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A two-element system controls instability at the *An3* locus in *Petunia hybrida*

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Introduction

Genic instability has been described for a large number of mutants in a variety of species. It is now generally accepted that this type of instability is caused by the action of so-called controlling elements. McClintock (1948) established that these elements can move around or transpose in *Zea mays*. She demonstrated that a controlling element, when present at the gene locus changes the expression of that gene and that, when the element leaves, its expression can be fully or partly restored. Two types of such instability were initially recognized in *Zea mays:* the autonomous type in which an element like *Ac* at the locus changes the expression of the gene; activity is restored when the element leaves the gene; the non-autonomous type where gene expression is suppressed if an element, like *Ds*, is integrated at the locus. Reversion towards activity, however, depends on the presence of a second element, *Ac*, somewhere else in the genome. In the absence of the *Ac* element the suppressed gene behaves as a stable recessive since the first element (*Ds*) cannot be excised on its own (Fedoroff 1983). These two-element systems (Fincham and Sastry 1974) were until now unique for *Zea mays*, although two cases have been reported which might point in the direction of two-element systems in other plants (Sand 1976; Sastry 1982). Here we report on a clear case of a two-element system in *Petunia hybrida* which is comparable to the system described above for *Zea mays*. Data are presented which indicate the partial suppression of gene action by a receptor element at the *An3* locus (chromosome IV); gene action can be restored in the presence of a regulator element linked with the gene *Fl* (chromosome II). In the long term it might prove that such two-element systems are not unique to *Zea mays*, but might merely be a general consequence of the wide-spread existence of controlling elements.

Key words: Flavonoid synthesis – *Petunia hybrida* – Controlling elements

Results

Reversion of an unstable *An1* allele (see Table 1 for explanation of gene symbols) to the wildtype pheno-

Table 1. Description of genes and their phenotypic effects

An1	controls the conversion of dihydroflavonols into anthocyanins
An3	controls the conversion of naringenin into dihydrokaempferol, which is the precursor for flavonols and anthocyanins; only trace amounts of flavonols and anthocyanins are found in *an3/an3* plants
an3-2	partly suppressed *An3* allele; suppression is apparently provoked by a *Ds*-like receptor element, which responds to an *Ac*-like element; linked with the gene *Fl*
Px	*phoenix;* during linkage analysis of the controlling element induced stable recessive *px* allele, the strain W159 appears to be heterozygous for the *an3-2* allele
Fl	*flavonol;* about a tenfold increase in flavonol amount is found in plants dominant for *Fl*.
W62	*an3/an3; Fl,Px/Fl,Px;* used as a tester strain; contains the *Ac*-like regulator element
W159	*an3-2/an3-2; fl,px/fl,px;* the *an3-2* allele gives rise to a phenotype intermediate between the standard dominant and recessive; the presence of the regulator element causes frequent reversion of *an3-2* to *An3-rev*

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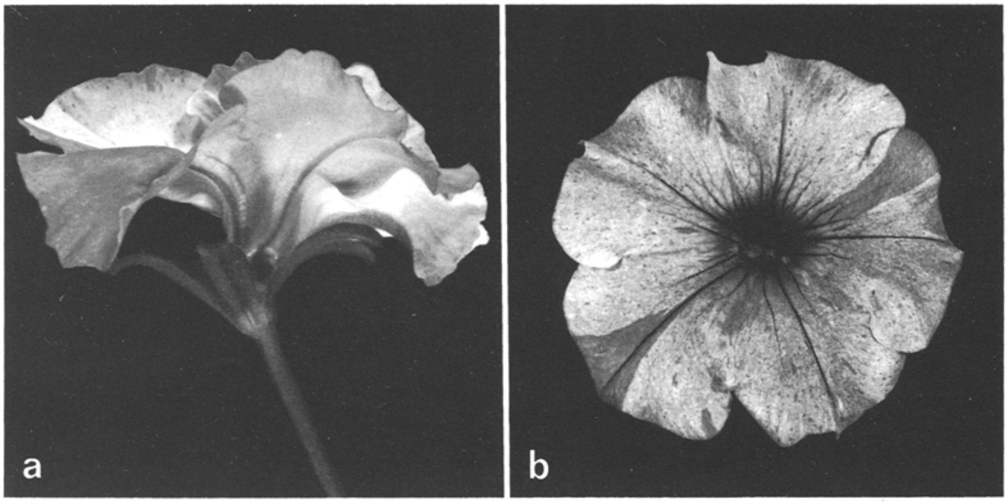

Fig. 1. **a** Phenotype of a *px/px* flower; note the twisted tube; **b** Phenotype of *an3-2/.* with the regulatory element, showing instability

Table 2. Progenies of F2 and BC1 crosses between the strains W62 *(FlPx;an3)* and W159 *(flpx;an 3-2)*

Cross	Progeny						Total
	an3/an3	*an3-2/.*				Others[a]	
		Fl,Px/..		*fl,px/fl,px*			
		Spotted[b]	Selfc.[c]	Spotted[b]	Selfc.[d]		
F1 × W62	119	133	1	–	–	–	251
F1 × W159	–	17	21	0	46	436	520
F2	68	135	39	0	42	289	573

