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Genetic Instability of Anthocyanin Production in *Impatiens balsamina*

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Summary. It is established that in a naturally occurring variegated *Impatiens balsamina* the phenotype is determined by a mutable allele p^m, of an anthocyanin-governing gene P^r. The special allele produces an acyanic phenotype like the stable recessive p but undergoes frequent changes to P^r in somatic and germinal cells (causing a variegated phenotype in the former) when a controlling element M is also present in the genome. It is suggested that p^m is a repressed P^r and M acts either by removing or inactivating whatever causes that repression. Such changes proceed in a unique fashion: either p^m changes to P^r or to an intermediate labile condition P' which then changes to P^r, resulting either in dark or pale, or dark superimposed on pale, sectors; a reverse situation was not observed. Colourless plants which occasionally appear in unstable lines seem to be due to loss of M although changes of p^m itself cannot be ruled out at present.

Key words: *Impatiens balsamina* – Genetic instability – Controlling element – Anthocyanin

1 Introduction

Many genes are known in plants which mutate with an unusually high frequency in somatic and germ cells. These 'unstable' or 'mutable' genes produce variegated phenotypes often with spectacular effects. Although such phenomena have attracted the attention of many geneticists even from the days of De Vries (1911), the most basic questions about unstable genes still remain unanswered. Furthermore, what we do know about these systems is largely confined to maize (see Fincham and Sastry 1974), *Antirrhinum majus* (Sastry et al. 1980; Jeffries and Sastry 1981) and *Mirabilis jalapa* (Spitters et al. 1975). Speculation that some features of unstable genes actually reflect gene-regulation mechanisms (see Fincham 1973 for instance) invite the search in other plants for a common basis.

Some years ago I found a garden balsam plant (*Impatiens balsamina*) with an interesting pattern of instability for anthocyanin production in stems and flowers (Figs. 1, 2). Initial observations on the original plant and its immediate progeny suggested a complex and novel pattern of gene changes, and a detailed study was undertaken. In this paper, results will be presented which identify the gene involved and suggest the existence of a controlling element.

2 Materials and Methods

Dominant alleles at three independent loci are known to condition anthocyanin production in garden balsam. Although, in the past, several different systems of symbols have been employed to designate these genes (see Paris et al. 1960) the system of Davis et al. (1958) will be used here since it is both simple and well established.

As can be seen from Table 1, the presence of P alone is enough for the production of coloured (pink) flowers. Apart

Table 1. A summary of the stem and flower colours of variegated genotypes[a]

Genotype	Stem colour	Petal colour
l h p	Green	White[b]
L H p	Green	Almost white
l h P^r	Red	Pink
l h P^r	Red	Red
L h P^r	Red	Purple
L H P^r	Red	Magenta

[a] Only genotypes which are relevant to the present paper are given here. For other genotypes, which are almost white, see Davis *et al.* (1958); colour photographs of various phenotypes are given in Clevenger (1964)

[b] In the present paper the description "colourless" should be taken to mean 'white' with reference to petals and 'green' with reference to stems

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from the recessive *p* two major alleles have been recognised at the *P* locus; of these, P^r produces intense pigment on the stems as well (especially on the lowest internode), whereas plants homozygous for P^g have green stems but coloured flowers. For the present investigation, seeds of *l l h h p p*, *l l h h* P^rP^r, *l l H H* P^rP^r and *L L H H p p* tester lines were kindly supplied by Dr. Sarah Clevenger. These are from the highly inbred genetic strains which have been extensively used by Dr. C. W. Hagen and his collaborators to study pigment development in *I. balsamina* (Hagen 1966). Other necessary combinations such as *L H* P^r, were prepared by crossing appropriate genotypes.

Vegetative propagation of balsam is simple and is accomplished by cuttings dipped in a rooting powder (Seradix B – May and Baker). If the branches are in flower at the time of cutting, they continue to produce flowers but not vegetative branches.

