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Evidence for transposition in Petunia

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Summary. In Petunia interaction is described between a *trans*-regulatory genetic element *Bi* and responsive anthocyanin alleles, resulting in a variegated flower colour pattern. Crosses segregated white-flowering plants that had lost the *Bi* element and could be shown to contain a responsive allele by the colour pattern resulting from interaction with the standard variegated parent. A weak mutant of the *Bi* element is described. Some pure lines of Petunia contained the *Bi* element, others did not. In two lines it could be mapped to chromosome I.

Key words: Petunia – Transposition

Introduction

Studying variegated plants of maize (McClintock 1965a; Fincham and Sastry 1974; Fedoroff 1983) yielded the first evidence for the existence of transposable genetic elements. Stable mutant types could be induced to become unstable by the introduction of unlinked genetic factors (two-element type of instability), and the target element these factors interact with, as well as the factors themselves, could be shown to change their map position. Ample evidence from *Drosophila* has firmly established the concept of transposition and movable elements (Green 1980; Rubin 1983). As to plants other than maize, no two-element system has been discovered in *Antirrhinum,* while in *Impatiens* a two-element system has been described, but could not be analysed as to linkage groups due to a lack of genetic markers (Sastry et al. 1980; Sastry 1982). In *Petunia,* instability at several loci (*An2, Rt,* Cornu 1977; *An1, An11,* Bianchi et al. 1978; Doodeman et al. 1984a, 1984b) has been described in terms of autonomous (one-element) variegation. However, it would be worthwhile to back up molecular-genetic studies in the Solanaceae by having a genetic test for distinguishing different families of elements, as has been done in maize (Peterson 1980; Friedemann and Peterson 1982).

The present study describes the genetic analysis of a two-element system in Petunia. The work is based on the long series of experiments by F. Bianchi and coworkers (1969–1983) on the unstable *an1* system (Doodeman et al. 1984a, 1984b) and its derived mutations. A general feature of the *an1* system is that reversion of flower colour was in most cases to the full-coloured (wild) situation. Interestingly, when pale sectors occur, no further change to darker colour takes place in the cells forming them (Bianchi et al. 1978). The system is utterly variable, with a very high incidence of mutations changing frequency and/or timing of variegation. Regeneration experiments could make clear that the processes involved in somatic reversion concern the same genes as in the germ line revertants (Bino et al. 1984).

Material and methods

Allele nomenclature

In the present system of variability it is difficult to speak of a discrete number of alleles of unstable systems because it is practically impossible to find a segregating progeny not containing new alleles. However, they can be distinguished as allele families, or types of alleles. In the present context An^+ is wild type (full-coloured), an^- fully mutant (white), *an** variegated (with full-coloured spots or, possibly, additional pale-coloured spots), *an(r)* a responsive allele (white, to be destabilized by the presence of a trans-regulatory element, *Bi*).

Within any family, different alleles will be indicated, when possible, by symbols and/or numbers.

The relevant loci have been located as follows: *An1* on chromosome VI (Bianchi et al. 1978), *An11* on chromosome III (Doodeman et al. 1984a). These genes regulate different steps in the anthocyanin synthetic pathway and complement each other.

Petunia pure line material

The collection of pure lines of the Institute of Genetics, University of Amsterdam, has been used for the present study (present address of the collection: Dept. of Genetics, Free University of Amsterdam). Line W138 (*an1*an1**) with red and pale spots is a derivative of line R27 (cultivar 'Roter Vogel') and its slightly variegated mutant line W17 ($an1^{s/+}$), with red spots. W138 contains the allele $an1^{s/p-+}$ (Doodeman et al. 1984a). Line W137 (*an11*an11**) is derived from a red revertant of W138 and contains the $an11^{s/+}$ allele (Doodeman et al. 1984b). Line W78 ($an1^{-}an1^{-}$) is also derived from R27 parentage. Line W162 is derived from a cross W138 × R4, the latter a stable red-flowering plant of light hue with kaempferol as the main flower pigment.

