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Genetic analysis of instability in *Petunia hybrida*

3. Periclinal chimeras resulting from frequent mutations of unstable alleles

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Summary. In *Petunia hybrida* frequent mutations of unstable alleles give rise to different types of periclinal chimeras. If genes expressed in the epidermis, such as the gene *An1* for flower colour, are concerned, mutations in the dermal layer of the shoot apex will result in changes in the phenotype but not in the offspring. Mutations in the subdermal layer will not lead to an altered phenotype, but to changes in the sporogenous tissues and, thus, to deviating segregations in progenies. Therefore, in crossing experiments with such an unstable mutant, it is always necessary to take the possibility into account that the plant may be a chimera, so as to prevent an incorrect interpretation of the recorded segregational ratios. Mutations of unstable alleles expressed in the mesophyll, such as gene *Yg3* for leaf colour, also give rise to chimeras. In such instances, however, a change in phenotype always involves a change in segregational ratios as well, since both the mesophyll and the sporogenous tissues are derived from the subdermal layer of the shoot apex.

Key words: Periclinal chimeras – Unstable alleles – *Petunia hybrida*

1 Introduction

Anatomical studies of *Petunia hybrida* have revealed that even in an early stage of development of the plant three mutually independent cell layers can be distinguished in the apical growing zone (Cornu and Bugnon 1971; Bianchi and Walet-Foederer 1974; Bianchi et al. 1974). The examination of shoot apices of flowering specimens showed that this situation is maintained in adult plants. In the outer two cell layers of the shoot apex periclinal division walls are never observed. This points to the presence of three mutually independent groups of initials; two for the one cell thick dermal and subdermal layers and one for the remainder of the shoot apex. Since in *Petunia* the epidermis of the whole plant is derived from the dermal layer while the larger part of the chlorophyll-containing mesophyll and the sporogenous tissues are presumably derived from the subdermal layer of the shoot apex (compare: Krüger 1932; Satina and Blakeslee 1941), one may assume that mutations in these layers may result in periclinal chimeras with deviations in the characteristics of the flower, the leaf or the offspring.

During the course of studies of flower colour genetics in *Petunia* a mutant was found that bears white flowers with red and pink spots and, occasionally, some unspotted white sectors, all this as the result of mutations of an unstable allele of the gene *An1* involved in anthocyanin synthesis. From the segregations observed in the progenies of this mutant, it could be computed that mutations from variegated ($an1^{s/p-+}$) to white (*an1*), to pink ($an1^{+/p}$) and to red ($an1^{+/+}$) occurred in 5.2%, 1.8% and 4.8% of the gametes, respectively (Doodeman et al. 1984a). The large number of spots in the white flowers and the percentages of mutated gametes point to a high mutation frequency of $an1^{s/p-+}$ both in the epidermis and in the sporogenous tissues of developing flowers.

Mutations do not remain restricted to flowers but can also occur in the shoot apex, and thus lead to sectors of mutated cells. If a floral primordium is initiated within such a sector, one may expect that self-coloured red or pink, or unspotted white flowers, will arise on plants that initially merely bore variegated flowers. Since gene *An1* only expresses itself in the epidermis of the corolla, such self-coloured flowers must be the result of mutations in the dermal layer of the shoot apex. Consequently, those flowers must be regarded as periclinal chimeras, for in most instances they will only be mutated in the epidermis, whereas the sporogenous tissues, as derivatives of the subdermal layer of the shoot apex, remain unchanged. Therefore, such red, pink, or white flowers, when used in crossing experiments, must be expected to yield progenies that do not essentially differ from those obtained from variegated flowers produced by the same individual plant.

Mutations occurring in the subdermal layer of the shoot apex may result in flowers that have remained variegated but became mutated in their sporogenous tissues. Crossing experiments carried out with such variegated flowers will yield unexpected segregations since they will produce an offspring one may expect from crossings involving self-coloured red- or pink-, or unspotted, white-flowering plants.

