EVOCATION OF INSTABILITY AT THE PAL LOCUS IN *ANTIRRHINUM MAJUS*

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SUMMARY

A new type of genetic instability in two *Antirrhinum* plants has been investigated. Results presented in this paper show:

(a) The instability is associated with a special allele of *Pal*, an anthocyanin-producing gene. The special allele is denoted as *pal^{rec-low}* to distinguish it from the hitherto characterised "standard" *pal^{rec}*, which has a much higher mutation frequency.

(b) A high degree of instability at *pal^{rec-low}* can be evoked by crossing it to other lines homozygous for *pal^{tub}* (colourless flowers with a narrow ring of pigment at the base of the floral tube) or *pal^{rec}* ("standard" unstable allele of *Pal*).

(c) The degree of released instability is inherited, although in earlier generations after the release there is a tendency for reversion towards the original state.

(d) There is no evidence for the presence of modifiers in the *pal^{rec-low}* plants which are capable of reducing the instability of standard *pal^{rec}*.

The evocation of instability in *Antirrhinum* is compared with paramutation in maize.

1. INTRODUCTION

ALTHOUGH the existence of instability at the *Pal* locus in *Antirrhinum* has been known for a long time (De Vries, 1911), its basic features were reported only recently in a series of papers by Fincham and Harrison (see Fincham, 1970, for references). In essence, various alleles of the *Pal* gene control anthocyanin formation in flowers and, less conspicuously, in leaves and stems. When the dominant allele, *Pal*, is present, the flowers are uniformly coloured. The recessive allele, *pal*, causes colourless flowers. When a special, unstable allele called *pal^{rec}* is present, flowers show coloured sectors of various sizes, shapes and intensities on a colourless background. Somatic changes of recessive *pal* (or a repressed form of *Pal*) to a dominant condition are suggested to be the basis of this variegation. For further details, the reader is referred to reviews by Fincham (1970, 1973) and a more recent paper by Harrison and Carpenter (1973).

Some years ago we observed that various degrees of instability at the *Pal* locus could be evoked in a particular strain in which the *pal^{rec}* allele appeared to have become more or less completely stabilised in a colourless form. On further examination, it was revealed that this process is unique in several respects and might be related to paramutation of *R* in maize, a phenomenon investigated by Brink and his students (Brink, 1973). This is the main thesis of the present paper.

2. MATERIALS AND METHODS

(i) *Strains*

Antirrhinum strains of two different origins have been employed. The first group which will be referred to as standard strains, comprises *pal^{rec}* and *pal^{tub}*; these strains were originally obtained from the John Innes Institute and are maintained in our laboratory in various combinations. The allele *pal^{tub}* produces colourless flowers, apart from the base of the floral tube which shows a narrow ring of pigment. This is a useful character, since it facilitates the separation of homozygous and heterozygous plants: *pal^{tub}pal^{tub}* (colourless flowers with ring), *pal^{rec}pal^{tub}* (variegated flowers with ring) and *pal^{rec}pal^{rec}* (variegated flowers without ring). Homozygous *pal^{rec}* plants show a higher mutation frequency than their *pal^{rec}pal^{tub}* counterparts. All the standard strains have been inbred for many generations; they are free from the stabilising modifier identified by Harrison and Fincham (1968).

The main experimental material, however, was derived from seed kindly donated by Dr Vishnu Swarup of the Indian Agricultural Research Institute, New Delhi. For several years he has maintained this variety, originally obtained from a local commercial seed firm, for breeding purposes. From the seed provided by Dr Swarup, 155 plants were grown at Leeds. These turned out to be a heterogeneous mixture of various phenotypes, a majority of them exhibiting varying degrees of variegation. Two plants (51-14-1 and 51-14-24) were, however, colourless and the tube-ring (characteristic of *pal^{tub}*) was not present. On further examination, a tiny spot was detected on one flower in each plant. Seed derived from these two plants provided the foundation material for the investigations reported in this paper. Although the material derived from the two plants was analysed separately, the results will be combined since there were no differences between the two sets of data. It will be shown that the peculiar properties of the low plants are controlled by a special allele at the *Pal* locus, so this allele will be referred to as *pal^{rec-low}* to distinguish it from the standard *pal^{rec}*.

Several plants with different levels of variegation obtained in the Delhi stock were also backcrossed to our *pal^{tub}pal^{tub}* line. One of these unstable lines was used in one set of experiments; this line will be referred to as *pal^{rec}Delhi*.

(ii) *Quantitative estimates*

Several methods have been tried to estimate the somatic mutation frequencies. Although actual counting of the mutant sectors appeared to be an accurate procedure, it was not considered to be practical when one had to score several hundreds of plants within a limited time. Ultimately we settled on a scale consisting of photographs showing increasing grades of variegation; on this scale, class 0 represents the flowers with no mutant sectors, class 8 means fully coloured flowers and the intervening classes represent the intermediate grades of variegation. By repeated scoring of coded samples we found our estimates were reproducible within an interval of 0.5 class so any differences less than that were not considered to be meaningful. In practice, several flowers in an inflorescence are scored individually and the mean scores for the plants are calculated from these

Plate I

An inflorescence showing shifts to higher mutation rates.