The hitherto undescribed line W162 has the following genotype (in brackets the chromosome involved): (I), *hf1hf1 Ph1Ph1 prxB2prxB2;* (II), *fl1 prxE1prxE1;* (III), *ht1ht1 prxA1prxA1 prxD1prxD1;* (IV), *prxC3prxC3;* (V), *popo hf2hf2;* (VI), *rtrt an1(r) an1(r);* (VII), *an4an4 prxF2prxF2.* The various markers are mentioned below. Because the parental material for W162 was still heterozygous for *prxC* and *Po*, these two markers did not segregate in all backcrosses to W162 (see results).

The lines M5, M7 and V35 are of recombinant descent with some ancestry in common; R27 and V23 (the latter from cultivar 'Blauzwerg', more or less equivalent to the classical multiflora nana compacta type 'Ratsherr') represent isolated types.

Genotype for variation

Line W138 is a derivative of a variegational type with white flowers showing a few scattered spots, and has itself many more spots – more than 15% of which are pale (in different shades). In all material described in the present paper the latter *an1** allele has been used. The situation as to timing is rather heterogeneous, with many early reversions (large sectors) as well as late reversions. The total picture is called the *an1* type of variegation (Fig. 1).

Line W138 has red spots in its pale sectors (homozygous type of variegation). When W138 is crossed to a stable white $an1^{-}an1^{-}$ plant, the pale sectors are unspotted in the hybrid (see Figures in Doodeman et al. 1984a), which is called the heterozygous type of variegation.

The same distinction can be made for W137 (*an11*an11**), but pale sectors are much less frequent (less than 10%). The percentage of pale spots is difficult to determine exactly when the spots are small; only spots exceeding an arbitrarily chosen minimum size were counted. In the *an11* type of variegation, moreover, most of the reversions come late and result in a fine spray of tiny red spots. The *an1* and *an11* types cannot be distinguished in an *ht1ht1* background where the anthocyanin content of the standard spots is too low for paler spots to be recognized (for factor *Ht1*, see below).

Both responsive alleles of the plain white mutants described presently will be indicated by the allele number – *1984.*

Genetic markers

In B1 and F2, the following markers segregated (in brackets the chromosome they have been located to): *Hf1* (I), *Fl* (II), *Ht1, An11, prxA* (III), *Dw, prxC* (IV), *Hf2, Po* (V), *Rt, An1* (VI), *An4,prxG* (VII). A description of these genes with references as to recognition of phenotypes can be found in de Vlaming et al. 1984, Wiering 1974 and Wijsman 1983.

The factors relevant to the crosses presently reported are *An1, An11, Ht1, Rt,* and *Hf1.* The *an* phenotypes can be recognized as white versus coloured (*An*), and in the same way *ht* (kaempferol as flavonol pigment) can be distinguished from *Ht* (quercetin as flavonol), and *Rt* (flower colour magenta or purple) versus *rt* (red or "gray"). In *anan* plants, flavonols are found, but no anthocyanins. The *Ht* factor not only influences flavonols, but anthocyanins as well, and in *htht* plants cyanidin concentration is low, resulting in a red colour of a very light hue.

Hf factors are involved in hydroxylation of the 5′ position of the anthocyanin molecule. *Hf1* does so completely, while *Hf2* does so in an incomplete way, resulting in a mixture of hydroxylated and unhydroxylated molecules. *Hf2* is only active in the flower limb, not in the flower tube. In some genotypes modifiers give rise to some unhydroxylated molecules even in the *hf1hf1* genotype. It can be difficult to recognize *Hf2* action chemically in variegated flowers of a "late", fine pattern with overall little anthocyanin content. Visually it is notoriously difficult to recognize *Hf2* in an *rtrt* background, as is necessarily the case in the crosses described below, in view of the extremely tight linkage of *an1* and *rt.* In these cases, as well as for all *an1an1* plants, the *Hf* genotype has been determined by crossing to *hf1hf1 hf2hf2* testers and examining the progeny.

