Minireview

On the origin of floral morphological novelties

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Abstract Floral morphological novelties, like homeotic changes of whorl 1 organs, can easily arise by modifying existing regulatory networks. Ectopic expression of B-function MADS-box genes in whorl 1 leads to a replacement of sepals by petals, as is found in the *Liliaceae*. In cases where leaf-like sepals or even inflated calyces develop, which ultimately envelop the mature fruit as in *Physalis*, ectopic expression of a vegetative MADS-box gene seems to be responsible. Current knowledge concerning the origin of such morphological novelties is reviewed.

Keywords: Ectopic expression; Transcription factor; MADS-box gene; Morphological novelty

1. Introduction

In the evolution of life forms, an ever-increasing level of complexity is observed. Among higher plants this becomes apparent in many traits, for example in the increasing complexity of leaf shape, inflorescence structure and flower form or structure just to name but a few.

Here, we will consider only certain floral structures and their diversity during evolution.

2. Floral architecture

Some basal angiosperms like the *Piperales* have no perianth organs covering developing sexual organs; in most higher eudicots a well-developed calyx and corolla protect these.

Most flowers of higher eudicots consist of four whorls of organs. The inner whorls bear the sexual organs, the females in the center whorl and the males in whorl 3. The corolla often made of individual petals in whorl 2 surrounds these. Petals serve in most cases as attractants for pollinators. In the outermost whorl the calyx consists of sepals, which protect the developing flower bud and in some cases the developing fruit is also formed. Fig. 1-1 shows a cross-section through a flower of *Antirrhinum majus*, one of the plant model systems, in which the individual organs are color-coded. Genes encoding MADS-box transcription factors define the identity of these four types of organs [1–4]. Other transcription factors, like TCPs, are involved in determining the symmetry of the flower and especially the symmetry of the petals in whorl 2 [5,6]. Some of these genes have been mapped molecularly on the eight chromosomes of *A. majus* [7]. Mutations within these genes can result in new floral structures.

The diversity in petal symmetry of the corolla will be considered next.

3. Symmetry changes of whorl 2 organs

Among 241 families of higher plants actinomorphy and medial zygomorphy were the most common symmetry types. Actinomorphy was found in 83% of dicot and 72.4% of monocot families, while medial zygomorphy was found in 33% of dicot and 44.8% of monocot families [8].

Flowers of *A. majus* consist of 5 petals, which are not of equal size and form resulting in bilateral symmetry. Apparently two genes, *Cycloidea* [5] and *Dichotoma* [6] encoding TCP transcription factors specify this zygomorphy. Mutations in either gene lead to semi-radial flowers, while double mutants are perfectly radial (compare Fig. 1-2a and -2b).

In peloric *Linarias*, the *CYC* gene is inactivated by methylation resulting in radial flower formation [9] (compare Fig. 1-3a and -3b). A comparison between the zygomorphic *A. majus* flower and the more actinomorphic flower of *Mohavea* has also been shown to affect the expression domain of *CYC* [10]. Apparently, the allelic states of *CYC* and *DICH* control the expression of these genes and thus determine floral symmetry.

In the *Leguminosae*, in which zygomorphy seems to have evolved independently, a phylogenetic investigation of *CYC*-like *TCP* genes revealed that numerous copies are present in the genome. Hence, orthology is difficult to establish and thus the role of *CYC*-like genes in zygomorphy remains obscure [11].

The discussion of whorl 1 organ identity requires a more detailed introduction.

4. Floral homeotic changes

Floral homeotic mutations, in which one type of organ is replaced by another type of organ, have been described in various species [12]. Interestingly, such mutations in most species affect organ identity of two adjacent whorls. The most prominent example is the filled rose, already described in 1790 by Goethe [13], in which all sexual organs are replaced by