Plate II

A series of flowers from different *paprec-low* lines in which the allele was showing different degrees of instability. The flower at the extreme right in the lower row is stabilized homozygous *paprec-low* (table 1).

values. This procedure has resulted in values like 5.3 or 6.9 instead of round numbers.

(iii) Pedigrees

Every plant is identified with three numbers, such as 51-14-24. The first represents the serial number of the experiment, the second the family number and the third the plant number within the family. Thus 51-14-24 means plant number 24 in the 14th family in experimental series 51. The sign $\otimes$ after such a number indicates that the plant concerned has been selfed. In crosses the first identification number denotes the female parent. Thus 51-14-24 $\times$ 51-5-76 $\rightarrow$ 51-79 $\rightarrow$ 51-79-32 $\otimes$ $\rightarrow$ 51-85 means that 51-14-24 and 51-5-76 were crossed, the F_1 was raised under the number 51-79, the plant with number 32 in this F_1 family was selfed and the progeny was grown under family number 51-85.

3. RESULTS

(i) Selfed progenies

Both 51-14-1 and 51-14-24 were selfed to provide seed for the S_1 generation; phenotypic clarification of this and three further generations is given in table 1. Apart from the plants shown in the table, about 150 plants from the S_2 generation were grown outside during the summer of 1970. Although these plants could not be scored in detail, they also showed a similar colourless phenotype with very rare mutant spots. From these observations it was clear that the near-colourless phenotype of the low line was stably inherited and nothing seemed to be segregating. No differences were seen even when the seed was obtained from the few flowers showing isolated mutational spots.

TABLE 1

Phenotypes of four selfed generations of 51-14-1 and 51-14-24

Generation	No. of parental plants	Colourless progeny	Plants with rare mutations	Total
S_1	2†	30	3	33
S_2	6	28	2	30
S_3	4	30	1	31
S_4	5	21	1	22

† The original two low plants (51-14-1 and 51-14-24).

(ii) Crosses with $pal^{tub}pal^{tub}$

Both 51-14-1 and 51-14-24 were reciprocally crossed with $pal^{tub}pal^{tub}$. From these crosses, 69 F_1 plants were grown to flowering. Later on, two more plants (51-306-2 and 51-318-4) from the S_2 generation were crossed with $pal^{tub}pal^{tub}$. Fifty-seven plants were raised from these crosses in a

different season. Data from these two sets of plants are presented in table 2 and demonstrate the following points:

- (i) Every one of the 126 F_1 plants scored showed a higher grade of variegation than the very sparsely spotted $pal^{rec-low}$ parent plants. This establishes that the low plants are homozygous for an unstable pal allele, whose mutability was to some extent released in every F_1 plant.
- (ii) There was a striking variation in the mutation frequencies of these plants, which varied from class 0.9 to 4.5.

TABLE 2

Phenotypic classification of F_1 plants from $pal^{rec-low}pal^{rec-low} \times pal^{tub}pal^{tub}$ crosses

Cross	Progeny	Plants falling in the classes of				Total plants	Mean $\pm$ S.D.
		1.0-2.0	2.1-3.0	3.1-4.0	4.1-5.0		
51-14-1 $\times$ 51-5-76 ^a	51-76	7	7	1	0	15	2.10 $\pm$ 0.88
51-5-11 $\times$ 51-14-1	51-77	3	5	5	0	13	2.76 $\pm$ 0.89
51-14-24 $\times$ 51-5-76 ^a	51-80	6	5	2	0	13	2.19 $\pm$ 0.39
51-5-43 $\times$ 51-14-24	51-81	7	16	5	0	28	2.70 $\pm$ 0.69
51-394-1 $\times$ 51-306-2 ^b	51-580	0	0	11	1	12	3.76 $\pm$ 0.99
51-394-7 $\times$ 51-306-2 ^b	51-581	1	1	10	0	12	3.67 $\pm$ 0.72
51-394-5 $\times$ 51-318-4 ^c	51-584	0	6	13	1	20	3.35 $\pm$ 0.53
51-394-6 $\times$ 51-318-4 ^c	51-585	0	5	7	1	13	3.34 $\pm$ 0.55
Total		24	45	54	3	126	
Mean $\pm$ S.D.		1.40 $\pm$ 0.40	2.84 $\pm$ 0.26	3.64 $\pm$ 0.28	4.50 $\pm$ 0.00		2.95 $\pm$ 0.90

^a Except in crosses 1 and 3, the low plants were used as male parents.

^b In crosses 5 and 6, the low parent is the same, but the pal^{tub} parents are different.

^c In crosses 7 and 8, the low parent is the same, but the pal^{tub} parents are different.

Variation from plant to plant is expected in the earlier crosses and back-crosses into a new strain of different genetic background. But a proportion of plants showed a significant and unusual "internal variation" caused by frequent shifts from one mutation frequency within a plant (plate I). As a result of this, different parts of the flowers, different flowers and flowers on different branches showed considerable variation. The shifting process became more striking and its significance more apparent as these studies progressed.