Results

Responsive allele of an1

Independent white-flowering mutants ($an1^{-}an1^{-}$) were isolated from progenies derived from W138 and systematically tested (7 cases): crossed to W138, six gave an F1 showing the heterozygous variegated pattern in the flowers. However, in a cross of W138 to line R4 (full-coloured), the F2 segregated for the following phenotypes: full-coloured, variegated, white, in a 12 : 3 : 1 ratio (Table 1). The white segregants were crossed to W138 to give an F1 with homozygously variegated flowers (Fig. 1), and it was concluded that they contained an undescribed allele, *an1–1984*, of type *an1(r)*, which is responsive to a trans-regulating element inducing variegation. Some variegated plants were self-pollinated and segregated 3 : 1 (76 : 17 for variegated and stable white). White-flowering plants when selfed again produced only white progeny (eventually isolated as line W162) and when crossed to *an1* an1** again a homozygous-type variegated F1 resulted.

To confirm the responsive character of *an1 (r)*, W162 was crossed to line W137, the latter containing an instable *an11*, a locus on chromosome III tightly linked to *Ht1* (less than 16.5 ± 4.5 cM, Doodeman et al. 1984b; 14 ± 3.4 cM, unpublished results). In the F2

Table 1. Crosses involving *an1(r)* as well as its *trans*-regulatory element

	Phenotypes		White	X^2	*P*
	Full-coloured	Variegated			
F2 generation ($X^2_{12:3:1}$)					
F2 (W138×R4)	136	43	11	1.91	0.43
F2 (W162×V23)	311	62	30	3.61	0.17
F2 (W162×V35)	169	40	17	0.71	0.70
B1 generation ($X^2_{2:1:1}$)					
(V22×W162)×W162	63	21	25	6.61	0.02
(V35×W162)×W162	33	16	8	3.66	0.07
(R27×W162)×W162	63	28	29	0.31	0.61
(M5 ×W162)×W162 } $X^2_{1:1}$	64	0	60	0.13	0.72
(M7 ×W162)×W162 } $X^2_{1:1}$	42	0	42	0.00	1.00

Table 2. Phenotypes in the F2 (W137×W162)

Genotypes:	*Ht1Ht1*, variegated *an11** × *ht1ht1*, white *an1(r)*			
Phenotypes:	Quercetin plants (*Ht1-*)		Kaempferol plants (*ht1ht1*)	
	Self-coloured	124	Self-coloured	125
	Variegated (*an1* type)	23	Variegated	27
	Variegated (*an11* type)	12	White	5
	Variegated (sectors with variegation)	8		
	White	6		

Ht1- means either *Ht1Ht1* or *Ht1ht1*
Variegation in the much lighter coloured *ht1ht1* plants with kaempferol cannot easily be distinguished on the basis of the frequency of pale sectors

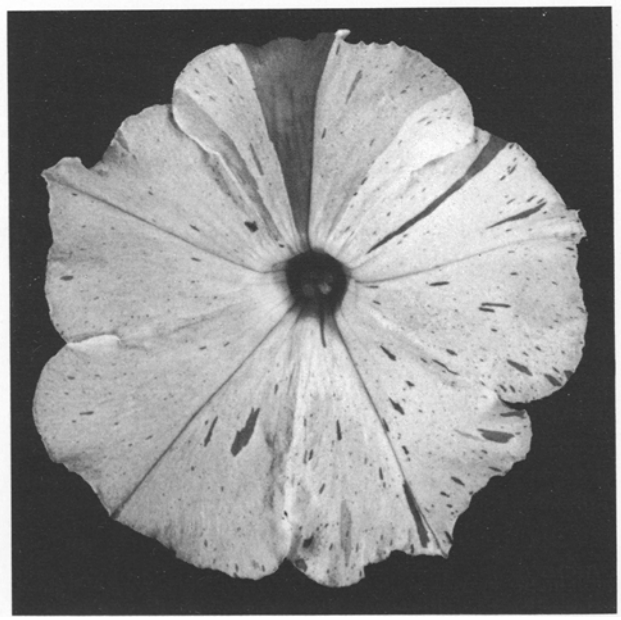

Fig. 1. Flower partly pink (*left*) and partly white. Both parts are variegated with red and pale red spots (type *an1*) showing the homozygous type of variegation. Cross: F1 (W162×W138) = *an1(r)an1**

