plants.

A sketch of the ancestral flower

Though many characteristics show some variability between different ANITA groups, the general comparison of ANITA taxa has enabled several conclusions to be reached on the likely condition of the ancestor of the living flowering plants. It has been suggested that the ancestor of the extant angiosperms would have been a woody plant of damp and shaded habitats, probably growing on disturbed substrates such as the banks of streams (11). The work of Peter Endress and colleagues (8, 9) indicates that the flowers of the angiosperms' ancestor were probably small, bisexual and insect-

pollinated. These flowers would have contained a spiral arrangement of floral organs, including a perianth that was not distinctly divided into petals and sepals. The carpels of the ancestral flower (9) would have matured before the dehiscence of its anthers (protogyny). These carpels would have remained free, rather than being syncarpically fused. The stigmatic surface of the ancestral carpel would have been covered by multicellular ridges or protrusions, rather than by unicellular papillae. Following pollination, pollen tubes would have grown through secretion-filled apertures or canals in the carpel apex, rather than through cellular structures, to reach the ovules. The ovules of the angiosperms' ancestor would have occurred singly, or in small numbers, in each carpel. These ovules would have been anatropous, covered by 2 integuments and would have contained a large nucellus. Evidence from Nuphar (Nymphaeaceae) (30) and Illicium (Illiciaceae) (31) suggests that the embryo sac of the angiosperms' ancestor would have contained 4 nuclei, rather than the 7 nuclei of most later-diverging groups. Double fertilization would have been present in the angiosperms' ancestor, and would have resulted in the production of a diploid, rather than a triploid, endosperm.

Colouring in the genes

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Molecular genetic studies of model plants, such as *Arabidopsis thaliana* and *Antirrhinum majus*, have identified many genes that control aspects of flowering and flower development. These include the induction of flowering, the specification of floral organ identity, and tissue differentiation within the different floral organ types. Many genes controlling flower development in model angiosperms might be expected to share gene orthologues with ANITA grade angiosperms: orthologues are genes in different species that evolved from the same gene present in the last common ancestor of those species. By analysing the occurrence and expression patterns of flower development orthologues in ANITA angiosperms, it is possible to determine the molecular processes that are likely to have controlled flower development in the ancestor of flowering plants.

For example, the gene CRABS CLAW (CRC) is a member of the YABBY family of transcription factors that controls gynoecium and nectary development in A. thaliana (2, 5). CRC is expressed in the abaxial (outer) side of the developing ovary wall. One of the effects of crc mutations, in double mutant combinations with other mutated genes, is a breakdown in adaxialabaxial (inner-outer) polarity in the ovary wall (10). An orthologue of CRC from Amborella trichopoda, AmbCRC, has recently been shown (12) to be expressed in the ovary wall in a very similar manner to CRC in A. thaliana (Fig. 2A and B). These data suggest that the CRC and AmbCRC gene lineages have been performing the same role in ovary wall development throughout the radiation of the extant angiosperms (Fig. 2C). Interestingly, an orthologue of CRC from rice, DROOPING LEAF



Figure 2. Evidence that the CRABS CLAW gene lineages in Arabidopsis and Amborella have conserved a common role in carpel development since the ancestor of the flowering plants. A. Non-radioisotopic in situ hybridization showing abaxial-specific ovary wall expression of AmbCRC (dark colouration) in carpels of an Amborella trichopoda female flower bud in longitudinal section (c = carpel, t = tepal, r = receptacle). Colouration in tepal tissue is natural colour, present also in control samples (not shown), rather than hybridization to the probe. B. Non-radioisotopic in situ hybridization showing abaxial-specific ovary wall expression of CRC (dark colouration) in the gynoecium (g) of an Arabidopsis thaliana flower bud in transverse section. C. Mapping expression data of CRC orthologues onto a simplified phylogeny of the angiosperms. Rice data is based on Yamaguchi et al. (35).

(DL), controls the boundary between the third and fourth floral whorls, and also plays a role in leaf development (35). If we accept the ANITA lineages as basal in the flowering plant phylogeny, then the most likely explanation for the novel role and expression pattern of DL is that these evolved specifically in the monocot lineage, after the separation of the monocots from the remaining lineage leading to eudicots and other groups (Fig. 1 and 2C).

By similar "evo-devo" style approaches, the evolutionary lineages of a further number of flower development genes have been retraced back to the ancestor of the flowering plants (1, 12, 33, 34, 36), or even further back to the ancestor of all extant seed plants (1, 4, 14, 19, 27, 38). Detailed expression studies may be used to determine the likely extent of conservation of function between gene orthologues from model plants, ANITA angiosperms and gymnosperms. In addition, mutants of model plants may be genetically transformed using constructions containing orthologous genes from distantly related taxa to demonstrate the possible conservation of protein activity (28, 32, 38). In addition to these approaches, it would be very useful to generate gene-knockouts in basal angiosperms and gymnosperms to directly assess the roles of flower development orthologues in these groups. For this reason, genetic transformation procedures in nonmodel plants, and also virus-based gene silencing systems (6), will probably form essential future research tools for evo-devo studies.

The mysterious origin of flowering plants

Molecular phylogenetic studies confirm that, having first separated from the lineage of extant gymnosperms around 300 million years ago (MYA), some unknown, gymnosperm-like lineage passed the next ~140 MY without leaving any obvious indication of its presence in the fossil record (Fig. 1). Then, in the early Cretaceous Period, this lineage evolved into the ancestor of the flowering plants, which subsequently diversified to form the estimated 300 000 species of angiosperms alive today. One of the most often cited quotations in evolutionary biology is that of Charles Darwin, in a letter to James Hooker, the director of the Royal Botanic Gardens at Kew (24), who described the above sequence of events as an "Abominable Mystery!"

It is possible that molecular data, in addition to other approaches, may help to provide solutions to Darwin's abominable mystery. Notably, several

theories have been proposed to explain how the flower became bisexual from a unisexual gymnosperm reproductive axis. One of these, the Mostly-Male Theory (13, 15), proposes the bisexual flower to be derived essentially from male structures, upon which ectopic ovules had developed. An alternative theory, Out-of-Male/Out-of-Female (29), proposes that bisexuality in the flower arose either by the feminisation of male reproductive organs, or the opposite of this, due to the displacement of expression boundaries of B-function or B-Sister genes along a previously unisexual axis. Such alternative theories generate different predictions of sex-specific gene expression in angiosperms, as compared with gymnosperms. It is possible that data from largescale EST sequencing projects of early-diverging taxa (1) may be used to choose between such alternative explanations for the origin of the flower. Comparative molecular data may also help to explain the evolution of other characteristics that are unique to the angiosperms, including the carpel, the outer ovule integument, endosperm tissue and perianth organs.

In addition to determining the molecular evolutionary events responsible for the origin of the flowering plants, we should also consider the ecological pressures that might have contributed to the sudden rise of this group. Perhaps the most plausible ecological explanation for the success of the angiosperms relates to their possible coevolution with newly evolved groups of insects that acted as novel pollination vectors (18). However, selective pressures relating to changes in the browsing patterns of herbivorous dinosaurs and early mammals, or to changes in atmospheric CO₂ levels, may also have been factors in the origin and divergence of the angiosperms, as critically reviewed by Barrett and Willis (3). It is clear that we should integrate evidence from many disciplines, including molecular and anatomical studies of living groups, the analysis of fossils, and ecological and evolutionary theories, if we want to paint a fuller picture of early flowering plant evolution.

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