The observations reported so far raise an interesting question: why does the low strain show a near-colourless phenotype even though it is homozygous for an unstable allele of pal ? If the experience with standard pal^{rec} is anything to go by, then the original, homozygous $pal^{rec-low}$ plants should have produced greater instability than the heterozygous plants. Several alternative explanations can be considered, but the one that comes to mind immediately is a possible presence of a homozygous mutation-reducing modifier in the low stock. This idea needs special consideration, since such a modifier for standard pal^{rec} has been found by Harrison and Fincham (1968).

(iii) F_2 progenies

To seek evidence for the above suggestion, F_2 progenies from ten different F_1 plants were studied; the scores of these F_1 plants varied from 1.0 to 3.5. As can be seen from table 3, only a limited number of plants for each F_2

TABLE 3
Mean scores of F_2 families and the respective F_1 parents

Pedigree		F ₁ score	F ₂ score				Total plants	F ₂ mean score ± S.D.
			<i>pal^{rec-low}pal^{tub}</i>		<i>pal^{rec-low}pal^{rec-low}</i>			
			Plants scored ^a	Score ± S.D.	Plants scored	Score ± S.D.		
F ₁ ^c	F ₂	F ₁ score	Plants scored ^a	Score ± S.D.	Plants scored	Score ± S.D.	Total plants	F ₂ mean score ± S.D.
51-76-8	51-309	1.00	22 (10)	1.68 ± 1.59	18	0.81 ± 0.71	40	1.28 ± 1.33
51-77-15	51-316	1.00	13 (5)	2.41 ± 1.86	7	2.69 ± 1.74	20	2.51 ± 1.78
51-80-11	51-321	1.00	14 (11)	2.81 ± 1.61	6	2.60 ± 1.83	20	2.75 ± 1.63
51-76-3	51-307	1.10	26 (10)	0.63 ± 0.45	11	0.56 ± 0.43	37	0.60 ± 0.43
51-76-9	51-310	1.20	19 (19)	0.93 ± 0.55	5	0.40 ± 0.46	24	0.82 ± 0.57
51-80-13	51-322	1.40	7 (3)	1.79 ± 1.34	3	2.03 ± 2.22	10	1.86 ± 1.52
51-77-1 ^b	51-314	1.90	9 (11)	4.04 ± 2.17	5	3.70 ± 2.41	14	3.92 ± 2.17
51-76-12	51-311	2.25	25 (17)	2.20 ± 1.57	9	1.74 ± 1.84	34	2.14 ± 1.68
51-76-5	51-308	3.00	33 (9)	3.24 ± 1.61	17	3.19 ± 2.45	50	3.22 ± 1.97
51-77-4	51-315	3.50	9 (4)	3.82 ± 2.10	13	3.86 ± 2.36	22	3.85 ± 2.21

^a Plants with *pal^{tub}* character (*i.e.* pigment at the base of corolla tube) but without mutant spots were considered as *pal^{tub}pal^{tub}* and they were not included in the above calculations; the number of such plants is shown within the brackets.

^b 51-77-1 showed sectoring from low mutability to high mutability.

^c For families from which F_1 originated, see table 2.

TABLE 4
Distribution of homozygous *pal^{rec-low}pal^{rec-low}* plants in F_2 populations†

F_2 pedigree	F_1 score	Plants falling in the classes of							Total plants
		0.0-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	
51-309	1.00	10	6	2	0	0	0	0	18
51-316	1.00	1	2	2	0	1	1	0	7
51-321	1.00	1	2	1	1	0	0	1	6
51-307	1.10	10	1	0	0	0	0	0	11
51-310	1.20	4	1	0	0	0	0	0	5
51-322	1.40	2	0	0	0	1	0	0	3
51-314	1.90	1	0	0	1	2	0	1	5
51-311	2.25	4	0	3	1	0	1	0	9
51-308	3.00	4	3	0	1	4	3	2	17
51-315	3.50	3	0	1	1	1	5	2	13
Total		40	15	9	5	9	10	6	94

† Using this method of classification, homozygous *pal^{rec-low}* plants described in table 1 fall into the first column (0.0-0.9).

family were scored. The original intention was to see if there would be any plants homozygous for the *pal^{rec-low}* with much higher mutability than the original plants. The desired homozygotes can be easily identified by the absence of the *pal^{tub}* pigment ring (see Materials and Methods). The presence of such genotypes with high mutation frequency would support the modifier hypothesis. In table 4 the homozygous plants are grouped

according to their scores; the situation is obviously not simple, but the presence of plants with very high scores is strikingly clear. This finding suggests the presence of a modifier or modifier complex in the low plants which affects the mutation rate of the unstable allele. Following the same line of argument, one may also say that the high plants, recorded in the 5.0-5.9 and 6.0-6.9 columns, are free from the modifier.