Seed from the unstable plants shows a high degree of post-harvest dormancy which lasts about six months. The dormancy can be broken by splitting the seed mechanically after soaking in water for 48 h. Hagen's genetic testers are free from such dormancy.

Fig. 1. A typical flower on a variegated plant: both pale and dark (some of which are superimposed on pale) sectors on a colourless background can be seen. Similar phenotypes were observed on both homo- and heterozygous plants

3 Results

3.1 Initial Observations[1]

The original variegated plant was found in a large ornamental garden in New Delhi; it produced flowers with several magenta sectors on a clear colourless background (Fig. 1). The stem, especially the first internode was also characterised by large anthocyanin sectors (Fig. 2). The plant was immediately isolated and only capsules set after isolation were harvested. A small progeny of 34 plants was grown and the following observations were made:

(a) Six plants had red stems from the seedling stage and the rest were green. Plants with coloured stems bore only self coloured flowers which were either red or magneta, (but in a later study plants with pink and purple flowers were also found, all with coloured stems. On selfing, all coloured plants produced coloured and variegated offspring in a ratio of approximately 3 : 1).

(b) Only plants initially scored as green seedlings showed instability for flower colour. On these, one could find self-coloured flowers, completely white flowers and flowers with variegation. Moreover, two types of sector, dark and pale (Table 2), were discernible on almost all variegated flowers. Quite frequently dark sectors appeared superimposed on large pale sectors (Fig. 1) but it is interesting to note that the reverse situation was never observed. Two exceptional cases were noticed among the green plants. These two plants bore only white flowers and the stems remained green without any coloured sectors. Progeny of the exceptional plants, grown from selfed seed, remained green and produced only white flowers.

(c) Plants with the unstable phenotype showed an interesting variation in the petals; in some plants the dark sectors were magenta, in some they were red, in some they were purple and in some they were pink. The characteristic colours of the pale sectors on these plants is given in Table 2. The colour of sectors on the stems did not, however, vary.

(d) Plants varied both in the frequency and size of petal sectors. The frequency of sectoring on the stems reflected approximately the situation on the petals.

Fig. 2. Stem variegation on a plant with "unstable" flowers

Table 2. Deduction of genotypes of mutable plants by comparing their dark sectors with tester genotypes

Dark sectors		Pale sectors	Postulated mutable genotype
Colour	Genotype[a]		
Magenta	*L H* P^r	Purple	*L H* p^m
Red	*l H* P^r	Pink	*l H* p^m
Purple	*L h* P^r	Mauve	*l h* p^m
Pink	*l h* P^r	Pale pink	*l h* p^m

[a] Tester genotype which produces a colour similar to the dark sector

1 Colour photographs of various unstable phenotypes referred to in this paper can be seen in Sastry et al. (1980)

Even after several generations of selfing, carried out during the period of present investigation, different types of variegated plants described in the paragraph (c) above still produced progeny containing a large number of variegated plants, a few self-coloured and occasional uniformly colourless phenotypes in no particular or predictable proportions.

3.2 Phenotypic Comparisons

From what we know about the genetics of anthocyanin production in balsam, the appearance of coloured sectors on a colourless background is only possible if the instability involves the change of recessive *p* to a dominant *P* form. If, on the other hand, *L* or *H* or even both had been unstable with a stable *P* allele, then either sectors should have appeared on a coloured background (in plants with dominant *P*) or they should not have appeared at all (in plants with *pp*). The fact that floral variegation is associated with the instability on the stems further suggested that an allele of *P* was involved since only that gene is known to produce anthocyanin in the stems. Keeping these points in mind, the mutable allele was provisionally designated as p^m.

A visual comparison was made between the flower colours of standard genetic strains (Table 1) and dark sectors on the four classes of mutable plants referred to in the preceding section, paragraph (c). It seemed possible to account for the observed dark sectoring as due to the genotypes postulated in Table 2, p^m being an unstable allele frequently mutating to P^r. The pale sectors are not obviously explained in this scheme.

