SOMATIC CROSSING-OVER IN *ANTIRRHINUM MAJUS*

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Received 23.viii.76

SUMMARY

Many aberrant somatic sectors on the corollas of heterozygous *Antirrhinum majus* can be related to a number of genetic causes while twin-spots are attributed to somatic crossing-over, hitherto, considered a relatively rare occurrence in higher plants. The administration of caffeine increases the frequency of single aberrant sites and also of twin-spots. The inclusion of the unstable genes *nivea-recurrens* and *pallida-recurrens* has no effect on the frequency of twin-spots. Homozygosity following somatic crossing-over is presumed for several loci and some evidence is presented for deficiency aberrations. The use of precise precursors in the biosynthetic pathway to anthocyanidin formation provided confirmatory evidence for the identification of the genes involved in the somatic aberrations.

1. INTRODUCTION

MOSAIC regions on various parts of plants have been recorded in a range of species and related to several genetic causes (Jones, 1937). Mosaics have also been identified and investigated in *Drosophila* (Stern, 1936), the domestic fowl (Serebrovsky, 1925), housefly (Nöthiger and Dübendorfer, 1971) and the mouse (Gruneberg, 1966); but it is particularly in higher plants that renewed interest and critical analyses have recently established the genetic causes with more confidence. The somatic variations reported in *Nicotiana tabacum* (Carlson, 1974; Dulieu, 1975), *Glycine max* (Vig, 1975), *Tradescantia* (Mericle and Mericle, 1967), *Lycopersicon esculentum* Ross and Holm, 1960; Christianson, 1975), *Arabidopsis thaliana* (Hirono and Rédei, 1965), *Crepis capillaris* (Dubinin and Nemtseva, 1969), *Gossypium* sp. (Barrow, Chaudhari and Dunford, 1973; Barrow and Dunford, 1974), *Pisum sativum* (Blixt, 1972) and *Salvia splendens* (Hendrychova-Tomkova, 1964) have been variously attributed to point mutation, gene conversion, non-disjunction, translocation, deletion and somatic crossing-over. The appearance of twin-spots (fig. 1) in heterozygotes is diagnostic of either non-disjunction or crossing-over; the two components would be expected to be of dissimilar size because of chromosome imbalance if initiated by non-disjunction but could be of similar size if the two components were homozygous and resulted from somatic crossing-over.

Analysis of twin-spots by Vig (1973, 1975) and Evans and Paddock (1976) in *Glycine max* and *Nicotiana tabacum*, the *in vitro* culture of twin-spot components in *Nicotiana* by Deshayes and Dulieu (1974) and Carlson (1974) and the mutational work on the petals and stamen hairs of *Tradescantia* by Mericle and Mericle (1967) and Christianson (1975) show that crossing-over is certainly involved in at least some aberrant somatic sites. However, if only one component of the twin establishes a cell lineage or if only one homozygote is recognisable against the heterozygous background then twin-

spots will not be observed although the aberrant site may be the result of crossing-over.

The present study on somatic variants in *Antirrhinum majus* provides further evidence for such aberrations in plants and suggests that somatic crossing-over is one of the main causes. Chemical agents, such as mitomycin and caffeine, which affect the frequency of crossing-over were advantageously used by Vig (1975) in *Glycine max* and their relationship to the production of twin-spots was substantiated. Mitomycin C was also found to increase the frequency of somatic crossing-over in fungi (Holliday, 1964). Caffeine has been shown to increase both point mutation and crossing-over in *Glycine max* and is considered, similarly with mitomycin, to allow the reunion of chromosome breaks, whereas puromycin causes segmental losses and no subsequent rejoining after breakage, with no associated increase in twin-spots (Vig, 1973).

In addition to the caffeine treated lines of *Antirrhinum* (heterozygous for several loci) a comparison was made of similar heterozygotes that also contained the unstable *pallida-recurrens* allele in hypostatic combinations in order to assess its effect on the production of somatic aberrations. At one time it had been considered possible that the mutability of the unstable gene was related to meiotic and mitotic crossing-over. It was shown that there was no evidence for this happening meiotically (Harrison and Fincham, 1965) but the mitotic possibility was unresolved. Since Stern (1936) showed that somatic crossing-over occurs in male *Drosophila* there can be no extrapolation from meiotic to mitotic processes in this connection.

The utilisation of precise precursors to overcome genetic blocks in the biosynthetic pathway to anthocyanin production (Stickland and Harrison, 1974; Harrison and Stickland, 1974) has provided a technique for identification of the genetic block within a mosaic site of twin-spot component, provided that the area contains several hundred cells.

2. MATERIALS AND METHODS

The inbred *Antirrhinum majus* stocks *incolorata*, *eosinea*, *nivea*, *nivea-recurrens* and *pallida-recurrens* have been maintained at this Institute for many years and agree with the description given by Stubbe (1966). Fuller descriptions of the *recurrens* alleles are given in Harrison and Carpenter (1973). The plants were sown and grown in standardised John Innes composts and then either grown outside or in a glasshouse where the natural light was supplemented during the winter months with G.E.C. Solarcolour sodium lights.

The method of counting sites varied from (a) scoring every flower during the flowering season and recording every site on the facial surfaces of the corolla lobes to (b) selecting certain plants and scoring all the sites on the facial surface of one upper lobe. The particular counting method is designated in the tables. The type of corolla lobe and surface chosen for counting is shown in close-up in fig. 6.

The caffeine treatment was effected by the immersion of young developing inflorescences in either 0.25 per cent or 0.5 per cent aqueous solutions for 24 hours; the largest buds of the treated inflorescences were approximately 15 mm long. The temperature during treatment was glasshouse temperature which ranged during the tests from 10°C (night) to 24°C (day). Subsequent

flower development in the glasshouse experienced a similar range of temperature.

The use and origin of anthocyanin precursors for the identification of somatic sites were as described in Stickland and Harrison (1974) and Harrison and Stickland (1974).

3. RESULTS

(i) *The effect of recurrens alleles on the frequency of somatic recombination*

The effect on the incidence of putative somatic crossing-over and other genetic events by the inclusion of the highly mutable *recurrens* alleles of *pallida* and *nivea* was assessed in several heterozygous populations of *Antirrhinum majus*. Any influence of the *recurrens* alleles would appear from a comparison of the frequencies of twin-spots and single sites in the families *incolorata* × *pallida-recurrens* and *incolorata* crossed to the stable non-*recurrens* alleles *pallida-tubocolorata*, *pallida-tincta* and *nivea* : *eosinea*.