The data of table 2 give some indication that part of the variation with respect to mutation frequency is heritable since there is a significant correlation ($0.66, 0.01 < P < 0.05$) between F_1 plants and the means of their F_2 progenies.

TABLE 5

Effect of pal^{tub} , extracted from $pal^{rec-low}pal^{tub}$, on pal^{rec} : phenotypes of $pal^{tub}pal^{tub}$ (ex $pal^{rec-low}pal^{tub}$) $\times$ pal^{rec} pal^{rec} (entries 1-18) and $pal^{tub}pal^{tub} \times pal^{rec}pal^{rec}$ (controls, entries 19 and 20)

Entry	Cross	Plants scored	Mean
1	51-587-15 $\times$ 51-544-8	6	5.50
2	51-325-4 $\times$ 51-365-2	20	5.50
3	51-325-7 $\times$ 51-369-2	16†	5.56
4	51-586-6 $\times$ 51-544-10	10	5.60
5	51-313-14 $\times$ 51-365-2	21	5.67
6	51-586-5 $\times$ 51-544-10	9	5.67
7	51-317-12 $\times$ 51-365-2	9	5.72
8	51-317-7 $\times$ 51-365-2	24	5.73
9	51-586-3 $\times$ 51-544-10	8	5.81
10	51-313-4 $\times$ 51-365-2	18	5.83
11	51-586-12 $\times$ 51-544-10	9	5.83
12	51-586-13 $\times$ 51-544-10	10	5.90
13	51-313-15 $\times$ 51-365-2	19	5.92
14	51-325-3 $\times$ 51-369-2	20	6.12
15	51-586-17 $\times$ 51-544-10	10	6.25
16	51-587-10 $\times$ 51-544-8	3	6.33
17	51-587-13 $\times$ 51-544-8	9	6.33
18	51-586-9 $\times$ 51-544-10	8	6.34
Total		229	5.83
19	51-824-15 $\times$ 51-681-d	20	5.63
20	51-824-15 $\times$ 51-681-c	21	5.38
Total		41	5.50

† One plant showing shifts for mutability.

(iv) *Effect of pal^{tub} derived from $pal^{rec-low}pal^{tub}$ on pal^{rec}*

If the low phenotype is caused by an interaction of a modifier (or a modifier complex) and a pal^{rec} -like allele, as the data presented in the previous section seem to suggest, then some $pal^{tub}pal^{tub}$ plants in progenies segregating for $pal^{rec-low}pal^{tub}$ genotypes should also carry the modifier. To test this, 18 $pal^{tub}pal^{tub}$ plants derived from various $pal^{rec-low}pal^{tub} \times pal^{tub}pal^{tub}$ crosses (see section viii) were crossed with standard $pal^{rec}pal^{rec}$; the F_1 phenotypes were compared with $pal^{rec}pal^{tub}$ in which the pal^{tub} was derived from a standard $pal^{tub}pal^{tub}$ tester.

From the mean scores obtained from different crosses presented in table 5, it can be seen that only four crosses gave values lower than the control values

(entries 19-20); analysis of variance showed that even these differences are not statistically significant ($F = 0.40$, $P < 0.05$, d.f. 5 and 87). So there is no evidence that any of the 18 $pal^{tub}pal^{tub}$ segregants inherited anything from the low strain which could influence the mutation frequency of pal^{rec} .

The crosses were also examined for shifts in level of variegation since several $pal^{rec-low}pal^{tub}$ families from which the $pal^{tub}pal^{tub}$ types were derived showed frequent shifting. Only one such plant was found in a total population of 229. Although its progeny have not yet been analysed, the presence of this single plant provides little support for the idea of a shift-causing modifier, especially since such plants are occasionally seen in the standard pal^{rec} strain.

(v) $pal^{rec-low}pal^{rec-low} \times pal^{rec}pal^{rec}$ crosses

The negative evidence reported in the previous section is in itself not very conclusive, since it can always be argued that the postulated modifier is linked to pal^{rec} , in which case very few of the homozygous pal^{tub} segregants might carry it. To obviate this difficulty, an alternative approach has been taken: six $pal^{rec-low}pal^{rec-low}$ plants from the fourth selfed generation (table 1) were crossed with $pal^{rec}pal^{rec}$. The phenotypes of the resulting $pal^{rec-low}pal^{rec}$ plants were compared with those of (a) $pal^{rec}pal^{tub}$ and (b) $pal^{rec}pal^{rec}$.

The mean score of 5.74 calculated from the 110 $pal^{rec-low}pal^{rec}$ plants is almost identical to that given by the $pal^{rec}pal^{tub}$ family (5.75) but significantly different from $pal^{rec}pal^{rec}$ (7.39); the detailed data are presented in table 6. These observations made it clear that if the low mutation frequency of the $pal^{rec-low}$ plants is due to modifiers that reduce the mutation frequency of pal^{rec} , these must be virtually completely recessive, yet the comparatively low scores shown by many of the F_1 plants shown in table 2 suggest a considerable, if variable, degree of dominance.