In view of the high mutation frequencies of $an1^{s/p-+}$, it may be expected that occasionally chimeric flowers will also arise in which both the epidermis and the sporogenous tissues are mutated independently of one another.

Due to frequent reversions an unstable allele of gene *Yg3* for leaf colour gives rise to plants with yellowish-green leaves bearing green spots. Occasionally branches with normal, green leaves develop on such plants. These branches must be the result of reversions in the subdermal layer of the shoot apex since gene *Yg3* becomes expressed in the chlorophyll-containing mesophyll derived from that layer. Since the sporogenous tissues are also formed out of this layer, it may be expected in this instance that flowers produced on such chimeric, green branches, after selfing, will yield progenies party consisting of plants with normal, green leaves.

The experimental results discussed in the present paper are intended to demonstrate that the different types of chimeras mentioned above do in fact occur regularly in plants containing a highly unstable allele. For an interpretation of the crossing results obtained with such plants, one always has to bear in mind that, in the crosses in question, chimeric flowers may have been involved.

2 Materials and methods

The experiments were carried out with mutant PZ 5158 L-1 and with a number of descendants obtained from selfings of this mutant. PZ 5158 L-1 is heterozygous for a highly unstable allele of the gene *An1* for flower colour that gives rise to white flowers with red and pink spots and, occasionally, unspotted, white sectors. A list of the different types of plants with their phenotypes and genotypes found in the progenies of PZ 5158 L-1 and its descendants is given in Table 1.

Plants homozygous for the unstable allele $yg3^r$ were also included in the experiments. This allele gives rise to plants with yellowish-green leaves. Somatic reversions towards wild-type green occur frequently and become visible as darker green spots on the leaves.

Self-pollinations are always carried out by applying pollen of one flower to the stigma of another flower of the same plant still in the late bud stage. Prior to pollinations, the immature anthers of the flower used as the female parent are removed.

All plants were reared in a greenhouse. Supplementary lighting was provided during the winter by means of a Philips HPI (TH 00.5SE) installation to a day length of at least 14 h.

3 Experimental results and discussion

3.1 Differently coloured, chimeric flowers resulting from mutations in the dermal layer

On plants with genotype $an1^{s/p-+}an1$ bearing white flowers with red and pink spots and occasionally with unspotted white sectors, self-coloured red or pink, or unspotted white flowers are frequently found. Table 2 lists the results of experiments in which plants bearing such differently coloured flowers were crossed with stable white-flowering plants (*an1an1*). Out of these 45 crosses, 37 capsules yielded progenies that one could expect in this type of cross (Table 2 A) on the basis of the percentages of mutated games found in the original $an1^{s/p-+}an1$ mutant (Doodeman et al. 1984a). The variegated descendants and the majority of the white-flowering plants must be ascribed to segregation of the alleles $an1^{s/p-+}$ and *an1,* respectively. A small number of the white-flowering plants and all of the pink- and red-flowering descendants must be the result of mutations of the unstable allele $an1^{s/p-+}$ to *an1,* to $an1^{+/p}$ and to $an1^{+/+}$ in the sporogenous tissues of the parent plant. The frequencies of representation of the different types of plants found in the progenies of the variegated, of the red, and of the white flowers are comparable. These results confirm the assumption that mutations occurring in the dermal layer of the shoot apex remain restricted to the epidermis and do not exert any influence upon the genotype of the sporogenous tissues derived from the second layer.

Since it has been demonstrated that in the second layer of the shoot apex the same mutational changes may occur as in the first layer, mutated sectors may conceivably also be formed in the subdermal layer. If a

Table 1. Phenotypes and genotypes of the different descendants of mutant PZ 5158 L-1

Phenotype		Genotype
'White-red-spotted pink':	white flowers with red and pink spots; within the pink spots smaller and darker pink and red dots	$an1^{s/p-+}an1^{s/p-+}$
'White-red-pink':	white flowers with red and pink spots	$an1^{s/p-+}an1$
White:	unspotted white flowers	*an1an1*
'Spotted pink':	pink flowers with darker pink and red spots	$an1^{s/p-+}an1^{+/p}$
Pink:	self-coloured pink flowers	$an1^{+/p}an1$ or $an1^{+/p}an1^{+/p}$
Red:	self-coloured red flowers	$an1^{+/+}$ ——

