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### PLANT BIOLOGY

# Unusual suspects in flower color evolution

The expression of phased small interfering RNAs caused monkeyflower color evolution

By Marie Monniaux

Pigmentation in flower petals mainly relies on anthocyanins and carotenoids, two classes of pigments whose biosynthetic and regulatory pathways are well characterized (1) and constitute theoretically ideal targets for evolution to create new color patterns. However, evolution of flower color sometimes works in mysterious ways. On page 576 of this issue, Liang *et al.* (2) show that a major speciation locus in *Mimulus* (monkeyflowers) that causes change in flower color contains a noncoding inverted repeat region. This repeat produces a long noncoding RNA that is cleaved into small interfering RNAs (siRNAs) in a regularly spaced (“phased”) pattern, and some of these siRNAs regulate the translation of a carotenoid biosynthesis regulator.

The finding that flower color change was caused by an inverted repeat that produces phased siRNAs constitutes an unexpected molecular mechanism for flower morphological evolution.

Flowers display a stunning diversity of shapes, sizes, and colors, which makes them amenable to studying the mechanisms that underlie morphological evolution. Flower color is a deceptively simple trait that has been extremely labile during evolution: Sister species from several genera can show substantially different pigmentation patterns, and repeated shifts in flower color are observed within a single genus. In most cases, changes in flower color during evolution are simply caused by regulatory or coding mutations in the genes that control pigment biosynthesis. For example, the evolution of red floral color in *Petunia* involved changes in multiple players of the anthocyanin biosynthesis pathway (3).

However, this is not always the case, according to the findings of Liang *et al.*, who

looked for the molecular determinant for carotenoid deposition in petals of monkeyflowers. Out of the many different coloration phenotypes found in this genus (see the photo), Liang *et al.* focused their study on two of them: flowers with pale pink petals (low levels of anthocyanins and no carotenoids) and yellow nectar guides (high local amounts of carotenoids) and flowers with bright red petals (a combination of high levels of both anthocyanins and carotenoids).

A single locus called *YELLOW UPPER* (*YUP*) was found to govern carotenoid deposition in monkeyflowers but was never finely mapped (4). *YUP* is a classical example of a speciation locus because exchanging *YUP* alleles between species is sufficient to directly influence pollinator visits and cause reproductive isolation of emerging species (5). By crossing two monkeyflower species that had not been used in previous studies, Liang *et al.* narrowed down *YUP* to an interval containing eight protein-coding genes. However, they found that reducing the expression of these

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The petal pigmentation of *Mimulus* (monkeyflowers) involves a long noncoding RNA that generates small interfering RNAs.

ight genes did not affect carotenoid deposition. The authors instead identified a long inverted repeat that was being actively transcribed in this region; this repeat produced abundant secondary siRNAs in a phased pattern, called phasiRNAs (6). PhasiRNAs can originate from a long noncoding RNA that is cleaved into secondary siRNAs. Cleavage sites in the RNA that contains phasiRNAs are regularly spaced, which generates a phased accumulation of siRNAs. But of the pool of phasiRNAs that accumulates, one siRNA is more abundant, and, in the case of *YUP*, the major siRNA matched the sequence of *REDUCED CAROTENOID PIGMENTATION 2* (*RCP2*), which encodes a major activator of carotenoid biosynthesis (7).

Liang *et al.* found that a functional *YUP* locus would produce abundant siRNAs that target *RCP2* and thereby reduce carotenoid accumulation in the petals of the pale pink flowers. The authors demonstrated that the siRNA targets *RCP2* mainly by reducing its translation. Moreover, they provided a satisfactory explanation for why carotenoids accumulate in the yellow nectar guides, which serve as guiding signals for bumble bee visitation: They found that another activator of carotenoid biosynthesis, *RCPI*, is present in nectar guides and that *RCPI* represses *YUP* transcription so that the phasiRNAs are not produced. In species with

bright red-colored petals, mutations in the *YUP* sequence have disrupted the phased pattern of phasiRNA production, and, as a result, the major siRNA that targets *RCP2* is not produced and carotenoid accumulation is not repressed.