TABLE 6

Effect of $pal^{rec-low}$ on pal^{rec} : F_1 scores from the $pal^{rec-low}pal^{rec-low} \times pal^{rec}pal^{rec}$ crosses are summarised in entries from 1 to 6. Entries 7 and 8 provide the scores from $pal^{rec}pal^{tub}$ and $pal^{rec}pal^{rec}$ plants respectively.

Entry	Cross	Plants scored	Mean score $\pm$ S.D.
1 ^a	51-578-1 $\times$ 51-544-10	13	5.85 $\pm$ 0.32
2	51-578-4 $\times$ 51-544-10	15	5.80 $\pm$ 0.25
3	51-579-1 $\times$ 51-544-10	17	6.06 $\pm$ 0.24
4	51-579-6 $\times$ 51-544-10	16	5.45 $\pm$ 0.53
5	51-582-6 $\times$ 51-544-10	31	5.68 $\pm$ 0.70
6	51-582-7 $\times$ 51-544-10	18	5.67 $\pm$ 0.42
All families		110	5.74 $\pm$ 0.54
7	51-564 ^b	38	5.75 $\pm$ 0.59
8	51-544 and 51-461	65	7.39 $\pm$ 0.23

Statistical analysis: $F = 89.35$ ($P < 0.01$); d.f.₁ = 7; d.f.₂ = 205.

^a Entries 1 and 2 contained, respectively, two and one plants showing extreme shifting; they were not included in these calculations.

^b Composite of three crosses.

Two other points should be mentioned: first, there were no exceptional plants with low mutability among the 110 scored and, second, only three plants showed shifts.

(vi) *Effect of $pal^{rec-low}$ on $pal^{rec}Delhi$*

A possibility that has not been considered so far is that pal^{rec} in standard strains may be insensitive to a special modifier carried by the low plants. Very specific interactions between controlling elements and the alleles controlled are well known in maize (Fincham and Sastry, 1974). The appropriate tester allele for a modifier in $pal^{rec-low}$ might be expected to be

TABLE 7

Effect of $pal^{rec-low}$ on $pal^{rec}Delhi$: scores of $pal^{rec-low}pal^{tub}$ and $pal^{rec-low}pal^{rec}Delhi$ plants from $pal^{rec-low}pal^{rec-low} \times pal^{tub}pal^{rec}Delhi$ crosses.

Entry	Cross	<i>pal^{rec-low} pal^{tub}</i>		<i>pal^{rec-low} pal^{rec}Delhi</i>	
		No. scored	Mean	No. scored	Mean
1	51-578-3 $\times$ 51-569-6	8	2.30	5	4.00
2	51-578-1 $\times$ 51-569-6	7	2.50	8	4.56
3	51-582-6 $\times$ 51-569-6	13	2.58	7	4.79
4	51-582-4 $\times$ 51-569-6	5	1.90	12	5.17
5	51-582-3 $\times$ 51-569-6	6	3.00	11	5.27
6	51-582-2 $\times$ 51-569-6	7	2.81	11	5.50
7	51-579-2 $\times$ 51-569-4	9	3.89	6	6.00
8	51-579-1 $\times$ 51-569-4	11	4.00	3	6.33
Total		66	2.87	63	5.20

$pal^{rec}Delhi$, inasmuch as this was the mutable allele present in other plants of the population in which $pal^{rec-low}$ was found. Accordingly, two plants from a $pal^{rec}Delhi pal^{tub}$ line were crossed with eight different homozygous $pal^{rec-low}$ plants. The $pal^{rec}Delhi$ has a fairly high and uniform mutability, although not as high as our standard pal^{rec} . The phenotypes of $pal^{rec-low}pal^{tub}$ and $pal^{rec-low}pal^{rec}Delhi$ plants from the crosses mentioned are given in table 7. Since these experiments were intended as a preliminary investigation to see whether $pal^{rec-low}$ had any drastic effect on $pal^{rec}Delhi$, controls such as $pal^{rec}Delhi pal^{tub}$ were not grown. With this qualification one may state that the $pal^{rec-low}$ genome has not severely reduced the mutation rate of $pal^{rec}Delhi$. The variation between $pal^{rec}Delhi pal^{rec-low}$ plants from crosses 1 to 6 (table 7) may also be noted; in these crosses only one $pal^{rec}Delhi pal^{tub}$ parent was used. This variation cannot be explained on the basis of presence or absence of a single modifier. Again, excepting one possible contaminant,

FIG. 1.—Distribution of mutability scores in inbred progenies of $pal^{rec-low}pal^{tub}$ plants. In each family heterozygous and homozygous plants were scored separately on the scale described in Materials and Methods. For the diagram plants were grouped into classes 0.0-9, 1.0-1.9, 2.0-2.9, etc., and labelled, respectively, 0, 1, 2, etc. Open bars represent $pal^{rec-low}pal^{tub}$ heterozygotes and solid bars $pal^{rec-low}$ homozygotes. The exact score of each selfed plant is shown on the arrow connecting it with its progeny. The number of plants scored is shown for each family (*heterozygotes, **homozygotes) with the mean score in brackets. The F_1 family was no. 51-76 (table 2). A parallel series of progenies stemming from F_1 family 51-80 showed a very similar pattern of results.

none of the *pal^{rec-low}pal^{rec}Delhi* plants showed exceptionally low mutability. Seven plants in the *pal^{rec-low}pal^{tub}* class showed clear shifts; there was only one such possible case, and that one was unclear, in the *pal^{rec-low}pal^{rec}Delhi* class.