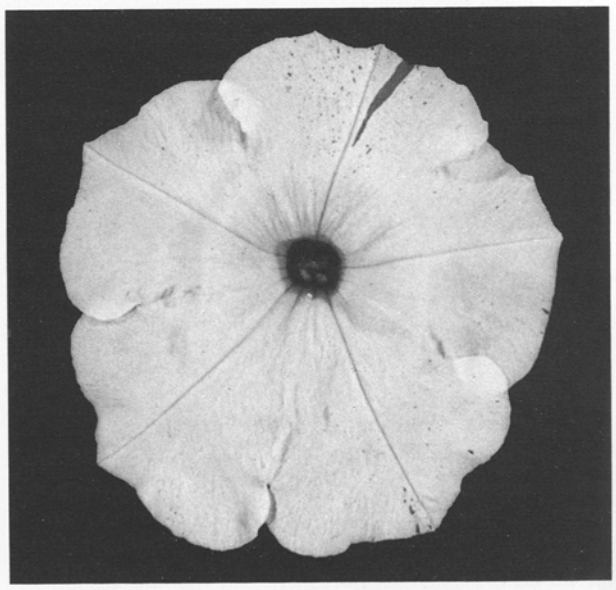

Fig. 2. Flower with sectors of variegation (as well as some isolated spots). Cross: F2 (W162×W137) = *an1(r)an1(r) an11(r)an11(r)* with one or two *Bi* elements (the number has not been determined in the plant sampled)

Table 3. Linkage relationships in backcrosses of several pure lines to W162 in order to detect the presence of *trans*-regulatory elements

Cross: (W137 × W162) × W162			
Genotype:	Genotypes and no. of progeny (*anan*)		
$\frac{Htlanl1(r), Bi, Anl}{htlAnl1, bi, anl(r)} \times \frac{htlAnl1, bi, anl(r)}{htlAnl1, bi, anl(r)}$	*Htlhtl*	variegated	74
	htlhtl	variegated	84
$X^2_{2\times2} = 0.80$; $P = 0.37$	*Htlhtl*	white	45
Conclusion: independent assortment	*htlhtl*	white	64
Cross: (V23 × W162) × W162			
Genotype: $\frac{Hfl, Bi, Anl^+}{hfl, bi, anl(r)} \times \frac{hfl, bi, anl(r)}{hfl, bi\ anl(r)}$	Genotypes and no. of progeny (*anan*)		
	Hflhfl	variegated	19
	hflhfl	variegated	2
$X^2_{2\times2} = 38.5$; $P < 0.01$	*Hflhfl*	white	0
Conclusion: 96% linkage of *Hfl* and *Bi*.	*hflhfl*	white	25
Cross: (V35 × W162) × W162			
Genotype: $\frac{Hfl, Bi, Anl^+}{hfl, bi, anl(r)} \times \frac{hfl, bi, anl(r)}{hfl, bi\ anl(r)}$	Genotypes and no. of progeny (*anan*)		
	Hflhfl	variegated	13
	hflhfl	variegated	3
$X^2_{2\times2} = 14.2$; $P < 0.001$	*Hflhfl*	white	0
Conclusion: 88% linkage of *Hfl* and *Bi*.	*hflhfl*	white	8
Cross: (R27 × W162) × W162			
Genotype: $\frac{Hfl, Bi, Anl^+}{hfl, bi, anl(r)} \times \frac{htl, bi, anl(r)}{hfl, bi\ anl(r)}$	Genotypes and no. of progeny (*anan*)		
	Htlhtl	variegated	14
	htlhtl	variegated	14
$X^2_{2\times2} = 0.84$; $P = 0.78$	*Htlhtl*	white	11
Conclusion: independent assortment	*htlhtl*	white	18

