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The *Sfm* system of gene control in *Antirrhinum majus*

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Abstract. In *Antirrhinum majus*, *pal-rec*, an allele of the *pallida* series, is considered to be involved in anthocyanin production. The *pal-rec* gene normally mutates autonomously from the recessive condition to the dominant giving fully pigmented *Pal* spots on a colorless background. The *Sfm* element, when inserted at the *pal* locus, destabilizes the activity of the *pal-rec* gene in such a way that the repressor (*Sf*) component abolishes gene activity in almost all of the cell lineages, whereas the mutator (*m*) acts to release full gene expression in some of the cells. Such a flip-flop control of the *pal-rec* results in what is known as shifting.

Key words: Floral instability – Transposable elements – Shifting factors – Mutability shifts – *Antirrhinum majus*

Introduction

One of the striking features of controlling elements is their determination of diverse patterns of mutability expression. These patterns are the consequence of two events; namely, timing and frequency of mutation. Large sectors of mutability shifts, which contribute to a relatively greater amount of tissue during ontogeny, result from mutations occurring early in flower development. Mutations occurring late in development contribute to a smaller amount of tissue that express the activity of the gene for a shorter period. Shifting frequency is related to the number of mutational events controlled by a regulatory element. The combination of timing and mutation frequency results in specific phenotypic patterns of mutability, which were previously referred to as "states" of the receptor or regulatory elements in maize (Fincham and Sastry 1974).

The investigation of *Antirrhinum majus* presented here shows that shifts in a heterozygote do occur and on a recessive background, result in different sectors of mutability that can vary in size, shape, and intensity, and depend upon an interaction between the element *Sfm* and the gene. The integration of repressor element into the *pal-rec* gene almost completely suppresses its activity, while presumably its accurate excision through mutations of the regulatory element enables the gene to express itself.

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Materials and methods

The *Antirrhinum* strains used were originally obtained from the John Innes Institute several years ago and have been since maintained at Leeds. The first group comprises *pal-rec-sd pal-rec-sd* and *pal-tub pal-tub* strains and will be referred to as 'standard' and 'tester' genetic strains respectively. The second group, maintained as a sub-line of *pal-rec-sd pal-rec-sd,* shows regular shifts and will be reffered to as *pal-rec-JI Pal-rec-JI.*

The *pal-tub* tester is a recessive homozygous and produces colorless flowers except for a characteristic ring of pigment that appears at the base of the flower tube. This pigmented ring facilitates the separation of homozygous and heterozygous plants, i.e., *pal-rec-sd pal-tub* (pigmented flowers with a pale ring at the base of the flower tube) and *pal-rec-sd pal-rec-sd* (mutable flowers without a pigmented ring at the base of the flower tube). Highly unstable homozygous *pal-rec-sd* plants show a higher mutation frequency than their heterozygous *pal-rec-sd pal-tub* counterparts. Also, the former are free from the stabilizing modifier (*St*) identified by Harrison and Fincham (1968) which, when present, considerably reduces the levels of mutability.

Quantitative estimates of floral instability were obtained by scoring individual flowers against a standard scale consisting of 0–8 classes. On this scale class 0 represents flowers with no mutant spots, and fully colored phenotypes fall in the highest class; the intervening classes represent intermediate grades of instability.

Results

Changes in the degree of mutability within an individual plant – shifting as it is referred to here – is not that uncommon in plants carrying *pal-rec-sd*; what is uncommon is that shifting that is inherited. For several years the John Innes Institute has maintained a sub-line of *pal-rec-sd pal-rec-sd* that shows regular shifting, this is now denoted as *pal-rec-JI pal-rec-JI* (Fig. 1).

The consistency of this shifting and the possibility of a shift-causing factor evoked this investigation on whether the factor, if present, would affect standard *pal-rec* strains. To check this, *pal-rec-JI pal-tub* plants were crossed with *pal-rec-sd pal-rec-sd* plants. The shifting parents were also crossed with *pal-tub pal-tub* testers. The number of shifting plants from all three types of progeny (*pal-rec-JI pal-rec-sd, pal-rec-sd pal-tub-JI,* and *pal-rec-JI pal-tub* are given in Table 1.