Single aberrant sites may result from a number of causes additional to the establishment of a single cell lineage from one component of a cross-over

TABLE 1

Flower pigmentation in various genotypes of Antirrhinum majus

Genotype	Phenotype of flower
<i>nivea/nivea</i>	acyanic (albino)
<i>nivea/Nivea</i> (<i>niv/Niv</i>)	medium/dark magenta
<i>Nivea/Nivea</i>	dark magenta
<i>incolorata/incolorata</i>	acyanic (ivory)
<i>incolorata/Incolorata</i> (<i>inc/Inc</i>)	medium magenta
<i>Incolorata/Incolorata</i>	dark magenta
<i>pallida-recurrens/pallida-recurrens</i>	flaked with pigment where <i>pallida-recurrens</i> mutates to <i>Pallida</i>
<i>pallida-recurrens/Pallida</i> (<i>pal-rec/Pal</i>)	dark magenta
<i>pallida-tubocolorata/pallida-tubocolorata</i>	acyanic except for pigmented ring around base of corolla tube
<i>pallida-tubocolorata/Pallida</i> (<i>pal-tub/Pal</i>)	dark magenta
<i>pallida-tubocolorata/pallida-recurrens</i>	flaked corolla and pale pigmented ring at base of corolla tube
<i>pallida-tincta/pallida-tincta</i>	acyanic, occasionally with pale flush
<i>pallida-tincta/pallida</i> (<i>Pal-tincta/Pal</i>)	dark magenta
<i>eosinea/eosinea</i>	pelargonidin pigment (<i>i.e.</i> pink)
<i>eosinea/Eosinea</i> (<i>eos/Eos</i>)	cyanidin pigment (<i>i.e.</i> magenta)

event potentially capable of producing a twin-spot. Twin-spots can, of course, only be identified when the gene involved is semi-dominant *e.g.* *incolorata* (see table 1 for gene expression).

A comparison of the two sets of data in table 2 shows that there was no significant effect of an unstable *recurrens* allele in the genotype on the frequency of single and twinned aberrant sites. There was a 10-fold increase in the number of single sites over twinned; and, as stated earlier, the single sites could arise from a number of different genetic events.

Precise data regarding the position of centromeres and the loci of many genes is lacking in *Antirrhinum majus*. The comparison of non-*rec* and *rec* genotypes in table 2 is based on different sets of known heterozygous loci.

TABLE 2

Frequencies of aberrant sites on flowers of non-recurrens and recurrens genotypes

Non-recurrens genotype	Aberrant sites†/number of flowers	
	Twinned	Single
<i>niv eos Inc</i>	11/2510	132/2510
<i>Niv Eos inc</i>		
<i>niv Eos Inc pal-tub</i>	9/11603	215/11603
<i>Niv Eos inc Pal</i>		
<i>Eos inc Pal</i>	19/13711	131/13711
<i>Eos Inc pal-tincta</i>		
<i>Eos inc Pal</i>	8/7210	66/7210
<i>Eos Inc Pal</i>		
	47/35034	544/35034
Mean number of sites per flower	0.0013	0.0155
<i>Recurrens genotype</i>		
<i>eos Inc pal-rec Lμ*</i>	8/9212	85/9212
<i>Eos inc Pal</i>		
<i>Eos Inc pal-rec Lμ*</i>	16/14854	91/14854
<i>Eos inc Pal</i>		
<i>eos Inc pal-rec Hμ*</i>	14/15324	288/15324
<i>Eos inc Pal</i>		
<i>Eos Inc pal-rec Hμ*</i>	11/6973	114/6973
<i>Eos inc Pal</i>		
<i>Eos Inc pal-rec vHμ*</i>	40/13886	233/13886
<i>Eos inc Pal</i>		
	89/60249	811/60249
Mean number of sites per flower	0.0015	0.0135

* $L\mu$, $H\mu$, $vH\mu$ —low, high and very high mutation rate as described in Harrison and Fincham (1968).

† Counts made macroscopically; they are lower than those recorded in tables 4, 6, 8, 9 and 10 which were made microscopically.

The comparison can only be approximate; for the non-*rec* set there was a mean of 2.8 known heterozygous genes per flower and for the *rec* genotypes the value was 2.3. The dissimilarity in known gene content together with accompanying unknown heterozygous genes affecting pigmentation can be expected to give some differences between the two sets of data which are in remarkably good agreement.

(ii) *Meiotic crossing-over in pallida-recurrens heterozygotes*

The original purpose of this test (Harrison and Fincham, 1965) was to assess any effect of crossing-over on the mutability of *pal-rec* to other more stable alleles in the series. The results are of interest here since it has already been shown in the previous section that no relationship between mitotic crossing-over and the presence of unstable *pal-rec* could be established. The converse is also shown, namely that no inhibition of meiotic crossing-over occurred when the unstable *rec* allele was included in the genotype; this is relevant when considering the possibility of an inhibiting effect occurring somatically.

The closest available markers flanking the *pal* locus were *Eluta* (*El*) (dilution of flower pigment) and *divaricata* (*div*) (morphological restriction of face area).

Crosses were made of the type:

$$\frac{el\ pal-rec\ div}{El\ pal-tub\ Div} \times \frac{el\ pal-tub\ div}{el\ pal-tub\ div}$$

and seeds were obtained from 52 capsules. Plants showing whole plant mutation of *pal-rec* were not significantly more frequent in the cross-over classes (with respect to the flanking markers) than in the non-cross-over class *el div* (7.2 per cent as compared with 8.1 per cent). Indeed, it was clear from the tendency of mutants to be concentrated in clusters in a minority (13) of the capsules, with no mutants occurring in the majority (39 capsules), that at least the greater number of mutations occurred prior to meiosis. The large three-point test progeny was divided into the various phenotypically distinct classes (table 3) (*El/el* is not easily scorable in plants homozygous for *pal-tub*).

TABLE 3

Progeny classes in a three-point test for meiotic crossing-over

Pattern of crossing-over*	Genotype	Number
n.c.o.	<i>el pal-rec div</i> (including 180 <i>Pal</i> mutants)	2914
1	<i>el pal-tub Div</i> } †	3792
n.c.o.	<i>El pal-tub Div</i> }	
1	<i>El pal-rec div</i> (including 65 <i>Pal</i> mutants)	844
2	<i>el pal-rec Div</i> (including 14 <i>Pal</i> mutants)	254
2	<i>El pal-tub div</i> } †	293
1, 2	<i>el pal-tub div</i> }	
1, 2	<i>El pal-rec Div</i>	7

* n.c.o., no crossing-over.

1, crossing-over between *el* and *pal*.