3.3 Tests for Allelism

As a first step in identifying the gene or genes concerned with the instability, all types of variegated plants listed in Table 2 were crossed with the tester lines *l l h h p p*, *L L H H p p*, *l l H H $P^r P^r$* and *l l h h $P^r P^r$*. If, for instance, *H* were the locus of instability, variegation would be seen only in progeny of crosses to plants carrying the recessive *h*. In the hybrids where the tester contributed a dominant and stable *H* allele, variegation caused by mutable *H* would be masked. These crosses were also designed to test the hypothesis that the differently coloured sectors in different plants (Table 2) were caused by the plant's constitution at *H* and/or *L* and they are not involved in creating instability per se.

As can be seen from the observations summarized in Table 3, variegated plants appeared only in those crosses where the tester parent contributed *p* (crosses in group A). Data from the B and C groups of crosses are also informative in the sense that when the p^m allele was masked by the tester P^r, the variegated phenotype was not manifested. Thus, allelic tests confirmed the earlier

Table 3. Tests for allelism. Results from reciprocal crosses

Variegated parent[a]	No. of crosses	No. of F_1 plants[b]			
		Variegated	Self-coloured	Colourless	Total
A) Crosses with *p* testers (*l l h h p p* and *L L H H p p*)					
Magenta	18	53	22	7	82
Red	8	24	4	0	28
Purple	15	37	14	11	62
Pink	14	89	17	3	109
Total	55	203	57	21	281
B) Crosses with *l* tester (*l l H H $P^r P^r$*)					
Magenta	6	0	37	0	37
Red	7	0	33	0	33
Purple	4	0	12	0	12
Pink	5	0	15	0	15
Total	22	0	97	0	97
C) Crosses with *l* and *h* tester (*l l h h $P^r P^r$*)					
Magenta	8	0	43	0	43
Red	8	0	36	0	36
Purple	5	0	23	0	23
Pink	10	0	44	0	44
Total	31	0	146	0	146

[a] Colour of dark sectors

[b] In some crosses variegated and/or self-coloured hybrids showed different colours if the concerned variegated parent was heterozygous for *H* and/or *L* (see also Table 4); the description variegated etc., applies to both stems and flowers

postulate that of three known anthocyanin loci, only an allele of *P*, which was designated as p^m, is directly involved in producing the instability; if the other two were involved at least some of the hybrids produced by *l l h h* $P^r P^r \times$ variegated combinations should have showed dark sectors superimposed on the coloured background produced by P^r. An examination of several thousand flowers of such F_1 plants has revealed no such superimposed sectors.

In group A crosses (Table 3) three phenotypes, variegated, self coloured and colourless, were distinguished. If the variegated parents were $p^m p$ instead of $p^m p^m$, then one would expect half the hybrids with *pp* tester to have white flowers and green stems; however only a few such plants were present – 21 as opposed to 203 hybrids – suggesting variegated parents were homozygous for p^m. Also, the fact that neither the original unstable plant nor its progeny produced a quarter of colourless plants on selfing further confirms their homozygosity for the mutable allele.

Heterozygous variegated plants ($p^m p$) were strikingly different from their homozygous parents in having a large number of branches bearing wholly white flowers. (See Sastry et al. (1980) for quantitative assessment.) As a consequence of this, the number of colourless plants mentioned in the preceding paragraph could have been a slight overestimate since several plants which were mutable as judged by the presence of variegated stem sectors, showed floral variegation only on the branches situated in the axils of cotyledons. Plants which did not produce these branches could have been scored as colourless individuals. It also appeared that branches with variegated flowers produced more wholly white flowers in heterozygous than in homozygous plants.

Flowers borne on heterozygous plants, like their homozygous counterparts, frequently showed pale sectors (Table 5) and dark flakes could be seen superimposed on several of these sectors. This demonstrates that a single p^m allele in a $p^m p$ plant can undergo sequential changes first to the 'pale' and then to the full dominant 'dark' condition.