Liang *et al.* also found that the sequence of *YUP* strongly matches that of a *CYTOCHROME P450* (*CYP450*) gene found a small distance away, suggesting that *YUP* originated from the duplication and inversion of part of this gene. *CYP450* enzymes take part in a large range of biosynthetic reactions during plant development (for example, they participate in the production of membrane sterols, plant hormones, or defense compounds) but were unlikely to be specific players in flower color evolution, and they share no phylogenetic relationship with *RCP2*. Pinpointing the unexpected origin and mode of action of the *YUP* locus would have been impossible without a well-established model genus such as *Mimulus*, which comes with genomic information and efficient transgenesis protocols.

Liang *et al.* also found *YUP* to be physically linked to two regulators of anthocyanin production in petals—*PETAL LOBE ANTHOCYANIN* (*PELAN*) (8) and *SISTER OF LIGHT AREASI* (*SOLAR*) (2)—in a small region that they propose acts as a superlocus that controls petal pigmentation. A superlocus (or supergene) is a group of genes that participate in a related function and are physically close on a chromosome; these genes are therefore inherited as a single locus. This allows a favorable combination of alleles to be maintained through generations, which is particularly important to avoid the dissolution of pollination syndromes (the suite of traits jointly recognized by pollinators) over generations. A superlocus in *Petunia* contains genes that control petal visible color, ultraviolet absorption, floral scent production, pistil length, and stamen length (9). In monkeyflowers, the *YUP-PELAN-SOLAR* superlocus controls most of the carotenoid and anthocyanin accumulation in the petals, which could maintain the two distinct petal pigmentation phenotypes that are recognized by different pollinators.

That noncoding regions of the genome, such as the *YUP* locus, underlie morphological evolution is not conceptually new, but evidence for such mechanisms is rare (10). Intriguingly, recent studies have identified other unusual molecular mechanisms that are involved in floral evolution. For example, similar to *YUP*, the recent appearance of a yellow pigmentation pattern in the petals of *Antirrhinum* (snapdragon) is caused by the *SULF* locus, an inverted duplication that generates siRNAs that target a pigment biosynthesis gene (11). In monkeyflowers, a

reduction in anthocyanin accumulation in pale pink petals was found to be caused by an alternative start codon in the 5' untranslated region of *PELAN*, which causes reduced translation (12). This was unforeseen because most of the cis-regulatory changes that have been found to shape phenotypic variation affect transcription (10). In *Capsella*, the reduction of petal size has recently evolved through mutations in the *CYP724A1* gene, a member of the *CYP450* gene family that is involved in the synthesis of brassinosteroids, a plant hormone that controls cell proliferation (13). The mutations were found in the exons of *CYP724A1* and increased splicing efficiency and transcription (13). Excessive levels of brassinosteroids limit cell proliferation, which results in smaller petals. That exonic mutations affect splicing efficiency is rarely reported because they are more likely to affect protein function, and this effect could have been completely overlooked.

Therefore, it appears that each step of the regulation of gene expression, in all its complexity (including recruitment of transcription factors, initiation of transcription, splicing, mRNA stability, and translation efficiency), might be a possible target for morphological evolution. In particular, mutations that affect protein translation or stability have been largely overlooked (14), but researchers of evolution now have to address these types of challenges for the field to move forward.

Is there something special about flower evolution that explains why it involves uncommon molecular mechanisms more frequently than the evolution of other traits in plants? It is probably more likely to be the case that flowers are visually attractive [and scientists are as sensitive to this as insects can be (15)] and their morphology is extremely diverse and directly affects reproductive isolation and speciation; therefore, they constitute an excellent system to explore the molecular determinants of morphological evolution. This has revealed the unforeseen but likely widespread ways that diversity can be generated through evolution. ■

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