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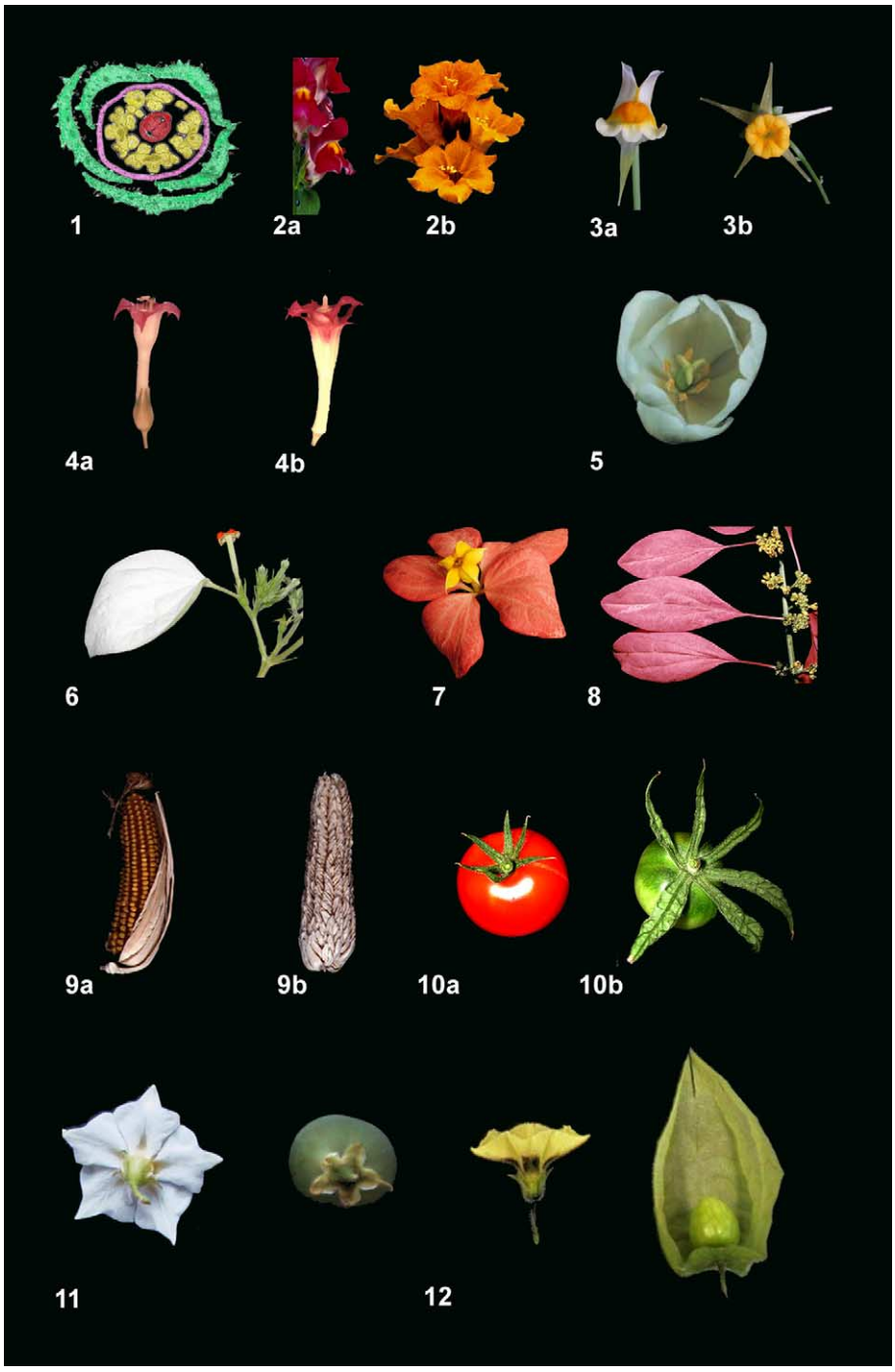