2, crossing-over between *pal* and *div*.

† *El/el* is not easily scorable in plants homozygous for *pal-tub*.

Assuming that the recombinant classes *El pal-tub Div* and *el pal-tub div* were equal to the distinguishable reciprocal classes, recombination frequencies in the intervals *el*→*pal* and *pal*→*div* are 20.83 per cent and 6.66 per cent respectively. The small number of double cross-over indicates a high degree of interference, the coefficient of coincidence being 0.12. The recombination intervals agree well with those given by Kuckuck (1938).

(iii) Identification of hypostatic genes

The sectors and twin-spots recorded in the several heterozygous families revealed the presence and nature of some of the hypostatic genes. Whether the uncovering of the recessive gene was due to somatic crossing-over or some other genetic cause was not always clear, particularly when a completely dominant allele was involved since no twin-spots could then occur.

(a) *Incolorata/incolorata*. The heterozygous flowers are medium magenta and the twin-spot illustrated in fig. 1 shows the homozygous acyanic component (*inc/inc*) and the darker component (*Inc/Inc*). Untwinned homozygous sectors are shown in fig. 4 and an unusual lobe with many aberrant

acyanic sites in fig. 6. This spangled lobe is reminiscent of the "mutable" lobe described by Cuany, Sparrow and Pond (1958) in their irradiated heterozygous line of *Antirrhinum majus* which unfortunately was genetically unsuitable for the identification of any induced twin-spots.

(b) *Pallida/pallida-recurrens*. The twin-spot shown in fig. 2 clearly identifies the homozygous flaking *pal-rec* sector and the more darkly pigmented *Pal/Pal* component to the left.

(c) *Eosinea/eosinea*. Only single components (*eos/eos*) of any twin-spot would be identifiable: the homozygous *eos* tissue synthesises the pink pelargonidin anthocyanidin (fig. 5) while the homozygous or heterozygous *Eos* synthesises cyanidin with no quantitative difference. Unless genetically acyanic the *Antirrhinum majus* flowers are normally either cyanidin or pelargonidin synthesisers; no mixtures of the anthocyanidins occur. However, chromatographic analysis has shown that, as in the case illustrated here, both anthocyanidins can be synthesised in the same flower, but not in the same cells.

(iv) *Precursor analysis of aberrant sectors and twin-spots*

The mutant genes used in this study are all involved in the biosynthetic pathway to anthocyanidin synthesis. The *niv* gene blocks all flavonoid synthesis; *inc* blocks the flavanones; the *eos* locus controls the production of pelargonidin (*eos/eos*) or cyanidin (*Eos*); and *pal* causes the late block occurring after flavanon synthesis. The insertion of specific precursors into *Antirrhinum majus* with blocked pathways (Stickland and Harrison, 1974; Harrison and Stickland, 1974) has enabled anthocyanidin synthesis to proceed. For example, the administration of dihydrokaempferol to an *Antirrhinum* flower blocked solely by *niv/niv* and/or *inc/inc* can synthesise cyanidin if the flower is *Eos*, or pelargonidin if *eos/eos*. The plant can add the extra hydroxyl to the B ring needed for cyanidin synthesis from dihydrokaempferol (1 OH). If the precursor given is dihydroquercetin (2 OH) then only cyanidin can be synthesised irrespective of the *eos/Eos* constitution. Since the dihydroflavone naringenin cannot initiate synthesis in an *inc/inc* and neither dihydroquercetin nor naringenin can initiate synthesis in a flower homozygous for recessive *pal* alleles, the aberrant sectors produced in plants heterozygous for *niv*, *inc*, *eos* and *pal* can be analysed by precursors. Thus, an acyanic site that might be *niv*, *pal* or *inc* can be identified by the administration of naringenin and dihydroquercetin. If no synthesis occurs with naringenin the flower sector must have a block in synthesis after the flavone stage *i.e.* it is either *inc/inc* or *pal/pal*; if the sector is then tested with dihydroquercetin and synthesis occurs, it must be blocked at the *inc* stage and not at the late *pal* point; similarly, if there is no synthesis following the administration of dihydroquercetin then the *pal* block is operating and the sector is homozygous for one of the recessive *pal* alleles. Although pelargonidin sectors can be analysed by chromatographic methods it is also possible to observe the extra synthesis of cyanidin in addition to the naturally occurring pelargonidin if dihydroquercetin is administered.

This technique has enabled many sectors to be related to precise genetic blocks and thus provides evidence as to which of several genes in a composite genotype is involved in the somatic aberration.

(v) *Deficiencies*

Until corolla tissue culture in *Antirrhinum majus* allows a cytological/genetical analysis of somatic aberrations the causes of the various mosaic types must, to some extent, remain conjectural. Amongst the heterozygous *inc/Inc* and *pal-rec/Pal* plants were several possible examples of deficiencies: either intrachromosomal deficiencies caused by deletion at the relevant loci or whole chromosome loss following non-disjunction. The heterozygous *inc/Inc* medium magenta coloured flowers can exhibit white or dark magenta sectors presumably by crossing-over, as described and illustrated in fig. 1; this, as in the photograph, can show as a white and dark magenta twin-spot against a medium magenta background or if the dark magenta (*Inc/Inc*) component fails to establish a cell lineage then as a single acyanic area. The reciprocal situation is more difficult to detect since variations in the intensity of pigmentation obscure the identification of dark magenta sites against medium magenta unless visually aided by the presence of an accompanying white component. Somatic segregants of different growth rates are unlikely to occur but if the heterozygote loses part of the *Inc* region or if the whole chromosome is missing then slower growth might be expected. Fig. 4 shows two flowers, one (a) with a white sector, presumably homozygous *inc*, in which the sector is showing a normal growth pattern; the other (b) has a distorted sector on the central part of the bottom lobe of the corolla with a much reduced area; this would suggest that the sector is hemizygous for *inc* (both synthesised cyanidin with dihydroquercetin). Similarly, in fig. 3 the heterozygous *pal-rec/Pal* flower has a flaking sector which could have arisen from somatic crossing-over to give a homozygous *pal-rec* sector; the upper companion flower also has a flaking sector but in this case a notch at the periphery of the flower indicates a slower growth rate; also, the frequency of flaking is reduced quite significantly. If only one *pal-rec* allele is present then the number of mutations to *Pal* is approximately half of the homozygote. Counts on these two flower sectors agree with this view: a 9 sq. mm area on the normally growing sector contained 179 mutant sites while a similar area on the slower growing and probably deficient sector had 80.