Table 2. Progenies obtained from crosses of differently coloured flowers on plants with genotype $anl^{s/p-+}anl$ with stable white-flowering plants ($anlanl$)

	Colour of flower[a]	No. of families	Descendants[a]									Conclusion about genotype[a]: $\frac{\text{epidermis}}{\text{sporogenous tissue}}$
			White-red-pink		White		Pink		Red		Total	
			No.	%	No.	%	No.	%	No.	%	No.	
A	White-red-pink	9	297	*44.9*	352	*53.3*	5	*0.8*	7	*1.1*	661	$\frac{anl^{s/p-+}anl}{anl^{s/p-+}anl}$
	Red	24	613	*47.5*	653	*50.6*	5	*0.4*	19	*1.5*	1,290	$\frac{anl^{+/+}anl}{anl^{s/p-+}anl}$
	White	4	64	*44.8*	77	*53.8*	1	*0.7*	1	*0.7*	143	$\frac{anlanl}{anl^{s/p-+}anl}$
B	White-red-pink	1[b]	–		69	*100.0*	–		–		69	$\frac{anl^{s/p-+}anl}{anlanl}$
	Red	4[b]	–		173	*100.0*	–		–		173	$\frac{anl^{+/+}anl}{anlanl}$
C	Red	1	–		60	*47.2*	–		67	*52.8*	127	$\frac{anl^{+/+}anl}{anl^{+/+}anl}$
D	White	2	–		54	*49.5*	55	*50.5*	–		109	$\frac{anlanl}{anl^{+/p}anl}$

[a] See Table 1 for a description of phenotypes and genotypes
[b] Descendants from the same parental plant

χ^2 test:

A Test on homogeneity of the segregations in the progenies from the 'white-red-pink' flowers and in those from the red flowers:
$\chi^2_{2\times 3}=1.220$ df=2 $P=0.54$ (the classes red and pink combined)
The progenies from the white flowers could not be included in this test on account of the small number of mutant descendants, but the segregation observed in these families does not give reason to assume significant differences with those observed in the other families

C $\chi^2_{1:1}=0.386$ df=1 $P=0.53$
D $\chi^2_{1:1}=0.009$ df=1 $P=0.92$

floral primordium is initiated within such a sector, the entire sporogenous tissue of the developing flower will have a different genotype. The aberrant segregations found in the remaining 8 families shown in Table 2 B–D, are obviously attributable to the presence of such chimeric flowers on the plants used in the crosses in question.

The 5 capsules that yielded the progenies given in Table 2 B (consisting of white-flowering plants only) were all produced by the same plant. In this individual the genotype of the tissues derived from the subdermal layer must have been $anlanl$ as the result of a mutation of $anl^{s/p-+}$ towards anl in an early stage of development. Additionally, a mutation towards red ($anl^{+/+}$) must have independently occurred within the dermal layer, which resulted in a branch bearing self-coloured red flowers.

In one instance a red flower of a variegated-flowering plant yielded a progeny consisting of a mixture of red-flowering and white-flowering plants (Table 2 C). The genotype of the two outer layers of the floral primordium from which the flower in question developed must have been $anl^{+/+}anl$ following two mutations of $anl^{s/p-+}$ towards red that must have occurred independently of one another.

The two white flowers that yielded white- and pink-flowered descendants exclusively (Table 2 D), must have been periclinal chimeras resulting from a mutation of $anl^{s/p-+}$ towards white in the dermal layer and one towards pink in the subdermal layer of the same shoot apex.