The degree of stem variegation was lower in heterozygous than in homozygous plants although there was considerable variation from plant to plant.

With the aid of observations given in Table 2, it was suggested that variation at the *L* and *H* loci may cause the different colours of the sectors produced by p^m.

Table 4. Predicted F_1 (Variegated testers with P^r, B and C in Table 3) phenotypes with reference to *L* and *H* variation; all predictions have been realised without an exception

Variegated parent		Tester	F_1 phenotypes expected[b] To be present	To be absent
Sector-colour	Genotype[a]			
Magenta	*L l H h* $p^m p^m$	*l l h h* $P^r P^r$	Magenta (*L l H h* $P^r p^m$) Red (*l l H h* $P^r p^m$) Purple (*L l h h* $P^r p^m$) Pink (*l l h h* $P^r p^m$)	None
		l l H H $P^r P^r$	Magenta Red	Purple Pink
Red	*l l H h* $p^m p^m$	*l l h h* $P^r P^r$	Red Pink	Magenta Purple
		l l H H $P^r P^r$	Red	Magenta Purple Pink
Purple	*L l h h* $p^m p^m$	*l l h h* $P^r P^r$	Purple Pink	Magenta Red
		l l H H $P^r P^r$	Magenta Red	Purple Pink
Pink	*l l h h* $p^m p^m$	*l l h h* $P^r P^r$	Pink	Magenta Red Purple
		l l H H $P^r P^r$	Red	Margenta Purple Pink

[a] All variegated parents have been found to segregate for sector-colour and hence they were considered as heterozygous for *L* and *H*

[b] Plants with different colours have all been added up and presented in Table 3 as a single class as self-coloured

This is further supported by the information obtained from the crosses. Assuming the genotypes in Table 2 are correct, certain predictions can be made regarding the phenotypes of the crosses. For instance, plants with pink sectors were assumed to be $l\,h\,p^m$. If this is correct, crosses with $l\,l\,h\,h\,p\,p$ should show pink sectors, crosses with $l\,l\,h\,h\,P^r\,P^r$ should show only self coloured pink flowers, crosses with $L\,L\,H\,H\,p\,p$ should produce plants with magenta sectors and crosses with $l\,l\,H\,H\,P^r\,P^r$ should show only uniformly red flowers. Such expectations have been worked out for all combinations reported in (B) and (C) of Table 3 and compared with their phenotypes: Table 4. In all cases, the predicted and observed results agreed showing that variation in dark sector-colour can indeed be accounted for, as suggested in Table 2. Also, in group A crosses (Table 3) when the tester $L\,L\,H\,H\,p\,p$ was used, sectors in variegated hybrids and uniformly coloured petals in self-coloureds were magenta, as expected.

For raising F_2 populations, seed from self-pollinated flowers was harvested separately from different F_1 plants and from different branches if they varied within a plant.

3.4 F_2 Data

Answers to two complementary questions were sought in the F_2 data. First, since both homozygous and heterozygous plants showed mutations of flower colour along three distinct pathways – colourless to pale, pale to dark and colourless to dark – would it be possible to obtain plants which showed only one type of instability? Such plants should be found in F_2 populations if different genetic factors are responsible for such changes. In the previous section, it has been shown that both *L* and *H* are stable, but this does not preclude the presence of a hitherto unknown gene in an unstable condition. The second question concerned the possible presence of an independent factor which provokes instability at p^m; such factors are reffered to as 'controlling elements'.

Progeny from $15\,p^m p$ plants with a varied constitution at *L* and *H* loci have been used for F_2 studies. One point that was immediately apparent from these progeny tests was that, within the limits of the number of plants examined, different pathways of instability (colourless → pale → dark etc.,) were not separable. Neither individual plants showing only pale sectors on a colourless background, nor showing only dark sectors on a pale background were found.