Fig. 1. An inflorescence of gene constitution *pal-rec-JI pal-rec-JI,* showing shifting of high mutation rate

Confirmation of a shifting factor

To confirm if something other than *pal* is involved in producing shifting, *pal-tub pal-tub* segregants produced by selfing *pal-rec-JI pal-tub* plants were crossed with *pal-rec-sd pal-rec-sd* plants. If there is a dominant *pal* independent factor in the John Innes *pal-rec-JI pal-tub* line, then one should expect three types of *pal-tub pal-tub* plants: in homozygous *pal-rec-sd* crosses they should produce (a) 100% shifting (homozygous for the factor), (b) shifting and non-shifting types (heterozygous for the factor), or (c) all non-shifting plants (absence of the factor. These types should be in a 1:2:1 ratio.

Initially, to investigate these proportions, six *pal-tub pal-tub* segregants from shifting *pal-rec-JI pal-tub* genotypes were crossed with *pal-rec-sd pal-rec-sd.* It can be seen from the data presented in Table 1 that all six segregants caused *pal-rec-sd* to shift (Fig. 2). As one would expect from a test-cross of a heterozygous genotype, only half of the progeny on the whole showed shifting. It was proposed to use the gene symbol *Sfm* to denote the John Innes shifting factor segregating with *pal-tub pal-tub* (Sastry et al. 1981).

To supplement the data several *pal-tub pal-tub* plants obtained by selfing 45-522-7, 45-526-4 and 45-526-15 from the six used in the pilot experiment were crossed with *pal-rec-sd pal-rec-sd;* the progeny were scored for shifting, and based on that data the crosses (hence the *pal-tub pal-tub* parents) were classified as (a) 100% shifting, (b) 50% shifting, and (c) nonshifting (Table 2). The

Table 1. Number of shifting and non-shifting plants observed in the crosses *pal-rec-JI pal-tub* × *pal-rec-sd pal-rec-sd* (1) and the *pal-rec-JI pal-tub* × *pal-tub pal-tub* tester (2)

	Number of progeny raised	Number of heterozygous plants shifting[b]	Number of homozygous plants shifting[b]
A			
(1)	6	12 (43)	1 (45)
(2)	6	5 (36)	–
	Number of progeny raised	% of shifting plants[b]	% of non-shifting plants[b]
B	Effect of *pal-tub pal-tub* (segregants) from John Innes shifting line of homozygous *pal-rec-sd*		
	6	50.85 (59)	49.06 (56)
	2[a]	00.00 (00)	100.00 (40)

Statistical analysis: χ^2 for shifting and non-shifting plants = 0.08, $P > 0.05$; for test cross vs control: $t = 0.93$; $df = 6$, $P > 0.05$