Fig. 1. 1: Cross-section through a flower of *A. majus* featuring the four whorls of organs (color-coded): 5 sepals (green), 5 petals fused to form a tube (pink), 4 stamen (yellow) and 1 stamenoid (yellow) and the bilocular carpel (red). 2a: Wild type flowers of *A. majus* featuring bilateral symmetry, 2b: phenotype of a double mutant *cyc/dich* featuring radial symmetry and an increased number of floral organs. 3a: Zygomorphic flower of *Linaria vulgaris*, 3b: epigenetic *cyc* mutation of *L. vulgaris* resulting in an actinomorphic flower. 4a: *N. tabacum* wild type flower, 4b: transgenic *N. tabacum* expressing the B-function genes, *DEF* and *GLO*, from *A. majus* ectopically. The resulting flower features a novelty: petals instead of sepals in whorl 1 and thus two whorls of petals. 5: Flowers of *Tulipa gesneriana* consist of 2 whorls of petals. 6: In *M. frondosa* a white leaf replaces one sepal. 7: In *M. erythrophylla* red leaves replace the 5 sepals. 8: In *W. coccinea* a red leaf replaces one sepal. 9a: All kernels on a cob of *Z. mays* are naked, 9b: all kernels on the cob of the dominant *Tunicate* mutation are covered by glumes. 10a: *L. esculentum* has a deeply dented calyx, 10b: a *lemads-mc* mutant features leaf-like sepals. 11: Flower and fruit of *S. tuberosum* feature small sepals (front half of the calyx removed to display the fruit). 12: Flowers of *P. floridana* feature small sepals, which upon fertilization start growing and ultimately enclosing the mature fruit.

reiterating perianth organs, i.e., petals and sepals. A complementary type of mutant is also observed, in which sexual organs replace all perianth organs. Last but not the least, a third type of homeotic mutant is seen, in which the organs of whorls 2 and 3 are replaced by other types, sepals replace petals and carpels replace stamen. It is worth mentioning that one mu-

tational step can change the identity of organs in two adjacent whorls. This was the basis for establishing the ABC model of floral organ identity [14] according to which an A-function is responsible for sepal formation, A + B for petals, B + C for stamen and C-function for carpel formation.

Most ABC-function genes encode MADS-box transcription factors. These proteins form dimers [15,16] and/or tetramers of different composition [17,18]. This has recently been summarized in a model called "Floral Quartets" [19]. Different "Quartets" are involved in the establishment of floral organ identity.

5. Sepal identity changes: petals instead of sepals in whorl 1

The synthesis of new "quartets" in a given whorl should lead to a change in the identity of the organs in that whorl. For example, if B-function genes, like *Def* and *Glo*, were expressed ectopically in the first whorl, then petals should replace sepals and hence such "mutants" should reveal two whorls of petals as has been shown for *Nicotiana tabacum* [20] (compare Fig. 1-4a and -4b).

Has nature taken advantage of this possibility, for example in the evolution of the *Liliaceae*, members of the monocots? Lilies and tulips, instead of featuring flowers with four whorls of different organs, have two whorls of petals, so called tepals in their perianth (Fig. 1-5). Previously, it has been suggested that these might be the result of ectopic expression of B-function [4,21]. Indeed, molecular analysis has recently provided evidence for this assumption in the case of *Tulipa gesneriana* [22].

Thus, in the evolution of floral structures like in the *Liliaceae* the presence, absence or composition of particular floral quartets have "engineered" their novel floral morphology.

6. Sepal identity changes: leaf-like and colored sepals

Leaf-like and colored sepals are found within the *Rubiaceae*. While most species within this family have mostly reduced calyces, in the genus of *Mussaenda* species feature an altered calyx. Either one or all of the sepals are changed to leaf-like structures with different colorations (Fig. 1-6 and -7 for *Mussaenda frondosa* and *Mussaenda erythrophylla*, respectively). Similar changes are also observed in *Warszewiczia coccinea* (Fig. 1-8).

Clearly these traits are morphological novelties. However, since these species are either evergreen shrubs or small trees from tropical areas in Asia and in Africa no molecular analysis of these features has been carried out so far.

7. Molecular analysis of the foliose-sepal-syndrome

Mutants featuring foliose-sepal-syndrome (FSS) (leaf-like sepals) or similar structures have been isolated in several model species like *Zea mays*, *Lycopersicon esculentum* and *Arabidopsis thaliana*.