(vii) *Further inbred progenies of pal^{rec-low}pal^{tub} plants*

Three *pal^{rec-low}pal^{tub}* plants from the families described in section (iii) have been selfed for 2 to 3 generations. Fig. 1 shows in detail the scoring of the progenies derived from two of these three plants; the results from the

FIG. 2.—Distribution of mutability scores in selfed progenies of highly mutating plants (score 4) and of plants with low mutability (score 1). Combined data from F_2 and later generations.

other lineage were very similar. It is clear that the F_2 generation shows segregation of factors controlling mutation frequency. F_2 high-mutable plants gave, in further generations, progenies which were predominantly of high mutability, with occasional low or very low plants. Predominantly high-mutable lines could be established either from homozygous *pal^{rec}* or from heterozygous *pal^{rec}pal^{tub}* plants. Low-mutable F_2 plants, on the other hand, gave almost entirely low or very low-mutable progenies (grade 1.0 or lower), even though they tended to be somewhat higher than the original *pal^{rec-low}* plants from which the breeding programme was started. A notable feature of the high lines, at least in the earlier generations of selfing, is the recurrence in each generation of the same pattern of variability, with a predominance of high plants and a small but variable number of low plants. This pattern is shown in fig. 2.

Three low plants which appeared in a second generation of high families were selfed and their progenies were examined to see if any higher plants were produced; all three progenies showed only very low frequencies. Data from these experiments are not shown in fig. 1.

It should be pointed out that the so-called "low" class includes different levels of mutability. In general, any plant or family with a grade of variegation of class 0.5 or less is referred to as low. However, if one compares the actual numbers of spots (the numbers of mutational events) it can be shown that most of these plants have higher mutation frequencies than the original homozygous *pal^{rec-low}* plants and their selfed derivatives shown in table 1.

Typical flowers from various selfed lines are shown in plate II. When plants of intermediate grades were scored they were examined in order to see whether their apparently intermediate phenotypes were due to close intermingling of high and low sectors (that is, to frequent shifts between two extreme states). Although this may have been the situation in some cases, most apparent intermediates could not easily be interpreted in this way.

TABLE 8

Mutability grades of various backcrosses: $pal^{rec-low}pal^{tub} \times pal^{tub}pal^{tub}$

Cross	Progeny family	Plants scored	Score	
			Parent	Progeny Mean $\pm$ S.D.
<i>First backcross^b</i>				
51-80-1 $\times$ 51-89-4	51-323	7	1.00	3.33 $\pm$ 1.02
51-80-11 $\times$ 51-89-4	51-324	6	1.00	3.70 $\pm$ 1.21
51-81-16 $\times$ 51-89-4	51-325	5	1.50	2.90 $\pm$ 1.24
51-81-2 $\times$ 51-89-4	51-326	8	2.00	3.31 $\pm$ 1.01
51-76-16 $\times$ 51-89-4	51-313	11	2.70	3.78 $\pm$ 0.89
51-77-4 $\times$ 51-89-4	51-317	13	3.50	3.64 $\pm$ 1.08
<i>Second backcross</i>				
51-325-1 $\times$ 51-331-8	51-595	14	2.00	3.64 $\pm$ 0.86
51-332-4 $\times$ 51-313-9 ^a	{ 51-586 51-587 }	26	2.10	3.50 $\pm$ 1.16
51-317-6 $\times$ 51-332-5		51-590	15	2.50
51-323-9 $\times$ 51-331-10	{ 51-593 51-594 }	18	3.79	3.96 $\pm$ 1.05
51-332-6 $\times$ 51-313-11 ^a		51-589	13	5.00
51-317-11 $\times$ 51-331-20	51-592	5	5.50	4.90 $\pm$ 0.89
<i>Third backcross</i>				
51-593-13 $\times$ 51-541-22	51-954	11	1.50	2.86 $\pm$ 0.71
51-586-1 $\times$ 51-514-4	51-947	6	2.00	4.25 $\pm$ 1.04
51-587-2 $\times$ 51-541-3	51-948	9	2.50	3.50 $\pm$ 0.83
51-587-5 $\times$ 51-541-2	51-949	14	2.50	3.57 $\pm$ 0.47
51-593-3 $\times$ 51-541-22	51-955	10	2.50	3.60 $\pm$ 1.02
51-593-9 $\times$ 51-541-22	51-953	10	3.00	3.80 $\pm$ 0.75
51-594-15 $\times$ 51-541-2	51-956	10	5.00	4.35 $\pm$ 0.47
51-594-2 $\times$ 51-541-2	51-957	9	5.00	3.83 $\pm$ 1.60
51-589-7 $\times$ 51-541-22	51-951	8	5.50	3.81 $\pm$ 0.88
51-589-13 $\times$ 51-541-22	51-952	8	5.50	4.88 $\pm$ 0.88

^a Except in these two crosses, *pal^{rec-low}pal^{tub}* was used as a female parent.