[a] Control cross

[b] Number shown in brackets indicates the number of plants scored in each case

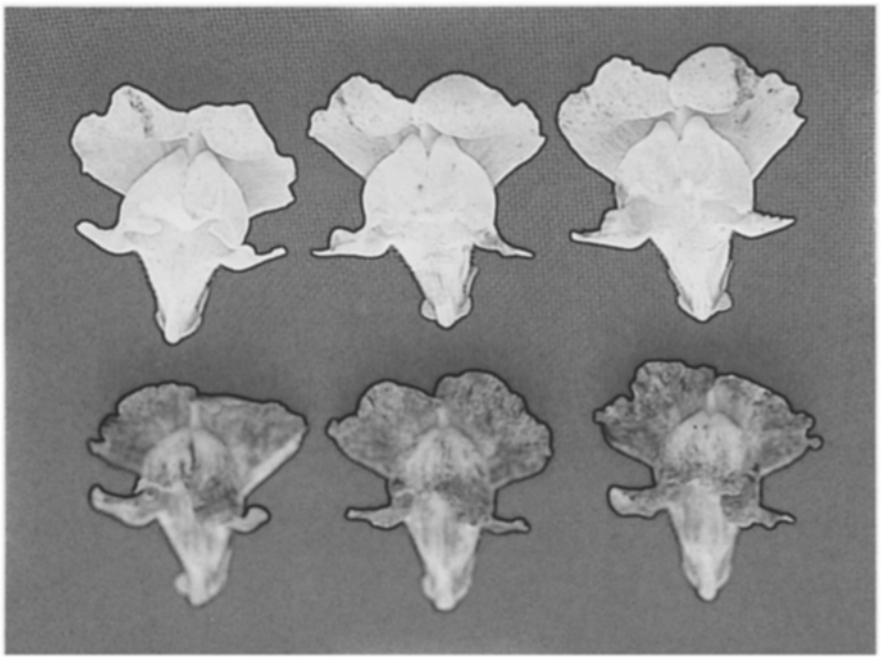

Fig. 2. *Top row: pal-tub pal-tub* segregants were crossed with *pal-rec-sd pal-rec-sd,* and the flowers from one representative demonstrate the activity of the *Sfm*-controlling element. *Bottom row:* Flowers from a *pal-rec-sd pal-tub* (tester) individual showing the absence of *Sfm* activity (control).

Table 2. Effect of homozygous *pal-tub pal-tub* with the shifting factor *Sfm* (tested in Table 1 and selfed) on *pal-rec-sd pal-rec-sd*

	Number of progeny raised	Number of shifting plants[a]	Number of non-shifting plants[a]
(a) Cross 1 with 100% shifting	5	92 (100.00)	00 (00.00)
(b) Cross 2 with 50% shifting	15	153 (51.95)	141 (48.04)
(c) Cross 3 with almost no shifting	6	5 (4.16)	96 (95.83)
(d) Cross 4 (control)	2	0 (00.00)	40 (100.00)

Statistical analysis: χ^2 for (a), (b) and (c) crosses (1:2:1)=0.69, $P>0.05$
χ^2 for (b) crosses only (1:1)=0.49, $P>0.05$

[a] Number shown in brackets indicates percentage of shifting and non-shifting individuals, respectively

Table 3. Effect of *St* on *pal-rec-JI*: *pal-tub pal-tub St St* plants were crossed with (a) *pal-rec-JI pal-rec-JI* plants (no known stabiliser) and (b) *pal-rec-sd pal-rec-sd* plants. Plants from both (a) and (b) were also pollinated with the *pal-tub pal-tub* tester

	Cross	Number of progeny raised	Number of plants scored	Mean score ± SD
(a)	*pal-rec-JI pal-rec-JI* × *pal-tub pal-tub St St*	15	300	0.64±0.15
(aa)	*pal-rec-JI pal-rec-JI* × *pal-tub pal-tub* tester	5	100	5.29±0.15
(b)	*pal-tub pal-tub St St* × *pal-rec-sd pal-rec-sd*	4	60	4.00±0.00
(bb)	*pal-tub pal-tub* tester × *pal-rec-sd pal-rec-sd*	2	30	6.62±0.17
	Effect of *St*:			
	on *pal-rec-JI* (aa-a)=class 4.65			
	on *pal-rec-sd* (bb-b)=class 2.62			
	Extra sensitivity of *pal-rec-JI*=class 2.03			

data clearly fitted a 1:2:1 ratio, showing that *Sfm* is dominant and segregating independently from the *pal* locus.

Effect of Stabilizer (St) on the mutability of pal-rec-JI

The Stabilizer (*St*) is known (Harrison and Fincham 1968) to reduce the mutability of the *pal-rec* gene to a low level. In the present experiment, its effect on the relative mutability of *pal-rec-JI,* as susceptible line to the effect of *Sfm*, was studied to see whether the unstable allele has changed its sensitivity and if not, how the process of shifting itself responds to *St*. In our experience, *St* alone or in the homozygous condition never causes shifting when combined with *pal-rec-sd*. However, the *pal-rec-JI*

line unlike *pal-rec-sd* turned out to be more sensitive to the action of *St* and reduced the level of mutability more than 4.5 classes when combined with *pal-rec-JI,* whereas *pal-rec-sd* registered a drop of about 2.62 classes. The higher sensitivity of *pal-rec-JI* prevented any observations on the effect of *St* on the mechanism of shifting itself since there were only very few mutant spots (Table 3).