(vi) *Cytological analysis of aberrant plants*

If the somatic aberration affects the heterozygous genes early in the ontogeny of the flower a large sector or the whole flower can be altered; still earlier onset can affect the whole plant and this may be analysable both germinally and somatically. This occurred in two plants in the *inc/Inc* F₁ families. Both plants were acyanic and the inference was that by crossing-over or deficiency they were *inc/inc*, *inc/inc*⁻ or *inc/-*. One plant was also sterile, suggesting a deficiency rather than crossing-over. Analysis of these whole plant aberrants might afford an explanation of some of the somatic aberrant sites that cannot, without an advance in tissue culture techniques, be examined cytologically. The plants were kindly examined by Mr G. E. Marks of the John Innes Institute. The sterile plant (22 per cent stainable pollen with acetocarmine) was heterozygous for a single interchange at least but the chromosomal reason for the semi-sterility (48 per cent stainable pollen) in the second plant is not yet known.

(vii) *Relative size of twin-spots*

If somatic crossing-over in heterozygous *Antirrhinum majus* plants were the principal mechanism producing twin-spots then components of approximately the same size would be generally expected. In *inc/Inc* flowers any segregants involving the locus would have an acyanic and a darkly pigmented component. Measurements of the acyanic/dark magenta twin-spots (1975 data) which were conjecturally *inc/inc* : *Inc/Inc* showed 71 in which the components were of equal size; 29 in which the acyanic component was larger and 23 in which the dark magenta component was larger. These measurements indicate that the acyanic cells were not at a growth disadvantage and that the majority, if not all, the twin-spots were more likely to be the result of somatic crossing-over; acyanic components arising by non-disjunction would have been at a disadvantage in establishing a cell lineage.

(viii) *Twin-spots and single sites on laterals and main inflorescence*

It has been previously noticed by J. R. S. Fincham and B. J. Harrison that the developmental stage of the plant affects the frequency and type of mutation (Fincham, 1970). In particular, plants heterozygous for *pal-rec/pal-tub* and *pal-rec/pal-tincta* show large sectors on the lateral side shoots than on the main inflorescence. Table 4 shows that this differential effect operated

TABLE 4
Frequencies of single and twin-spot sites on laterals and main spike
F₁ plants of genotype inc:Pal/Inc:pal-rec

	Sites	Single sites		Twins	Twin-sites	
		Flowers examined	Frequency/ Flower		Flowers examined	Frequency/ Flower
Main spikes	180	9188	0.020	30	9188	0.003
Laterals	454	51061	0.009	59	51061	0.001

*F₁ plants from crosses of inc:Pal × stocks not containing
pal-rec (Inc:Pal or niv:Inc:Pal or Inc:pal-tincta)*

	Sites	Single sites		Twins	Twin-spots	
		Flowers examined	Frequency/ Flower		Flowers examined	Frequency/ Flower
Main spikes	234	5924	0.040	22	5924	0.004
Laterals	310	30430	0.010	25	30430	0.001

for the expression of single- and twin-spot sites. Significantly more ($P < 0.001$) single and twin-spot sites occurred on the main inflorescence than on the laterals; a 3-4-fold difference was generally found. This result might arise from ageing processes within the plant or from the different environmental conditions operating at the time of development. Vig and Paddock (1970) found an increase in twin-spotting in *Glycine max* after cold shocks (3°C) and variability in the frequency of spots according to leaf position on the plant. However, the temperature records (table 5) of the

TABLE 5
Temperature range during experimental periods

	Screen temperatures (°C)	
	Mean daily	Range in 10-day period
1975		
July 4-13	Max. 21.9 Min. 13.2	24.4-18.9 15.6- 8.9
July 14-23	Max. 22.0 Min. 14.0	26.7-18.9 16.7-12.2
July 24-Aug. 2	Max. 23.4 Min. 11.3	29.4-17.8 16.7- 7.8

three 10-day periods are sufficiently comparable not to expect any major effect of temperature on the outside plants during the scoring of the main spike and laterals. Main spikes were mostly scored during the first 10-day period; main spikes together with laterals during the middle period with some overlap into earlier and later periods, while mainly laterals were scored in the last period.

An analysis of single and twin-spot sites recorded on the same or consecutive days on laterals and main spikes is in table 6. The number of flowers is relatively low but the difference between laterals and main inflorescence is consistent. The difference in expression of mosaics on laterals and main spike is probably due to an internal rather than an external cause.

TABLE 6
Site frequency on main inflorescence and laterals scored on same or consecutive days

		Number of flowers	Single sites	Twin-spots
F_1 <i>pal-rec:Inc/Pal:inc</i> 23-24/7/76	Main spike	608	19 (3.1%)	1 (0.2%)
	Laterals	960	21 (2.2%)	1 (0.1%)
F_1 <i>niv:Inc eos/Niv:inc Eos</i> 17/7/75	Main spike	673	64 (9.5%)	9 (1.3%)
	Laterals	388	15 (3.9%)	1 (0.3%)

The effect of internal environment on the frequency of mosaic formation can be seen by comparing the two surfaces of the corolla: on the forward facing surface (as shown in fig. 6) the number is very much higher. No comparative counts were made since the reverse surface was less amenable to counting but the conclusion is unequivocal and the decreased number of mosaics on the reverse side accords with the lower number of somatic mutations of *pal-rec* to *Pal*. This effect was also noted by Vig (1974) in *Nicotiana* where the frequency of aberrant sites was much greater on the upper surface of the leaf than on the lower.

It is perhaps relevant in the context of internal environment effects to record the difference in mutability of the *pal-rec* allele in the male and female germ line (Harrison, 1967). The conclusion was based on 4861 plants of *pal-tub* $\times$ *pal-rec* and *pal-tincta* $\times$ *pal-rec* (from 79 crosses) and compared with 2722 plants of a reciprocal set (from 48 crosses). Although there was some scatter in the percentage values of mutations within each class, the standard error of the mean percentages and a χ^2 test showed a difference

in mutability dependent on whether the mutable gene was in the pollen or ovules (table 7). ($P < 0.001$ if analysis is based on the minimum number of distinct mutations *i.e.* using the number of capsules in which one or more mutants occurred rather than the number of mutants; a capsule could give rise to a number of mutant plants but all could be the result of a single early mutation.)