7.1. *The Tunicate mutation of* Z. mays

One of the oldest mutants known is the dominant Tunicate mutation [23]. While in wild type maize each kernel is naked (Fig. 1-9a), kernels of the Tunicate mutant are entirely wrapped in glumes as is shown in Fig. 1-9b. This strange looking cob is then covered as usual by husks. Molecular analysis of Tunicate revealed that the MADS-box gene *ZMM19*, whose expression in the wild type is restricted to leaves and husks, is now expressed ectopically in all floral organs. The mutational event that led to this unusual expression pattern is rather complex and involved changes in the promoter region and the copy number of the affected gene. The duplicated loci could be separated by recombination resulting in less severe phenotypes. The strength of expression of *ZMM19* in male and female inflorescences is determined by the structure and sequence of the locus and correlates with the strength of the phenotype [24]. The ectopically expressed ZMM19 protein seems to promote growth of the glumes, which then ultimately cover the entire kernel (compare Fig. 1-9a and -9b). Even though glumes are not necessarily homologues to sepals, they might serve as a model for the type of genes involved in FSS of higher eudicots. This assumption is corroborated by our preliminary observation that *ZMM19* ectopic expression in *A. thaliana* leads to foliose sepal formation (Fig. 3). *ZMM19* is a member of the *STMADS11*-clade (Fig. 2) [25,26] of MADS-box genes and genes belonging to this clade may lead to a higher proliferate potential of vegetative tissues [27].

7.2. *Leaf-like sepals in a mutant of* L. esculentum

Recently, a mutant of *L. esculentum* affecting sepal size (compare Fig. 1-10a and -10b) and form has been published in which the *LeMADS-MC* gene is affected [28]. Unlike *ZMM19* of *Z. mays* described above, *LeMADS-MC* belongs to the SQUA-clade of MADS-box genes (see Fig. 2) [29].

These results seem to indicate that two different MADS-box genes can generate FSS, ectopic expression (see below) of a vegetative *STMADS16*-like gene (Figs. 1-9, -12 and 3) or a knockout mutation of a *SQUA*-like gene (Fig. 1-10b).

7.3. *Leaf-like Arabidopsis plain mutants and transgenic plants*

The above findings are corroborated by phenotypes of either *A. thaliana* mutants or by phenotypes of transgenic *A. thaliana* plants expressing heterologous genes of the *STMADS11* clade.

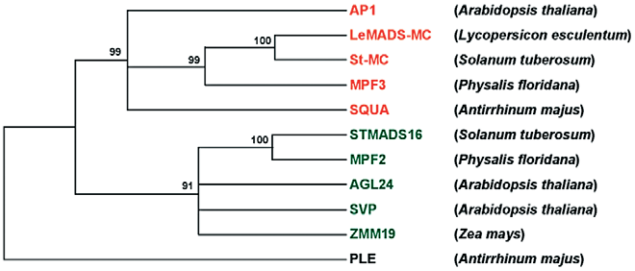

Fig. 2. A most parsimonious phylogenetic tree of MADS-domain proteins of the SQUA-subclade (shown in red) and the STMADS11-subclade (shown in green) using PLE as an outgroup. Only selected members of these subclades are shown. Instead of the name giving member STMADS11 another gene from *S. tuberosum*, STMADS16, is given as representative of this clade. The corresponding species are indicated in brackets. Sequence data were taken from GenBank. Sequences of St-MC, MPF3 and MPF2 are unpublished (He and Saedler). Detailed phylogenetic studies of MADS-box genes are given by Theissen et al. [4].

Fig. 3. Phenotypes of wild type and of various transgenic plants. A flower and a silique of the Columbia ecotype is shown as the wild type. Ectopic expression of *STMADS16*-like genes (*35S::ZMM19*, *35S::SVP* and *35S::AGL24*) in this *A. thaliana* ecotype lead to leaf-like sepals and evergreen flowers. *STMADS16*-like genes from *Solanaceae* have not been introduced into *Arabidopsis* yet. However, expression of *35S::STMADS16* in *N. tabacum* featured leaf-like calyces [26].

Knockout mutations of the *AP1* gene of *A. thaliana*, an orthologue of *SQUA*, showed a complex phenotype including large or foliose sepals [30]. *AP1* is considered an A-function gene and besides that also controls the expression domain of the C-function gene *AG*. *AP1* is also involved in the transition of vegetative to generative growth. If mutated the vegetative growth phase seems to extend into floral development generating foliose sepals.