In Table 2 no results are given of crosses with self-coloured pink flowers. Crosses were made with 10 pink flowers, but only 3 capsules developed and the seeds from these capsules did not germinate. For some unknown reason it is very difficult to obtain a viable offspring from pink-flowered plants bearing the allele $anl^{+/p}$ since most crosses carried out with such plants do not yield any seed. Apparently, this also holds true for pink flowers that occasionally occur on variegated-flowering plants. Nevertheless, offspring could be obtained from a 'spotted pink'-flowered branch of a 'white-red-spotted pink'-flowered plant homozygous for the allele $an1^{s/p-+}$. The genotype of the epidermal tissues of the chimeric branch must have been $an1^{s/p-+}an1^{+/p}$ as the result of a mutation of $an1^{s/p-+}$ towards pink ($an1^{+/p}$). The pink flowers bear red and darker pink spots due to mutations of the second, unstable allele. Table 3 shows the results of self-pollinations of 4 'spotted pink' flowers of the chimeric branch and of 21 'white-red-spotted pink' flowers of plants with the same genotype. The segregations found do not significantly differ and demonstrate that mutations of $an1^{s/p-+}$ towards pink in the dermal layer of the shoot apex do not exert any influence upon the genotype of the subdermal layer.

The cases described so far concern plants that only partially had the character of a chimera, but repeatedly plants are found that must be chimeric throughout. In Table 4 the results are listed of selfings of a red-flowering plant and of an unspotted white-flowering plant produced after self-pollinations of a plant with the genotype $an1^{s/p-+}an1$. The results found can be explained by assuming that originally the genotype of both plants was $an1^{s/p-+}an1$. In a very early stage after fertilization had taken place, mutations must have occurred of $an1^{s/p-+}$ towards $an1^{+/+}$ and $an1$, respectively, which resulted in the development of plants that carry the mutation in question in the entire epidermal layer, whereas the genotype of the subdermal layer remained unaltered. A comparison between the segregations found in the families in question and in the offspring of plants with genotype $an1^{s/p-+}an1$ confirm this assumption.

The results summarized in Table 4 clearly show that for an interpretation of crossing results with mutants descended from plants with an unstable allele, it is always necessary to bear the possibility in mind that such a mutant might be a periclinal chimera. Therefore, in addition to any type of crossing experiment carried out with such a mutant, self-pollinations should always be made so as to ascertain the true character of the individual.

3.2 Chimeric flowers with unchanged phenotype and mutated sporogenous tissues resulting from mutations in the subdermal layer

The different mutations of the unstable allele $an1^{s/p-+}$ observed in the epidermis of the corolla may also occur in the subdermal layer of the shoot apex. Consequently, one may also predict that chimeric flowers occur in which no change in the colour of the corolla has occurred but whose sporogenous tissues have a mutated genotype resulting from mutations in the second layer of the shoot apex.

In Table 5 results are given of 70 selfings of plants with genotype $an1^{s/p-+}an1$, in each case 2 'white-red-pink' flowers of the same individual have been crossed. The 66 families shown in Table 5A and segregating according to expectation have been discussed in an earlier paper (Doodeman et al. 1984a). The remaining 4 families were omitted at that time because no plants homozygous for $an1^{s/p-+}$ ('white-red-spotted pink') were found. The segregations found in the families in question are given in Table 5B–D. They can readily be explained by assuming that in each case one of the flowers used in the crosses was a chimera, due to mutations of $an1^{s/p-+}$ in the subdermal layer of the shoot apex to $an1$, to $an1^{+/p}$ and to $an1^{+/+}$, respectively. Consequently, the selfings carried out must in fact be regarded as crosses of $an1^{s/p-+}an1$ with $an1an1$, or with $an1^{+/p}an1$, or with $an1^{+/+}an1$. The segregations of the different types of plants occurring in each family

Table 3. Progenies obtained from selfings of differently coloured flowers on plants with genotype $an1^{s/p-+}an1^{s/p-+}$

