Genetic control of diverse inflorescence architectures

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2007

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SUMMARY

Plants and animal display an amazing variation in architecture of their body that is thought to result from evolution. Although it is widely accepted that evolution proceeds via mutation and selection, it is largely unknown how this has lead to the wide variation in size, architecture and appearance that is seen today. The classical view was that major changes resulted from the accumulation of many mutations that are each of small effect. However, genetic evidence indicates that needs not be true, as in many species mutants have been found in which genetic alterations in a single master gene can have profound impact on body shape. However, a coherent picture how such alterations contributed morphological variation is still lacking. Plants provide an interesting and promising system to study the evolutionary diversification of body shape. Higher plants diverged relatively recently and exhibit a large variation in body architecture, in particular of their inflorescences in several plant species, some of which are amenable for genetic experiments.

Although the inflorescence structures vary immensely between and within plant families, the specification of meristem/organ identity seems to be determined by conserved genes. Nonetheless, species differ from one another developmentally and, thus, also morphologically. To understand how similar molecules can generate diversity one needs to characterize and compare gene function and expression between different species. In our experiments we compared two distinct species with different inflorescence: the world's plant model system, *Arabidopsis*, and petunia.

Post-embryonically plant development is driven by groups of stems cell that are located at the apices of shots and roots, which are known as meristems. After the transition to flowering, the shoot apical meristem is not longer responsible for the stem growth and the formation of leaves, but for the formation of flowers and is called an inflorescence meristem (IM). The main difference between the inflorescence architectures of petunia and *Arabidopsis* is the fate of the IM. In *Arabidopsis*, the IM is indeterminate, retaining its meristematic properties

throughout the plants life span, and the flower meristems (FMs) are formed at the flanks of the IM, producing a raceme inflorescence structure. In petunia, the IM terminates in an FM, producing a terminal flower, and the continuity of the inflorescence growth is assured by the formation of a secondary IM (or sympodial meristem) at the side of the FM. The specification of flower meristem identity (FMI) in the FMs is essential for flower formation as well as the depletion of FMI in the IM is essential for the maintenance of meristematic fate. What happened between the regulation of the FMI and IM genes of *Arabidopsis* and petunia that lead to such morphological differences?

Evolutionary changes in developmental and metabolic systems result from morphological changes. These changes are generally the result of co-option events involving the acquisition of novel gene function (for review True and Carroll, 2002; Carroll, 2005; Prud'homme et al., 2007). Co-option can occur without duplication of the ancestral gene by changes in the protein coding sequence and/or by acquisition of a novel expression pattern.

In **Chapter 2**, I compared the genes that control the switch from vegetative growth to flowering and that specify floral meristem identity in *Arabidopsis* and petunia. I present evidence that the petunia and *Arabidopsis* FMI factors ALF and LFY (ABBERRANT FLOWER AND LEAF and LEAFY), as well as DOT and UFO (DOUBLE TOP and UNUSUAL FLORAL ORGANS) are exchangeable and able to trigger similar downstream events within each plant species. Our results suggest that the F-box proteins DOT and UFO are required for post-translational activation of the transcription factors ALF and LFY, possibly via ubiquitination, and that this is essential for the switch to flowering, specification of floral meristem identity and the activation of floral organ-identity genes. However, the differences observed between plant species rely on the differential expression patterns of *ALF/LFY* and *DOT/UFO* within their plant of origin. In *Arabidopsis*, FMI is acquired by expression of *LFY* (*UFO* is present in the IM at all times) at the flanks of the IM, while in petunia, the expression of the *ALF* and *DOT* at the apex of the IM promotes its conversion into a terminal FM. These observations suggest that the establishment of the expression domains of these FMI is one of the key steps for the determination of the inflorescence architecture. The different expression patterns

have evolved most likely by changes in the *cis*-regulatory regions that can cause the gene to be regulated by different transcription factors. However, we cannot exclude the possibility that the distribution of a *trans*-regulatory factor could have changed, while its target *cis*-regulatory sequences remain conserved. To distinguish between these two possibilities more experiments would have to be performed making use of the non-coding sequences of *ALF/LFY* and *DOT/UFO* and testing their activity in each plant species.

Other modes of co-option involve gene duplication. Gene duplication creates initially redundant paralogs and can be followed by divergence in the amino acid sequence and/or regulatory DNA sequences.

In **Chapter 3 and 4**, we present the isolation and characterization of *EVG (EVERGREEN),* which is required for the formation of the sympodial inflorescence meristem, the key feature that distinguishes cymose inflorescence from solitary flowers. The results show that *EVG* encodes a homeodomain transcription factor that appears to promote proliferation of the sympodial IM by activating the synthesis of auxin. Although *Arabidopsis* lacks sympodial branching it does contain 1 or 2 clear orthologs of *EVG* (named *WOX8* and *WOX9* or *STIMPY*) that encode proteins with (nearly) identical activity, but which have a very different expression pattern. Petunia contains at least one *EVG* paralogs, *SISTER OF EVERGREEN* (*SOE)* with an expression pattern different from *EVG* but more similar to *WOX9/STIP*. Our data suggest that *EVG* and *SOE* arose by a recent gene duplication, and acquired different functions by divergence of their expression pattern. On one hand, the protein function of both genes remained conserved, as both genes seem to be involved in promoting cell proliferation through the induction of auxin biosynthesis. On the other hand, the divergent regulation of *EVG* leads to the acquisition of a new gene function, as the expression pattern of *EVG* got restricted to the sympodial meristem, essential for petunia cymose inflorescence architecture. We believe that this sub-functionalization contributed greatly to the evolution of a novel trait, the sympodial growth.

To examine whether the formation of new meristems and organ primordia relies not on auxin transport alone, as is widely accepted, but also on the sites where it is synthesized, I examined another mutant, named *floozy* (*fzy*). In *fzy*

mutants the formation of floral primordia is disrupted at an early stage. **Chapter 5** shows that organ formation in *fzy* can be restored by local expression of a bacterial gene (*IAAM*) encoding an enzyme that can synthesizes auxin, implying that the absence of floral organs in *fzy* is due to reduced local auxin synthesis

Overall the results show how important changes in the mechanisms controlling gene expression have been for the evolution of plant inflorescence architecture. These conclusions are in accordance with the believe that variations in the level, pattern or timing of gene expression provide more variation for natural selection to act upon than changes in the gene product alone (Carroll, 2005; Prud'homme et al., 2007).

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