(Table 2), three types of variegation were found: variegation of the *anl1* type (like the parent W137); variegation of the *anl* type; and a new type of variegation with sectors showing coloured spots (Fig. 2). The genotype of the latter plants was interpreted as *anlanl anl1anl1*, white wherever reversions of both genes do not coincide. Crosses to both parents confirmed the supposed genotype that results in sometimes rather large patches of an *anl* reversion speckled with the later occurring *anl1* flecks. Occasionally, the basal reversion could be traced to an early *anl1* reversion; then the spots showed the percentage of pale as usual for *anl*.

W137, a two-element system

The appearence of the unstable *anl1** allele in the progeny of a red revertant of *anl** suggests that it belongs to the same instability system. Moreover, the sectors-with-spots type mentioned above seems to confirm this hypothesis. Therefore, in the backcross (W137 × W162) × W162, assuming that *anl1** is an autonomous allele, it was expected that in variegated plants the *anl(r)* allele would be activated by *anl1** itself and, in view of the cloes linkage between *Htl* and *anl1**, that most of the variegated plants would have inherited *Htl*. By contrast, from Table 3 it can be deduced that the variegation inducing factor is not linked to *Htl*. Apparently, W137 contains a two-element system consisting of a trans-regulatory element (called *Bi*, see below) combined with, but unlinked to, a responsive *anl1(2)* allele, to which the number *anl1–1984* can be applied. The locus of *Bi* in W137 is as yet undetermined. It is not linked to *Anl* because, if that were the case, most *anlanl* plants would be white, while variegation would be exceptional. By contrast, independent assortment is indicated by the segregation of variegated (*anl*) : white (among *Htl* plants) in a 23 : 6 ratio (Table 2). Nor is it linked to *prxC*(IV), segregation in the backcross being as 10 : 7 : 15 : 7.

The data of Doodeman et al. (1984 b) point to a relatively high proportion of white-flowered mutant progeny of line W137 (*anl1*anl1**). This may be due

to the loss of the *Bi* element, uncovering *an11(r)* *an11(r)* plants. These cases need further study.

Alleles of the Bi element

It is postulated that *an1–1984* and *an11–1984* contain a mutated genetic element unable to carry out the mutation function, but still interfering with the level of expression (null, low, or full). This bi-functional element is called the *Bi* element. When an external Bi^+ restores mutational activity, the type of expression is the same as it was before, *cis*-dominant as the receptor element is. A hybrid F1 (W138 × W162), of genotype *an*an1(r)* has been crossed to W78 ($an1^-an1^-$), a stable white derivative of *an1** parentage. A 1 : 1 segregation was anticipated of white and normally variegated plants. However, all plants were variegated although two types were found: one like the W138 parent, as well as another type, very weakly variegated. It is postulated that W78 is homozygous for a mutant bi^w element, which results in a reduced frequency of response of *an1(r)*. Normal and weak variegation segregated as 124:113.

Tests of lines

Eventually all pure lines of the Petunia collection have to be tested for the presence of one or more *Bi* elements. This is in particular true of those considered representative of certain old cultivars as well as those based on recent accessions from the wild. For the moment, the *Bi* content of five lines has been assayed. In backcrosses of lines M5 and M7 no variegated plants occurred (Table 1); either they are free of active forms of the variegation-inducing *Bi* element, or, possibly, *Bi* is tightly linked to $An1^+$, in which case only coloured plants would inherit *Bi*. In backcrosses of lines V23, V35, and R27, the segregation of full-coloured : variegated : white was as 2 : 1 : 1 (Table 1). Apparently, a single *Bi* element is present and unlinked to *An1*. This was confirmed by the analysis of the F2 for V23 and V35. The variegation has many pale sectors and is clearly of the *an1* type. This is expected in view of the *cis*-dominant character of the *an1* mutation.