^b Origin of the F_1 families is shown in table 2.

(viii) *Results of backcrossing to pal^{tub}*

F_1 plants (see table 2) were backcrossed successively to plants of the homozygous pal^{tub} stock. The results (table 8) showed that a fairly high level of mutability was maintained through the successive backcrosses, but with a good deal of persistent variability. This variability showed no more than a low degree of heritability; there were no significant differences between the mean scores of any of the families despite the widely varying scores of the parent plants. It seems that virtually the whole potential instability of the pal^{rec} derived from $pal^{rec-low}$ is released by one generation of crossing to pal^{tub} . Indeed, the mean scores of the F_1 progenies (table 2) are not consistently less than those of the backcross generations, and the somewhat lower average score in the F_1 is of uncertain significance in view of the fact that one is comparing progenies grown in different seasons.

(ix) *Effect of pal^{rec} on $pal^{rec-low}$*

If one assumes that the instability of the $pal^{rec-low}$ allele is evoked by pal^{tub} , or something in the pal^{tub} stock, then it may be asked whether other stocks can induce a similar change. To answer this question, nine $pal^{rec-low}$ pal^{rec} plants described in section (v) were selfed and 143 F_2 plants were raised. If pal^{rec} has no influence of the mutability of $pal^{rec-low}$, then the F_2 progenies should possess a quarter of plants without any (or with very few) mutant spots. On the other hand, if pal^{rec} pal^{rec} stock has a similar effect as the stock carrying pal^{tub} then the F_2 from the $pal^{rec-low}$ pal^{rec} should show considerable variation. The F_2 data presented in table 9 show the amount of variation displayed by the F_2 progenies in question and indicate that the pal^{rec} stock has a similar effect to that of pal^{tub} .

4. DISCUSSION

This project was undertaken to investigate the genetic basis of a repressed phenotype shown by two colourless plants and their selfed progeny. Throughout the investigations two alternative explanations were kept in mind:

(1) In these plants, pal^{rec} itself was in its "standard" state and the "low" phenotype was caused by the presence of a mutation-repressing modifier or modifiers.

(2) The $pal^{rec-low}$ is a special stable state of pal^{rec} from which it can change to a highly unstable state when placed in a different genotype, this release being self-perpetuating once it has occurred.

The first possibility was strongly anticipated in the beginning since a semidominant modifier (*Stabiliser*, *St*) which can repress the mutability of standard pal^{rec} was previously discovered by Harrison and Fincham (1968). When *Stabiliser* is segregated out from the genotype the mutability of pal^{rec} is completely restored and when such restored pal^{rec} plants are crossed with pal^{tub} pal^{tub} plants containing *St* the instability of pal^{rec} is again reduced. However, only the F_2 data discussed in the Results section (iii) supported the modifier hypothesis for $pal^{rec-low}$, and even here there were some difficulties. First of all, there was much variation, at least some of it heritable, among F_1 plants from the cross of $pal^{rec-low}$ to pal^{tub} . This cannot be explained by

homozygosity for a single modifier though it would be compatible with a more complicated system of duplicate modifiers, some homozygous and some heterozygous.

Secondly, the fact that plants with very high mutability appeared in the F_2 (see table 4) would suggest that the hypothetical modifier(s) were at least semidominant, like the one described by Harrison and Fincham (1968); they could not have been completely recessive, since the F_1 plants showed comparatively high mutability. As has been pointed out in the Results, the modifier(s) should be absent from high plants and these should breed true. Contrary to this expectation, high plant progenies almost always included a proportion of very low plants (fig. 2).

Again, the fact that all grades of mutability tended to occur in all progenies, though in different proportions, would require a very complex system of modifiers to explain it.

Several tests were made for the presence in the low plants of modifiers capable of suppressing the mutability of either *pal^{rec}* or *pal^{rec}Delhi*; none of the tests was positive.

The strongest evidence against the modifier hypothesis is provided by the shifts in mutability observed in various plants (plate I). Three main types of shifts have been encountered so far: (a) cases where the grade of mutability is more or less constant within each inflorescence but the inflorescences on the same plant differ from one another, (b) cases where the individual flowers are different from each other and (c) cases where different sectors of a flower differ. Although a detailed analysis of this phenomenon is not yet complete, it has not been possible to show so far that the shifting is associated with any genetic factor other than *pal^{rec}* itself. The shifting phenomenon shows that the level of mutability is itself subject to some kind of mutation during vegetative growth, and that the different levels observed cannot be wholly due to a stable modifier or modifier complex.