TABLE 7
Frequencies of gametic mutations of pal-rec in male and female germ lines

Female	Male	Mean % of mutation
<i>pal-rec</i> × <i>pal-tincta</i>	}	12.6 ± 3.2 (S.E.M.)
<i>pal-rec</i> × <i>pal-tub</i>		
<i>pal-tincta</i> × <i>pal-rec</i>	}	31.1 ± 7.7 (S.E.M.)
<i>pal-tub</i> × <i>pal-rec</i>		

(a) *Site counts on homozygous lines.* Two inbred lines were assessed for aberrant single sites and twin-spots. The first was a full-red line (*Pal*) that originated from a highly inbred homozygous *pal-rec*; the second was homozygous *pal-carnea* (a pale magenta). Both lines were inbred for the major genes used in the current work, but some residual heterozygosity is possible for unknown genes affecting pigmentation and these would differ between the two lines. Barrow, Chaudhari and Dunford (1973) concluded that, in their "homozygous" cotton, heterozygous sectors derived from homozygous cells. Some residual heterozygosity with no apparent phenotypic effect may have existed. They additionally considered it possible that a pseudo-allelic condition with crossing-over within the gene between two minute mutations could explain the observed segregation. A cross-over within a pericentric inversion could also give duplication and deficiencies for two colour genes expressible as twin-spots. Such possibilities add to the complexity in interpreting the aberrant areas solely in terms of known heterozygous genes in purposely made F_1 s. Single sites in homozygous lines arising from point mutations of unknown genes would be expected to occur at approximately the same frequency as in purposely made heterozygous lines. It can thus be considered that a background level of aberrations will be common to both homozygous lines and heterozygotes and the counts recorded in table 8

TABLE 8
Aberrant site frequency in three homozygous lines

		Flowers examined*	Single sites		Twin-spots
			Acyanic	Medium magenta	
<i>Pal</i> (Full red)	Stock 7	106	25	32	0
	Stock 8	42	12	12	0
<i>pal-carnea</i>	Stock 37	42	5	6	0
	Total	190	79	94	0
			173		

* Whole flower counted; for comparison with single lobe counts the whole flower count is divided by 4.

173 single sites/190 × 4 = frequency of 0.23 single sites per lobe compared with 0.96 (*inc/Inc*) in table 9; 1.47 (*Eos:Niv/eos:niv*) in table 10; 1.69 (*Niv:Pal:Sulf/niv:pal-tub:sulf*) in table 11.

provide an estimate for the background level. It is significant that no twin-spots were recorded and that the mean estimate of 0.23 single sites per lobe is approximately 1/7 of the frequency recorded in the F_1 s (table 9). The comparison should be made with the untreated controls in the caffeine data (tables 9, 10 and 11) since these plants had similar growing conditions to those for the homozygotes and were all counted microscopically.

TABLE 9
Effect of caffeine on the incidence of single and twin-spot sites

	Genotype <i>Incolorata/incolorata</i>				
	Single sites		Twin-spots		
	Acyanic	Pale magenta	Acyanic/ Dark magenta	Acyanic/Pale	Pale/Dark
Flowers examined					
UNTREATED					
70 (3 plants)	34	33	2	0	0
	67		2		
Mean number of aberrant sites per lobe†	0.96		0.03		
TREATED					
0.25% CAFFEINE (24 hrs)					
26 (3 plants*: D82 ^{3, 4, R8})	123	626	16	9	0
	749		25		
Mean number of aberrant sites per lobe	28.8		0.96		
Fold increase over untreated	× 30		× 32		
TREATED					
0.5% CAFFEINE (24 hrs)					
29 (4 plants*: D82 ^{1, 4, R11, R14})	1405	4589	79	24	4
	5994		107		
Mean number of aberrant sites per lobe	206.6		3.7		
Fold increase over untreated	× 215		× 123		

* Only those flowers from affected part of inflorescence recorded.

† Only upper left-hand lobes of corollas counted.

(ix) Caffeine treatment

The effect of caffeine on higher plants in increasing the frequency of somatic crossing-over and point mutation has been demonstrated in *Glycine max* by Vig (1975) and for chromatid exchanges in *Allium* by Kihlman *et al.* (1971). It appears to have little influence on the frequency of other genetic aberrations resulting from other causes. If caffeine increases the rate of crossing-over, treated heterozygotes for suitable genes should show an

increase in the number of twin-spots; sites resulting from point mutations will also increase but these will be single sites.

Table 9 shows that untreated heterozygotes of *incolorata/Incolorata* had a mean of 0.96 single sites per flower lobe and the lower figure of 0.03 twin-spots per lobe. (Only one lobe, the upper left facing the flower, was counted in detail using a binocular microscope.) This estimate would be approximately one-quarter of the aberrations on the whole front surface of the flower (see Methods) excluding the corolla tube.

TABLE 10

Effect of caffeine on the incidence of single and twin-spot sites

Flowers examined	Genotype <i>Eosinea:Nivea/eosinea:nivea</i>				
	Single sites		Twin-spots		
	Acyanic	Pale magenta	Acyanic/ Dark magenta	Acyanic/Pale	Pale/Dark
UNTREATED 121 (8 plants)	11	167	0	1	1
	178		2		
Mean number of sites per lobe†	1.47		0.02		
TREATED 0.25% CAFFEINE (24 hrs) 21 (3)*	105	1055	4	1	17
	1160		22		
Mean number of sites per lobe	55.24		1.05		
Fold increase over untreated	× 37.58		× 52.5		
TREATED 0.5% CAFFEINE (24 hrs) 10 (3)*	6	572	0	0	0
	578		0		
Mean number of sites per lobe	57.8		0		
Fold increase over untreated	× 39.32		—		

*† See Table 9.

The acyanic single sites could result from deficiencies or point mutation at the *Inc* locus or from the homozygous recessive component of a twin-spot. The pale magenta single sites could arise from unknown heterozygous genes affecting pigmentation in the purposely constituted *inc/Inc* genotype. Pale magenta areas were conspicuously present in much of the material examined and some twin-spots of pale magenta with an acyanic or dark magenta component were recorded. The origin of these aberrants can only be conjectured but whatever the cause it is increased by caffeine treatment and thus could be either the consequence of point mutation or crossing-over.

The sensitivity of the young buds to caffeine varied from no effect,

Plate I

FIG. 1.—Twin-spot comprising an acyanic *inc/inc* and dark magenta *Inc/Inc* components on the medium magenta background of the heterozygous *inc/Inc* flower.

FIG. 2.—Twin-spot of a flaking *pal-rec/pal-rec* component with the dark magenta *Pal/Pal* on medium-dark magenta background of the heterozygous *pal-rec/Pal* flower.

Plate II

FIG. 3.—*Upper*. Sector on *pal-rec/Pal* flower of 80 mutant sites/9 sq mm and peripherally indented; the low flaking and reduced growth indicates an absence (following non-disjunction or a deficiency) of the *Pal* chromosome or region which allowed the expression of the single unstable *pal-rec* allele.