Ectopic expression of *AGL24*-, *SVP*- and *ZMM19*-genes, all belonging to the *STMADS11*-clade (Fig. 2), results in FSS in *A. thaliana* (Fig. 3). Another member of the *STMADS11*-clade, *STMADS16* from potato, which is expressed only in vegetative tissue in *Solanum tuberosum*, if expressed ectopically in *N. tabacum* also leads to FSS [26].

Therefore, even in *A. thaliana* at least three types of MADS-domain proteins, AP1, SVP and AGL24, can control the size of sepals.

8. The inflated-calyx-syndrome (ICS): a morphological novelty

Recently, Knapp [31] provided a phylogenetic perspective on fruit diversity in the *Solanaceae*. Besides this, *Solanaceae* also feature diversity within other traits, for example within flowers. Among the 96 genera of *Solanaceae*, including some 2297 species, only a few genera feature inflated calyces [32].

While the size and the form of calyces in *Solanaceae* range from dented as in potato (*S. tuberosum*, Fig. 1-11), deeply toothed (Tomato: *L. esculentum*, Fig. 1-10a) tubular (*Datura stramonium*), to inflated as in the "Chinese Lantern" (*Physalis* sp., Fig. 1-12), the majority has small sepals as compared to the size of the petals. Species within the genera *Anisodus*, *Nicandra*, *Physaliastrum*, *Physalis*, *Physochlaina*, *Przewalskia* and *Withania* feature an ICS, which ultimately covers the developing and mature fruit (an example is given in Fig. 1-12). The function of the inflated calyx is not entirely clear, but in certain species like *Przewalskia tangutica* it seems to reduce the specific weight and thus might facilitate wind dispersal of the fruits [31].

Several questions dealing with the origin of ICS come to mind concerning:

- Closest non-ICS featuring relative(s).
- Site(s) of origin.
- Gene(s) involved.
- Mutational event(s).

For taxonomic reasons the closest relatives have to be searched within the *Solanaceae* [32]. This is corroborated by molecular analysis using *rbcL* and other genes in phylogenetic reconstructions. Clearly *Physalis* is closely related to *Solanum* [33].

The majority of the roughly 1000 *Solanum* species have small sepals compared to their petals. However, in a few species such as *Solanum aetiopicum* and in some *Solanum macrocarpon* (Eggplant) populations the sepals can be as long as the petals and may grow out to cover a major part of the fruit. While these *Solanum* species are native to Africa, the vast part of the 75 *Physalis* species, a closely related genus, grows in Central America (the majority in Mexico, 21 in Guatemala and 10 in Nicaragua and 9 in Panama) [32]. Another genus, *Przewalskia*, featuring ICS exclusively grows in alpine regions of China.

Does this suggest polyphyly of the ICS? Studies based on morphology [34] and on chloroplast DNA [35] suggest even polyphyly of *Physalis*, but this has to be verified molecularly using trait determining genes.

While most *Physalis* species are of Central American origin, *Physalis alkekengi* seems to have originated in the Old World (Eurasia) as is suggested by a picture of *Physalis* in Codex Aniciae Julianae dating before 512 [36].

Molecular analysis of the gene(s) involved in the trait might provide a more conclusive answer. However, a more detailed molecular phylogeny within the *Solanaceae* including many species of the above genera will be essential to define the closest relative of *Physalis*. For this purpose several collections can be used.

Recently, we have initiated research aimed at a molecular understanding of ICS based on findings in the above described model systems. Preliminary results indicate that indeed *MPF2*, a MADS-box gene belonging to the *STMADS11*-clade (Fig. 2), which in *S. tuberosum* is expressed vegetatively, in *Physalis floridana* is ectopically expressed in floral structures. Ectopic expression of this gene seems to be essential, but it is not sufficient. At least one other component, fertilization is required as well. In emasculated flowers no ICS develops even though *MPF2* is expressed in the sepals, suggesting that a signal emitted by fertilized ovules might be involved. In addition, a third factor, *MPF3*, which belongs to the *SQUA*-clade of MADS-box genes ([29], Fig. 2) might also contribute to ICS. Further molecular details, however, have to be unraveled in order to fully understand development and evolution of ICS.

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