[a] Comprises *an1/an1* and *hfl/hfl* classes, in which instability cannot be scored

[b] Grey-1-red, spotted grey-1-middle

[c] Selfcoloured grey-1-middle

[d] Selfcoloured grey-1-red

type can be accompanied by the occurrence of stable or unstable mutations at unrelated, unlinked loci (Doodeman et al. 1984). One such mutant, which was detected in the progeny of a selfed revertant, was the stable recessive *phoenix* (*px*) allele (Fig. 1 a). It was so named because of its peculiar growth habit: a new flower grows through the tube of an old one (de Vlaming et al. 1984). Genetic analysis showed the gene to be closely linked with the gene *Fl* in chromosome II (results not shown). No linkage with markers in other linkage groups was found.

On crossing *px/px* plants (strain W159) to the strain W62 (homozygous *Fl,Px;an3*) unexpected segregation was found in the progeny (results not shown). Some *px/px* plants gave rise to the expected grey-1-middle flowered progeny; others however showed a progeny ratio of 1 grey-1-middle to 1 red-grey, spotted grey-1-middle. A third group of *px/px* plants gave rise to a progeny of 100% red-grey, spotted grey-1-middle flowered plants (Fig. 1 b). The nine selfcoloured grey-1-middle flowered plants resulting from this last group are considered to be revertants of *an3-2* to *An3*. Since the interaction of regulator- and receptor element takes place after fertilization, these must be early mitotic rather than sporogenic events. Crossing the immediate forebears of the strain W159 to the strain W62 revealed no indication of the presence of an unstable allele at a locus other than *An1*.

Revertant flowers and their spotted counterparts of the same plant were analyzed for their flavonoid content. From the results (not shown) it is clear that both flavonols and anthocyanins are present in a lower amount in the grey-1-red parts compared to the grey-1-middle parts of the flower. Apparently the mutation partly suppresses the action of a gene in an early step of flavonoid synthesis.

Spotted *Fl* (W159×W62) plants were backcrossed to the strain W62 (Table 2). From the results it is deduced that the mutation involved caused a partial suppression of the gene *An3*. If a gene other than *An3* was involved, the *An3/an3* (called *an3-2/an3*) class in Table 2 should have shown a 1 spotted to 1 non-spotted segregation. The phenotypic data are in accordance with the fact that the *An3* locus is involved: in *an3/an3* plants both flavonol and anthocyanin synthesis are inhibited (de Vlaming et al. 1984). The gene *An3* controls the activity of the enzyme flavanone 3-hydroxylase (Froemel et al. 1985).

From F2 crosses (selfed *Fl*, W159×W62 hybrids) it was deduced that instability of the new *an3* allele was always associated with the presence of a dominant allele of the gene *Fl* (Table 2). All grey-1-red flowered plants (all of which are *fl,px/fl,px*) are selfcoloured, whereas all red-grey, spotted grey-1-middle plants are *Fl,Px/*... The 39 selfcoloured grey-1-middle flowered plants from these F2 crosses are assumed to be the result of sporogenic (or early mitotic) reversion of *an3-2* to *An3* (for an alternative explanation see below). The class "others" (Table 2) contains *an1/an1* white flowered plants in addition to *hf1/hf1* coloured flowered plants. The *hf1/hf1* segregants produce cyanidins as a main pigment. At least some of these plants showed instability. Since the difference between spot and non-spot area in flowers of these plants is often hardly visible, results on spotted versus non-spotted are given only for the *An1/.;Hf1/.;an3-2.* class. It is known that *Fl* greatly reduces cyanidin production (Gerats et al. 1982). If the element causing instability had been independent of *Fl,Px,* one quarter or around 34 plants of the spotted group of progeny plants from the F2 crosses should have been *fl,px.* We conclude that instability of the *an3-2* allele is under the control of an *Ac*-like element very close (in crossover units) to the gene *Fl.*

The linkage of the *Ac*-like regulator element and the gene *Fl* is confirmed in the backcross (W62×W159)×W159 (Table 2). All *fl/fl* plants are selfcoloured grey-1-red, whereas all red-grey, spotted grey-1-middle flowered plants are *Fl/fl*. Remarkably, a 1 : 1 segregation was found for the red-grey, spotted grey-1-middle to selfcoloured grey-1-middle flowered progeny class. If we assume this to be merely the result of reversion we have to explain the fact that in the *Fl:* W159×W62 2.4% of the plants were revertant (9 out of 371; results not shown), in the backcross (W159×W62)×W62 0.7% (1 out of 134; Table 2), in the backcross (W159×W62)×W159 55.3% (21 out of 38; Table 2) and finally in the F2 (W159×W62) 22.4% (39 out of 174; Table 2). This difference in number of revertants might be caused by the segregation of still another element, influencing the apparent reversion frequency of the *an3-2* allele. We are presently researching this possibility.

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