Lower. Normal growth of a *pal-rec/pal-rec* sector with parental flaking frequency (179 mutant sites/9 sq mm) caused by somatic crossing-over in the *pal-rec/Pal* flower.

FIG. 4.—(a) *Left*. Acyanic sector of *inc/inc* (synthesised cyanidin with dihydroquercetin) with normal growth on *inc/Inc* flower.

(b) *Right*. Acyanic central lower lobe of reduced growth (synthesised cyanidin with dihydroquercetin); probably due to deficiency (*inc/inc*⁻) or chromosomal loss (*inc/-*) on an *inc/Inc* flower.

Plate III

FIG. 5.—Large sector of *eos/eos* (pelargonidin pigment) on an *eos/Eos* (cyanidin pigment) flower.

FIG. 6.—Localised multiplicity of acyanic sites (+2 twin-spots) on one lobe of an *inc Eos:Pal/Inc eos:pal-rec* flower.

Plate IV

FIG. 7.—Acyanic sites and twin-spots on an *inc/Inc* (D82^{R11}) flower after 24-hour treatment at bud stage with 0.5 per cent caffeine.

FIG. 8.—Three twin-spots (*inc/inc* + *Inc/Inc*) on part of a corolla lobe from a caffeine treated (0.25 per cent) *inc/Inc* inflorescence (D82⁴).

FIG. 9.—Inflorescence of *inc/Inc* plant (D82^{R11}) treated with 0.5 per cent caffeine. Stunted flowers followed by a sequence with numerous acyanic sites and twin-spots.

Plate V

FIG. 10

5

6

FIG. 10.—Probable constitution of sectors on a *pal/rec/pal-rec* flower:

- A *Pal/Pal* (or any other *pal* allele).
- B *pal-carnea/pal-carnea* (or *pal-tincta* or *pal-tub*).
- C *pal-rec/pal-rec*.
- D *pal-tincta/pal-tincta* (or *pal-tub*) or *pal-tub/pal-tub*.
- E *pal-rubra/pal-rubra* (or *pal-carnea*, *tincta* or *tub*).
- F *pal-carnea/pal-carnea* (or *pal-tincta* or *pal-tub*).
- G *Pal/Pal* (or any other *pal* allele).
- H *pal-tincta/pal-tincta* (or *pal-tub*).
- I *pal-rec/pal-rec*.

through variable frequencies of increased aberrant sites to gross abortion. Fig. 9 shows an inflorescence of *inc/Inc* 42 days after a 0.5 per cent treatment; the central part has aborted flowers while the top has a large number of acyanic and pale magenta sites together with many twin-spots. A close-up of a flower on the same inflorescence but photographed 13 days earlier (fig. 7) exhibits so many aberrant sites that it resembles the effect of an unstable gene.

TABLE 11

Effect of caffeine on the incidence of single and twin-spot sites

Flowers examined	Genotype <i>Nivea: Pallida: Sulfurea/nivea: pallida-tubocolorata: sulfurea</i>					
	Single sites		Twin-spots			
	Acyanic	Pale magenta	Acyanic/ Pale magenta	Acyanic/ Dark	Pale/ Dark	Pale/ Medium
UNTREATED 119 (7)	28	173	0	0	2	0
	201		2			
Mean number of sites per lobe†	1.69		0.02			
TREATED 0.25% CAFFEINE (24 hrs) 27 (4)*	2651	1210	14	2	8	3
	3861		27			
Mean number of sites per lobe	143.00		1.04			
Fold increase over untreated	× 85		× 52			
TREATED 0.5% CAFFEINE (24 hrs) 14 (2)*	624	1890	0	0	11	0
	2514		11			
Mean number of sites per lobe	172.43		0.79			
Fold increase over untreated	× 102		× 40			

* † See Table 9.

An assessment of the size of the various types of site on one lobe of the flower in fig. 7 and an approximate count on the frequency of site types, gave the following results:

20 pale magenta largest site 210 cells
53 acyanic largest site 360 cells
5 twin-spots largest site 350 cells (total for both components)

Approximately 250,000 cells comprise the whole of the upper surface of the upper lobe of this particular flower. About 10 cell generations appear to have occurred following treatment to the sensitive primordia, which gives a maximum site size of 512 cells; the smaller sizes would increase in frequency

so long as the treatment or its effect continued during flower development.

The increase in twin-spots following caffeine treatment is exemplified in fig. 8 in which these are three twin-spots involving *inc-Inc* on part of one lobe.

Since the main effect of caffeine is usually restricted to a cluster of flowers on the inflorescence, representing those at the sensitive stage of

FIG. 11.—Frequency of single sites on a sequence of flowers on inflorescences of *inc/Inc* treated with 0.25 (D82R⁸) and 0.5 per cent caffeine (D82R¹¹). The effect of the 24-hour caffeine treatment begins in these particular inflorescences with a sequence of stunted flowers.

development at the time of treatment, only these flowers were selected for recording and comparison with the untreated controls. From table 9 and figs. 11 and 12 the 0.25 per cent caffeine effected a 30-fold increase in single sites and twin-spots while the 0.5 per cent treatment gave a 100-200-fold increase. The effect of caffeine is unequivocal.

The data in table 10 are less informative than those in table 9. The effect of the 0.5 per cent caffeine was to produce many aborted flowers and

the frequency of single sites indicates that the highly sensitive stage of development was not chosen. The 0.25 per cent treatment yielded a comparable increase in single sites and twin-spots to that achieved in the *inc/Inc* material (table 9). The *niv* gene suffers the disadvantage, in comparison with *inc*, of giving a less clear difference in pigment intensity between the heterozygous and homozygous dominant. The precise control of pigmentation by the *niv* gene is possibly affected by localised breakage or breakage within the gene complex. The previous work on *niv* and *niv-rec* (Harrison and Carpenter, 1973) showed a flushing phenomenon for this gene that may be

FIG. 12.—Frequency of twin-spots on a sequence of flowers on inflorescences of *inc/Inc* treated with 0.25 (D82R8) and 0.5 per cent caffeine (D82R11). The effect of caffeine in these inflorescences begins with a sequence of stunted flowers.

related to a paramutable condition. A large putative sector of *niv/niv* involving half an inflorescence arising in heterozygous *niv/Niv* was tested with the precursors naringenin and dihydroquercetin and the ensuing synthesis of anthocyanidin indicated the sector to be *niv/niv* but there was a slight flush of pigment present which was not expressed in the acyanic parent. If the control of *niv* is easily affected by localised breakage then some of the pale/dark twin-spots and pale sites may be related to this type of disturbance. The increase in the single and twin-spot sites following 0.25 per cent caffeine treatment was similar to that in the *inc/Inc* plants (table 9). The closeness in the absolute frequencies could indicate that *niv* and the unlinked *inc* have similar distances from their centromeres.