Colour of flower[a]	No. of families	Descendants[a]													Conclusion about genotype[a]: epidermis / sporogenous tissue
		White-red-spotted pink		White-red-pink		White		Spotted pink		Pink		Red		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	
White-red-spotted pink	21	1,658	*81.8*	116	*5.7*	2	*0.1*	41	*2.0*	1	*0.05*	210	*10.4*	2,028	$\frac{an1^{s/p-+}an1^{s/p-+}}{an1^{s/p-+}an1^{s/p-+}}$
Spotted pink	4	287	*89.1*	9	*2.8*	1	*0.3*	2	*0.6*	–		23	*7.1*	322	$\frac{an1^{s/p-+}an1^{+/p}}{an1^{s/p-+}an1^{s/p-+}}$
Total	25	1,945	*82.8*	125	*5.3*	3	*0.1*	43	*1.8*	1	*0.04*	233	*9.9*	2,350	

[a] See Table 1 for a description of phenotypes and genotypes

Test on homogeneity: $\chi^2_{2\times4}=5.836$ df=3 $P=0.12$ (the classes white, 'spotted pink' and pink combined)

Table 4. Comparison of progenies obtained from selfings of a white- and of a red-flowering descendant of mutant PZ 5158 L-1 (genotype: $anl^{s/p-+}anl$) with those obtained from selfings of that mutant

Phenotype plant[a]	No. of families	Descendants[a]													Conclusion about genotype[a]: epidermis / sporogenous tissue
		White-red-spotted pink		White-red-pink		White		Spotted pink		Pink		Red		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	
White	7	35	*22.3*	71	*45.2*	37	*23.6*	2	*1.3*	3	*1.9*	9	*5.7*	157	$\frac{anl/anl}{anl^{s/p-+}anl}$
Red	4	99	*18.0*	269	*49.0*	148	*27.0*	3	*0.5*	2	*0.4*	28	*5.1*	549	$\frac{anl^{+/+}anl}{anl^{s/p-+}anl}$
White-red-pink	34	772	*19.8*	1,782	*45.8*	1,045	*26.9*	27	*0.7*	33	*0.8*	231	*5.9*	3,890	$\frac{anl^{s/p-+}anl}{anl^{s/p-+}anl}$

[a] See Table 1 for a description of phenotypes and genotypes

Test on homogeneity: $\chi^2_{3\times4} = 5.016$ df = 6 $P = 0.54$ (the classes 'spotted pink', pink and red combined)

Table 5. Progenies obtained from selfings of white flowers with red and pink spots of plants with genotype $anl^{s/p-+}anl$

Colour of flowers[a]	No. of families	Descendants[a]													Conclusion about genotype of the sporogenous tissue of the 2 parental flowers[a]
		White-red-spotted pink		White-red-pink		White		Spotted pink		Pink		Red		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	
A White-red-pink															
observed	66	1,282	*19.8*	2,989	*46.0*	1,766	*27.2*	46	*0.7*	58	*0.9*	353	*5.4*	6,494	$anl^{s/p-+}anl \times anl^{s/p-+}anl$
expected[b]		1,260	*19.4*	3,013	*46.4*	1,799	*27.7*	52	*0.8*	65	*1.0*	305	*4.7*	6,494	
B White-red-pink															
observed	1	–		47	*48.0*	46	*46.9*	–		4	*4.1*	1	*1.0*	98	$anl^{s/p-+}anl \times anl/anl$
expected[b]		–		43	*44.1*	52	*52.6*	–		1	*0.9*	2	*2.4*	98	
C White-red-pink															
observed	1	–		32	*20.1*	42	*26.4*	31	*19.5*	47	*29.6*	7	*4.4*	159	$anl^{s/p-+}anl \times anl^{+/p}anl$
expected[b]		–		35	*22.1*	42	*26.3*	35	*22.1*	43	*27.2*	4	*2.4*	159	
D White-red-pink															
observed	2	–		64	*23.4*	65	*23.7*	–		1	*0.4*	143	*52.2*	273	$anl^{s/p-+}anl \times anl^{+/+}anl$
expected[b]		–		60	*22.1*	72	*26.3*	–		1	*0.5*	140	*51.2*	273	