Discussion

In *Antirrhinum majus,* the *Sfm* is envisaged as a dominant factor that shares a property common to all kinds of controlling elements, the blocking, partially or totally, of the expression of genes at whose loci they are inserted. For example, the *A1* locus of maize is one of a number of loci that contribute to anthocyanin production. Upon integration of *Spm*, a regulatory element, into the *A1* locus of mutants $a1^{m-5}$ and $a1^{m}$, anthocyanin production is eliminated, but frequent self-induced excision of the element during plant growth results in pigmented sectors (McClintock 1962). Similarly, with Peterson's *En* controlling element, which is identical to *Spm* (Peterson 1965), mutability arises when the regulatory element becomes inserted at a locus such as *A2*. The resulting phenotypic change is from a uniformly colored kernel to a colorless kernel with occasional spots of color. The colored spots apparently result from excision events at the *A2* locus that release the inhibiting effects of the regulatory element (Peterson 1977).

The *Sfm* in *A. majus* seems to have properties analogous to the *Spm* controlling element, since plants carrying standard *pal-rec-sd,* which governs standard levels of mutability, when made heterozygous with *pal-tub pal-tub* that had been extracted from the original John Innes shifting line through successive generations, produced progeny with shifting characteristics. The resulting progeny phenotype suggested a mechanism of gene regulation based on a two-component system: component-1, *Sf,* of the controlling element *Sfm*, is believed to suppress *pal-rec-sd* gene activity in most cell lineages; the mutator, *m,* component-2, may act to release full gene activity in some of the cells. The suppressor and activity releasing function of the *Sfm* may be inseparable as no separate 'receptor' has so far been identified. This is in contrast to a two-element system where an identifiable receptor element, such as *Ds* (McClintock 1952), or the inhibiting element *I* (Peterson 1960), which is adjacent to the affected locus, alters or inhibits gene activity. This receptor element responds to a second element, the regulatory element, in such a way that the gene activity could be restored (Peterson 1977).

However, the mutability shifts produced by the controlled activity of the *pal-rec-sd* gene could be due to an appropriate association between the *pal-rec-sd* gene and *Sfm*, and this association remains unbroken until *Sfm* undergoes a temporary transposition to somewhere else in the chromosome complement, thus freeing the previously controlled allele from suppression and allowing it to mutate autonomously such self-induced excisions of the *Sfm* controlling element were detected in plants that initially showed shifting characteristics but which upon selfing produced non-shifting high mutables. Interestingly enough, progeny raised from such non-shifting high mutables in turn produced a high frequency of shifting plants, suggesting the re-integration of the element in the *pal-rec-sd* gene.

These observations are consistent with those reported by McClintock (1965) and Peterson (1977) in maize, in which an association of the 'receptor' with the 'regulatory' element results in kernels showing colored spots on a colorless background. If this type of association is disturbed through self-excised transposition of the regulatory element, the normal activity of the *A2* allele is restored.

On the other hand, gene changes induced by mutations of a regulatory element in *Petunia hybrida* (Bianchi et al. 1978) suggested that spots on the corolla were the result of the quantitative nature of the differences between the mutations in both the regulatory and receptor elements. This could point to a combination of the two components of the regulatory element that is built up from intermediate repetitive DNA. Mutations may accordingly be considered to be the result of smaller or larger deletions in the repetitive DNA. A deletion in the 'mutator' may result in the complete inhibition of the structural gene for the production of flower pigment. The anthocyanin synthesis could recommence after the deletion had been restored by an amplification of the repetitive DNA during cell division. The larger the deletion, the smaller the number of spots on the corolla.

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