No twin-spots involving *eos* would be seen since *Eos* is completely dominant and sectors of homozygous *eos*, which would synthesise pink pelargonidin instead of the cyanidin in the remainder of the corolla, would if small, appear as pale magenta. However, since pale magenta sites of unknown origin occur in homozygous *Eos* flowers it is clearly unreliable to attribute pale magenta sites to crossing-over involving the *eos* gene unless the sector is sufficiently large to allow a chromatographic analysis: this has been possible and is illustrated in fig. 5 in which there is the unusual situation of a flower synthesising both cyanidin (*Eos/eos*) and pelargonidin (*eos/eos*).

The effect of caffeine on the *Niv* : *Pal* : *Sulfurea* (*Sulf*)/*niv* : *pal-tub* : *sulf* heterozygote (table 11) is again clear but this genotype has the disadvantage of lacking distinctive components for the *niv* combinations. No sectors for yellow (*sulf/sulf*) were identified; these would be seen as a mixture of yellow aurone and magenta cyanidin and would probably be recognisable only if the sectors were large. Some of the larger acyanic sites were tested with dihydroquercetin; those that synthesised cyanidin were recorded as *niv/niv* and those that failed as *pal-tub/pal-tub*.

(x) *Non-recurrens* sectors in homozygous *pallida-recurrens*

The unstable *pal-rec* allele when associated with homozygous recessive *stabiliser* (Harrison and Fincham, 1968) readily mutates somatically (and germinally) to the dominant fully pigmented *Pal* and less frequently to other members of the allelic series (Fincham and Harrison, 1967). If somatic mutations of *pal-rec* to alleles such as *tincta* (usually acyanic, sometimes tinged) or *carnea* (pale magenta flowers) occur early in the development of the corolla then, by somatic crossing-over, homozygous acyanic or palely pigmented sectors free from flaking should be identifiable at a frequency different from that expected from coincident mutations of both *rec* alleles. The somatic segregant *pal-rec/pal-rec* component re-formed from *pal-rec* "new *pal* mutant allele", will be indistinguishable from the normal flaking background. A preliminary survey of these types of non-flaking sectors in homozygous *pal-rec* has indicated the frequency to be higher than expected from independent mutation of both alleles. It must be recognised however, that since the *pal-rec* allele can be considered unusual in its high mutability it could be additionally aberrant in losing the unstable nature (Sastry, 1976) of one allele while the other mutated to another stable allele, thus simulating a homozygous sector; two dissimilar alleles arising from the two *pal-rec* alleles could also simulate a homozygous sector. The partial inactivation of *pal-rec* alleles described below would not easily account for the homozygous non-*rec* sectors since the low level of flaking would still be observable even against the pigmented backgrounds of *carnea* or *rubra*.

Variations in the frequency of somatic mutation of *pal-rec* are common and these vary from sectors to whole flowers, shoots or whole plants showing low mutability. This could be attributed to: (a) mutations involving the *stabiliser* control; (b) one or both alleles mutating to a stable acyanic form; (c) segregation of genes affecting instability; or (d) a change of *pal-rec* (high μ) to *pal-rec*^{low} (Sastry, 1976) but so low as to be unrecognisable as a flaking sector. A survey of 1065 plants of *pal-rec/pal-rec* (normally of high mutability) were grown outside during the hot summer of 1976. The

scoring for the frequencies of somatic mutations was on fully mature plants whose main spikes were still flowering as well as on the many laterals. 258 (24.2 per cent) plants were mutating throughout the entire plant at a high rate (mean of 2360 mutant sites per lobe); 707 (66.3 per cent) plants had mixtures of high, medium and low sectors and laterals; 15 (1.5 per cent) were wholly medium in flaking intensity (mean of 525 mutant sites/lobe; and 85 (8.0 per cent) plants were either entirely low (mean of 141 sites/lobe) or a mixture of low and medium frequencies. These massive somatic changes in mutability in populations of *pal-rec/pal-rec* have been seen on a previous occasion but usually the stock maintains its high flaking; the effect is only somatic, no germinal changes having been recorded. Although temperature is inversely correlated with flaking frequency (Harrison and Fincham, 1964) several other similar lines of *pal-rec* grown under the same conditions did not lose their high mutability and 24 per cent of the plants in the affected family maintained their normal flaking frequency. Somatic crossing-over is an unsatisfactory explanation for this phenomenon but it must be considered a possibility in some instances of sectoring for different flaking intensity, particularly when the line is heterozygous for stabiliser.

In this survey sectors occurred to alleles other than *Pal* and these were usually flaking at a frequency expected from the remaining single *pal-rec* allele: occasionally the flaking on a *carnea* type sector was low but recognisably present. A change of *pal-rec* ($H\mu$) to *pal-rec*^{low} in one allele and a mutation to a stable allele in the other does not readily explain the occurrence of the "homozygous" type sectors which were entirely free from flaking. An array of contiguous separate mutational events resulting in non-*rec* sectors in a *pal-rec/pal-rec* flower is shown in fig. 10; in some lines recently established this behaviour is particularly common and may be the result of a Dissociator type mechanism acting somatically and instigating increased chromosome breakage and reunion (McClintock, 1951). In this particular flower there are mutations of *pal-rec/pal-rec* to *Pal*, *rubra*, *carnea*, *malacea* (not shown in the figure) and *tincta* types that on visual assessment could all be homozygous. Except for the epistatic full red *Pal* sector which could be heterozygous *pal-rec* all the remainder lack both the normal high flaking *pal-rec* alleles.

The occurrence of such putatively homozygous non-*rec* sectors presents obvious problems in interpretation. If, for example, one allele mutated to stable *tincta* early in the development of the inflorescence then any subsequent mutation of the remaining *rec* allele would appear as an unflaking sector that would simulate a homozygous sector for any several alleles. However, this does not seem likely since the flaking frequency of the *rec* sectors is commensurate with two *rec* alleles mutating. Thus, the two *rec* alleles appear to have mutated simultaneously to new stable alleles probably of the same type. If the mutation of one allele triggers the other then it is a mechanism that occurs relatively frequently, more than would be expected by chance, and the occurrence is more frequent in some lines. In the present context of somatic crossing-over, if one allele mutates to a stable form and is then followed by chromosome breakage and subsequent crossing-over to give a homozygous sector it will be accompanied by its companion homozygous component that is the same as the general background and be unrecognisable. If this occurs then it does so frequently and we have already seen that mutations of *pal-rec* to *Pal* are not associated with either germinal or somatic crossing-over within the *el* to *div* region of the *pal* chromosome.