[a] See Table 1 for a description of phenotypes and genotypes

[b] Starting from the conclusion about the genotype of the sporogenous tissue of the parental flowers as given in the last column of the table, expected values were calculated using the percentages of mutated gametes as found in the progenies of the original mutant PZ 5158 L-1 (see chapter 1)

χ^2 tests:

A $\chi^2 = 10.181$ df = 5 $P = 0.07$

B $\chi^2 = 0.663$ df = 1 $P = 0.42$ (the classes white, pink and red combined)

C $\chi^2 = 1.757$ df = 3 $P = 0.62$ (the classes pink and red combined)

D $\chi^2 = 1.011$ df = 2 $P = 0.60$ (the classes pink and red combined) correspond with the segregations that may be expected in the three types of crosses mentioned (see Table 5 B–D). These results are in agreement with the assumption that in each case the entire sporogenous tissue of one of the parental flowers had a different genotype because the primordia of these flowers must have been initiated within a mutated sector of the subdermal layer of the shoot apex.

3.3 Significant deviations of expected segregations due to the occurrence of large clusters of mutated gametes

It has been established that such external factors as temperature and light intensity can have a marked effect on the mutation frequencies of unstable *An1* alleles (Bianchi et al. 1978). Also, certain genetic factors appear to exert an influence, e.g. the gene *In1* (Gerats et al. 1982; Doodeman et al. 1984a). However, crosses carried out with plants having the same genetic background under identical external conditions may nevertheless yield different segregational ratios. Such differences, which may also occur between progenies descended from the same parental plant, must be ascribed to the fact that the number of mutated gametes also depends on the point of time at which the mutations occurred during the development of the flowers. Occasionally a mutation may happen at a very early stage during the formation of the sporogenous tissue, thus giving rise to a large cluster of mutated gametes. If in a cross such a flower is used as the female parent, or, if pollen is taken from an anther with such a cluster, conspicuous differences may occur in the segregation found in the offspring, as compared with those found in other progenies from the same plant. When percentages of mutated games were computed from segregational ratios in progenies, such conspicuous deviations were not included in the calculation. The percentages obtained in this way appeared to be useful for making reliable predictions about segregations in crossing combinations that had not yet been carried out.

Large clusters of mutated gametes do not necessarily have to be the result of early occurrences of mutations during floral development. A mutation in a cell of the subdermal layer of the shoot apex may lead to the formation of a sector of mutated cells in that layer. If a floral primordium is initiated within such a sector, the flower arising from it will be a periclinal chimera that has sporogenous tissues with a mutated genotype. If only a part of the floral primordium develops within the mutated sector, a flower will arise that only in that part has the character of a periclinal chimera. Such an only partially chimeric flower, when used in a pollination experiment, may also give rise to a deviating segregational ratio on account of a large cluster of mutated gametes.

Table 6 shows the segregations observed in two families descended from the cross 'white-red-spotted pink' × stable white. In this type of cross, 4.8% of red-flowering descendants can be normally expected to appear, but in the two families in question, 37.3% and 16.7% of such plants were found. However, the numbers of red-flowering descendants observed are too small to permit the conclusion that the variegated flowers used in these crosses had to be taken for 'complete' chimeras resulting from mutations towards red in the subdermal layer of the shoot apex. Therefore, the recorded deviations must be ascribed to the incidence of large clusters of mutated gametes resulting from very early occurrences of mutations in developing flower buds, or from the incidence of partially chimeric flowers. From the results of hundreds of crosses carried out with plants with unstable alleles of genes for flower colour and also of other genes, it could be concluded

Table 6. Progenies obtained from crosses of 'white-red-spotted pink'-flowered plants with stable white-flowered plants