Thus the evidence seems much more compatible with the first hypothesis, namely that the original plants, 51-14-1 and 51-14-24, contained a special unstable *pal* allele, referred to here as *pal^{rec-low}*. I propose that this allele, when homozygous, produces a near-colourless phenotype, but that when the colourless plants homozygous for *pal^{rec-low}* are crossed with standard strains the instability of *pal^{rec-low}* is released to a degree which is different in different plants. The variation in F_1 plants may be partly due to differences in the timing of the release of mutability, giving various proportions of high and low mutability in intermingled sectors, but it seems also as if persistent states of intermediate mutability can be present in the germ cells or arise early in development. The occurrence in later generations of homozygous families with high mutability (fig. 1) demonstrates that a destabilised *pal^{rec-low}* does not necessarily revert to the original repressed condition when made homozygous, though occasional low plants did occur in these families.

One never sees plants with very low mutability among progeny of backcrosses to *pal^{tub}* although, as just noted, occasional low plants did appear in the selfed progenies. This suggests that the maintenance, or re-establishment, of the low-mutable state requires homozygosity of some factor present in the original *pal^{rec-low}* plants. It is tempting to speculate that this stabilising factor could be *pal^{rec-low}* itself, and that the instability is evoked by the association of *pal^{rec-low}* with another *pal* allele, either *pal^{tub}* or standard *pal^{rec}*. This interpretation would make the present system comparable to para-

mutation at the *R* locus in maize (Brink, 1973), where a change in state of *R^r* is specifically induced by its association in a heterozygote with the paramutagenic allele *Rst*. *R^r*, which produces a somewhat mottled phenotype, tends to suffer a reduction in pigment-producing potential on being kept homozygous for a number of generations (Styles and Brink, 1966). Conversely, its pigment-producing potential is progressively increased when it is kept heterozygous with *r* or hemizygous (Styles and Brink, 1966, 1968). In the case of *pal^{rec-low}*, however, it is still possible, however, that the repression of the potential for mutability is not a function of the *pal* locus itself but of

TABLE 9
Distribution of F₂ plants from pal^{rec-low}pal^{rec-low} × pal^{rec}pal^{rec} crosses

Family	No. of F ₂ plants falling in the classes of†								Total plants	Family mean score
	0.0-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-7.9		
51-965	4	4	3	4	2	2	2	0	21	3.11
51-966	5	3	4	2	4	1	1	0	20	2.89
51-967	8	0	0	3	9	3	2	0	25	3.48
51-968	4	1	0	2	8	3	4	0	22	4.26
51-969	8	1	0	3	4	1	0	0	17	2.55
51-980	4	1	2	1	1	0	0	0	9	1.98
51-983	1	1	2	1	2	1	1	0	9	3.72
51-984	0	0	3	3	2	2	0	0	10	4.30
51-985	0	0	0	0	4	6	0	0	10	5.40
Total	34	11	14	19	36	19	10	0	143	3.52
51-986 ^a	0	0	0	0	0	0	6	2	8	7.13
51-469 ^b	0	0	0	0	0	0	8	14	22	7.27
Total	0	0	0	0	0	0	14	16	30	7.20

† In all families, except in 51-986 and 51-469, some selection against seedlings showing high mutability was exerted to increase the number of low plants to flower.

^{a, b} 51-986 and 51-469 are homozygous *pal^{rec}pal^{rec}* families and were obtained by selfing two *pal^{rec}pal^{rec}* plants. They are presented here to show the nature of variation in the standard genotype.

another, nearly recessive stabilising element present in homozygous condition in the original *pal^{rec-low}* plants. The essential difference between such an element and the semi-dominant *Stabilizer* of Harrison and Fincham (1968) might be that, whereas the latter controls the mutation frequency of *pal^{rec}* in a direct way, determining a constant level of mutability of an essentially constant *pal^{rec}* allele, the element involved here is one with a second-order effect, controlling the frequency of mutability "shifts" from one *pal^{rec}* state to another.

To determine whether the stabilisation of the non-shifting, low-mutable state of *pal^{rec-low}* is a function of *pal* itself or of some other locus, we need to know whether it is possible to obtain a homozygous *pal^{tub}* stock lacking the ability to evoke *pal^{rec-low}* mutability. If such a stock were obtained, analysis of crosses between it and our existing *pal^{tub}* line should give information on the gene or genes controlling stability. If such a stock appeared to be unobtainable this would suggest that *pal^{tub}* itself (or another suitable *pal* allele)

was necessary for the evocation of the instability and the phenomenon could be described as paramutation.

Some changes at the *niv* locus (also in *Antirrhinum*) recently described by Harrison and Carpenter (1973) bear an interesting resemblance to the phenomenon described here. The dominant allele of *niv* controls an earlier step in pigment in pigment production. It was found that when *niv^{rec}*, an unstable allele, was crossed to stable *niv* using the former as male parent, a very high proportion of F₁ plants showed a colourless phenotype. This loss of variegation is stably inherited. The extent of the change depends on the particular *niv^{rec}* and *niv* lines used. A further comparison of Harrison and Carpenter's phenomenon and the system described in this paper could be highly informative.

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