Clearly, further investigation is needed before this peculiar behaviour is satisfactorily explained but the sectoring could be the result of a triggering mechanism of great interest or an additional manifestation of somatic crossing-over.

4. DISCUSSION

The examples of somatic crossing-over in higher plants vary in their degrees of conviction and the present data add one more plausible example. The occurrence of twin-spots of the precise type expected on flowers heterozygous for known genes could be additionally tested by administering anthocyanidin precursors. The synthesis or non-synthesis of pigment following imbibition of defined precursors in the acyanic component enabled the position of the block in the biosynthetic pathway to be identified. If the position of the block identified by precursor treatment agreed with the expected block arising from homozygosity of one of the included genes in the heterozygote then the genetic nature of the component could be assumed with greater certainty even if the mechanism by which it arose is still obscure. However, many possible mechanisms can be immediately eliminated; equal growth of both components makes non-disjunction an unlikely mechanism in the majority of the twin-spots recorded. Carlson (1974) cultured the components of 12 twin-spots in the leaves of *Nicotiana tabacum* and concluded that 11 were the result of mitotic crossing-over and one, possibly induced by the colchicine treatment, to non-disjunction. The increase in twin-spots following caffeine treatment accords with the work of Vig on *Glycine max* which showed an increase in point mutations and somatic crossing-over. Caffeine has been shown to increase the frequency of point mutations and crossing-over in plants but no other major type of aberrant mechanism consequent upon treatment has been identified although potentiating effects have been observed with chemical mutagens and ionising radiations. It is also significant that caffeine potentiation has been recorded mainly in plant and rodent cells which lack or only have inefficient excision repair mechanisms (Kihlman, 1974).

The mechanism by which caffeine (an inhibitor of oxidative phosphorylation) affects the frequency of somatic crossing-over is still conjectural. Methylated oxypurines perhaps induce fragmentation of the replicating chromosomes through an alteration in the permeability of the inner membrane of the nuclear envelope (Weber, 1968; Mizuno *et al.*, 1971) and Doman and Rauth (1969) have shown that there is an increase in lethal damage caused by UV and mitomycin C in mouse L cells following caffeine treatments during the interphase period of DNA synthesis. Kihlman *et al.* (1971) suggest "at least two mechanisms by which methylated oxypurines produce chromosomal aberrations in plants and mammalian cells: (1) a mechanism favoured by low temperatures which results in exchange-type aberrations in cells treated during prophase and G_2 and which, at least in plant cells, is dependent on the ATP level of the cells and (2) a mechanism favoured by high temperature which results in fragmentation of chromatids in cells treated during the period of DNA synthesis and which is independent of the ATP level in the cells". All inhibitors of DNA synthesis, however, do not increase somatic crossing-over and Vig (1973) suggest it is "not DNA *per se* but a specific step in its synthesis, or in the synthesis of protein or some

related phenomenon, which gives rise to somatic recombination in *Glycine max*". Complementary chromosomal exchanges following caffeine treatment cannot be due solely to the inhibition of DNA synthesis but additionally to a more specific event in DNA repair, such as an inhibition in the gap-filling process which could lead to an accumulation of gaps in the daughter strands and an increase in chromosomal aberrations.

Single site aberrations could arise from cross-overs in which one component failed to establish a cell lineage; in *Antirrhinum* this has been seen with some certainty but many of the single sites have doubtless arisen by other means; point mutation, non-disjunction, deficiencies, translocation are obvious possibilities. The number and type of single site aberrations recorded here indicate the many pathways to floral mosaicism although accurate identification of all types is not possible at this stage. Many somatic variants can, however, be identified with some certainty and these could produce viable new plant forms by vegetative propagation. Also, heterozygous material, if repeatedly propagated vegetatively, could be expected to diverge considerably from type. A recent survey of fruits from many apple varieties and seedlings at the John Innes Institute gave abundant evidence for twin-spotting and sectoring that would provide uncontrolled variability if it occurred in the scions used for multiplication.

The ambiguous sectoring behaviour sometimes seen in homozygous *pal-rec* may result from somatic crossing-over within newly formed heterozygous tissue. A situation could thus occur: *pal-rec/pal-rec* μ $\rightarrow$ *pal-rec/pal-carnea* $\rightarrow$ (by crossing-over) *pal-rec/pal-rec* (component as general background and indistinguishable in flaking intensity) + *pal-carnea/pal-carnea* (a homozygous unflaked palely pigmented component); or as an additional example, *pal-rec/pal-rec* μ $\rightarrow$ *pal-rec/pal-tincta* (lower flaking intensity) $\rightarrow$ (by crossing-over) *pal-rec/pal-rec* (normal flaking and indistinguishable) + *pal-tincta/pal-tincta* (acyanic unflaked component). The observed frequency of unflaked sectors in homozygous *pal-rec* appears higher than expected and unless some triggering of both *pal-rec* to mutate simultaneously to the same or similar stable alleles occurs the sectoring behaviour could be an additional manifestation of somatic crossing-over.

Evans and Paddock (1976) have made a comparative study of *Glycine max*, *Nicotiana tabacum*, *Gossypium barbadense* and *Lycopersicum esculentum*, the four crop species that have provided the most convincing evidence for somatic crossing-over in higher plants. Somatic crossing-over has been calculated by Carlson (1974) to occur spontaneously between the *Sulfur* marker and the centromere in *Nicotiana tabacum* at a frequency of 6.7×10^{-5} per mitosis in leaf tissue and 4.6×10^{-5} per mitosis in *in vitro* cultured cells. An estimate on the same material by Evans and Paddock was 0.77×10^{-5} and for *Glycine max* ($\Upsilon^{11}\Upsilon^{11}$) a frequency of 5.74×10^{-5} . Our tentative estimate for *inc/Inc* in *Antirrhinum majus* (from control in caffeine treatment) incorporating several approximations is 0.96×10^{-5} . The *Antirrhinum* data provide further evidence for the more general occurrence of somatic crossing-over, and, in spite of probable differences in gene to centromere distance, at a comparable frequency to that presented for the *Su/su* heterozygote in *Nicotiana tabacum* by Evans and Paddock.

Acknowledgments.—It is a pleasure to record our appreciation to Mr Tim Hardwick for summer vacational help and to Mr L. S. Clarke for the production of the photographs.

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