Family[a]		Descendants[a]									$\chi^{2\,d}$	P
		White-red-pink		White		Pink		Red		Total		
		No.	%	No.	%	No.	%	No.	%	No.		
C 5371 B	observed	60	*58.8*	3	*2.9*	1	*1.0*	38	*37.3*	102		
	expected:											
	$an1^{s/p-+}an1^{s/p-+} \times an1an1$[b]	90	*88.2*	5	*5.2*	2	*1.8*	5	*4.8*	102	229.027	≪0.01
	$an1^{+/+}an1^{s/p-+} \times an1an1$[c]	45	*44.1*	3	*2.6*	1	*0.9*	53	*52.4*	102	8.837	0.003
D 5612 F	observed	111	*80.4*	3	*2.2*	1	*0.7*	23	*16.7*	138		
	expected:											
	$an1^{s/p-/+}an1^{s/p-+} \times an1an1$[b]	122	*88.2*	7	*5.2*	2	*1.8*	7	*4.8*	138	38.526	≪0.01
	$an1^{+/+}an1^{s/p-+} \times an1an1$[c]	61	*44.1*	4	*2.6*	1	*0.9*	72	*52.4*	138	69.726	≪0.01

[a] See Table 1 for a description of phenotypes and genotypes

[b] Segregation as normally may be expected

[c] Expected values if the variegated flower used in the cross was a periclinal chimera as the result of a mutation of $an1^{s/p-+}$ towards $an1^{+/+}$ in the subdermal layer of the shoot apex

[d] The classes 'white-red-pink', white and pink combined

Table 7. Progenies obtained from selfings of flowers on a branch with normal, green leaves of a plant with yellowish-green leaves bearing green spots ($yg3^r yg3^r$)

Family	No. of capsules	Descendants					$\chi^2_{3:1}$	P
		Green		Yellowish-green with green spots		Total		
		No.	%	No.	%	No.		
G 5149	2	123	*74.1*	43	*25.9*	166	0.072	0.79

Table 8. Progeny obtained from a cross of a flower on a yellowish-green branch with green spots and a flower on a branch with normal, green leaves of the same plant

Family	No. of capsules	Descendants					$\chi^2_{1:1}$	P
		Green		Yellowish-green with green spots		Total		
		No.	%	No.	%	No.		
G 5151	1	39	*45.3*	47	*54.7*	86	1.674	0.20

that such large clusters giving rise to significant deviations in segregational ratios, are only of very rare occurrence.

3.4 Chimeras resulting from mutations of an unstable allele of gene Yg3 for leaf colour

In the course of the investigations into the inheritance of unstable alleles, new unstable mutations occurred, presumably as the result of transpositions of a genetic element inserted at the *An1* locus towards other loci (Doodeman et al. 1984a, b). One of these mutations affected the gene *Yg3* and gave rise to plants with yellowish-green leaves bearing sharply delimited green spots – a phenomenon which must be ascribed to reversions of the unstable allele, $yg3^r$, towards *Yg3*. These reversions do not remain restricted to the leaf, but also occur in the sporogenous tissues. This is manifest from the occasional incidence of plants with normal, green leaves in progenies obtained from selfings of plants homozygous for $yg3^r$. As might be expected, such green revertants appear to be heterozygous for the reversion.

On yellowish-green plants with variegated leaves lateral branches sometimes develop with normal, green leaves. Since the chlorophyll-containing mesophyll, in which gene *Yg3* becomes expressed, is derived from the subdermal layer of the shoot apex, those branches must be the result of reversions within this layer, which also yields the sporogenous tissues. Selfings of flowers on such a green branch resulted in progenies segregating into 3 green : 1 yellowish-green with green spots (Table 7). A cross of a flower produced on a green branch with one from a green-spotted, yellowish-green branch of the same plant resulted in an offspring of green and green-spotted, yellowish-green in a ratio of 1 : 1 (Table 8).

These results differ from corresponding selfings and crosses of self-coloured flowers borne on variegated-flowering plants. In those instances, mutations leading to changes in flower colour do not exert any influence upon the progenies obtained from flowers mutated in this way. The explanation must be sought in the fact that genes for flower colour only find their expression in the epidermal cells of the corolla. Consequently, changes in flower colour must be the result of mutations in the outer layer of the shoot apex. Such mutations will only be present in the epidermal tissues derived from that layer and not in the subdermal layer that yields the sporogenous tissues and, accordingly, the gametic nuclei.

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