In the crosses to V23 and V35, factors located on all chromosomes segregated. However, it was not possible to allocate a definitive genotype as to *Hf1* and *Hf2* to every plant. Moreover, in the white-flowering plants no anthocyanin factors are expressed. Therefore, genotypes had to be determined on the basis of test crosses.

There is evidence showing the linkage of *Bi* with *Hf1* (I) in the case of V23 and V35 (Table 3). As to V35, the effects of *Hf1* and *Hf2* could not be distinguished, though linkage to one of these polymeric factors was likely since no *hfhf* variegated plants were observed. To determine linkage, test crosses were made, and on the basis of the final genotypes, linkage of *Bi* to *Hf1* was found (Table 3). In R27, *Bi* is not linked to *An1* (VI), to *Ht1* (III) (Table 3), nor to *prxC* (IV), segregation in the latter case being 10 : 10 : 9 : 7.

Discussion

Ever since instability in higher organisms was attributed to the action of transposable genetic elements, variegation in plants has been suspected to be caused by them. For instance, Doodeman et al. (1984 b) attribute the high frequency of mutant types in certain Petunia, some of these again unstable, to transposition. However, the demonstration of transpositional phenomena requires that the new as well as the original instability should relate to the same family of elements and this can only be proved by the use of responsive alleles (Peterson 1980). For this purpose, stable white alleles of *An1* and *An11* (the latter a derivative mutation of the former) were investigated. Through the application of a responsive allele, we can now explain the origin of *an11** as a new unstable mutation induced by insertion of the *Bi* element that, in the process, itself mutated to a state (*bi*) dependent on external activation by Bi^+.

The *Bi* element in its trans-regulatory form was found in two states of activity. In this respect, it resembles the *Spm = En* element in maize (with the allele Spm^w, McClintock 1965 b). The location of the *Bi* element in lines V23 and V35 will be followed by screening other lines, first of all line R27 (Wijsman and Gerats, in preparation). Aberrations of the 3 : 1 ratio in F2's as found in Table 2 (*Ht1:* 173 : 157; *Bi:* 70 : 11) are of frequent occurrence in Petunia and can be ascribed to certation between pollen tubes with different parental chromosomes. However, concerning the shortage of certain phenotypes in some of the backcrosses of Tables 1 and 3, only ad hoc hypotheses can be offered. Systems of instability in Petunia in other genetic backgrounds, and discovered independently of the *an1/an11* system, involve *ph4** (line R62), *an3** (line W29), *dw** (line R12, Bianchi et al. 1974), *rt** and *an2** (under their former names *A* and *R*, Cornu 1977). Gerats (1985) has independent evidence for one other two-element that, however, does not seem to be dependent on *Bi*. Investigation of several lines and cultivars to ascertain the possible presence of the *Bi* element is in progress.

W137 has been shown to be a two-element line; it follows that its parental line W138 is likely to be the same, in view of the absence of linkage between *Bi* and $An1^+$ in W137. The probability that in W137 both the receptor factor and the regulatory element would have

transposed simultaneously seems small. Interestingly, Doodeman et al. (1984) have described that reversion of *an1** results in an allele called *an1*$^+$, less stable than the wild *An1*$^+$ allele. This suggests that at least part of the element still resides at the locus where internal rearrangements might inactivate its inhibition of *An1* gene expression. The unstable *an11** mutation is not the only unstable mutation derived from the *an** system (Doodeman et al. 1984 b), and further study of these should make clear whether there are autonomous mutations amongst them.

Petunia is now a pliable system for formal genetics (more than 100 genes have been mapped: de Vlaming et al. 1984 and unpublished data) and becoming important as a model plant for in vitro cultivation (Fraley et al. 1983), while in addition because of its Solanaceous connections some data may be extrapolated directly to economically important genera.

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