F_2 segregations were also of critical importance for determining whether or not the instability of p^m was regulated by a separate controlling element. In the present system, F_2 ratios are determined by the presence or absence of a separate controlling element, its linkage with p^m and whether p^m produces the self-coloured or a uniformly colourless phenotype in the absence of the controlling element. If the element regarding mutability is integrated within or closely linked to p^m, then selfed plants of $p^m p$ genotype should give progeny segregating for unstable and stable phenotypes in a 3 : 1 ratio. If, on the other hand, an independent controlling element is present and p^m produces a colourless phenotype in its absence, then one should look for a 9 : 7 (unstable : colourless) ratio. Finally, if p^m produces a self-coloured phenotype in the absence of the controlling element, then a 9 : 3 : 4 (unstable : coloured : colourless) should be expected.

As shown in Table 6 all but two (52–413 and 52–424) F_2 families contained some self-coloured plants. The totals of variegated, self coloured and colourless plants (508, 88 and 499 respectively) are, however, clearly incompatible with a 9 : 3 : 4 ratio ($\chi^2 = 271.2$; $P \ll 0.001$; 2 df). It is likely therefore that the self-coloured plants arose by mutation of p^m to P^r in the germ cells; adding the self-coloured and variegated classes gives a ratio of 596 : 499, which is an acceptable fit to 9 : 7 ($\chi^2 = 1.4$; $P = 0.2$–0.3, df = 1) but is clearly incompatible with 3 : 1 ($\chi^2 = 246.0$, $P \ll 0.001$, 1 df). Hence, if fully coloured plants are included in the variegated class, the data support the hypothesis of an independent controlling element, in the absence of which p^m ressembles the stable acyanic allele *p*. However, it will be apparent from Table 6 that F_2 families were heterogeneous (heterogeneity χ^2, calculated for a 9 : 7 ratio was 97.1 for df = 8; $P \ll 0.001$]; in fact, one family (52–404) gave a good fit to a 3 : 1 ratio of variegated to colourless ($\chi^2 = 0.35$, $P = 0.5$–0.7).

Table 5. Instability levels to dark and pale conditions in a sample of $p^p\,p^m\,M\,M$ and $p^m\,p\,1M$ plants. Estimates were obtained by scoring coded samples of 100 petals against a standard scale consisting of 0 – 9 classes; class 0 represented petals with no mutant spots and fully coloured phenotypes fell in the highest class

	Average score	
	Dark	Pale
Homozygous plants		
296-1	6.2	1.1
296-2	5.8	1.1
171-8-3	4.8	1.3
291-1	2.5	2.3
291-3	3.5	0.7
291-6	3.0	0.9
Heterozygous plants		
203-2	2.0	0.4
203-3	3.9	2.4
206-1	4.3	1.4
227-1	1.4	1.2

Table 6. Phenotypic nature of F_2 population produced by selfing 15 F_1 plants obtained in different variegated × *pp* tester crosses referred to in Table 3, group A

F_1 genotype	F_2 family pedigree	Coloured	F_2 phenotypes[a] variegated	Colourless	Total
L L H H p^m *P*	52-404	20	117	51	188
L L H H p^m *p*	52-405	6	23	57	86
L L H H p^m *p*	52-406	18	63	16	97
L L H H p^m *p*	52-407	8	30	38	76
L l H H p^m *p*	52-408	13	31	16	60
L L H H p^m *p*	52-409	1	31	18	50
L L H H p^m *p*	52-410	4	1	24	29
L L H h p^m *p*	52-411	1	12	4	17
L L H h p^m *p*	52-412	5	34	74	113
L L H H p^m *p*	52-413	0	2	15	17
L L H H p^m *p*	52-421	7	48	38	93
L l H H p^m *p*	52-422	1	9	24	34
L L H H p^m *p*	52-423	1	3	20	24
l l H h p^m *p*	52-424	0	2	20	22
L l H H p^m *p*	52-425	3	102	84	189
Total		88	508	499	1095

[a] Segregation for *L* and *H* was also scored but is omitted here for the sake of clarity

3.5 Reconstitution of the Variegated Phenotype

Since the F_2 data could not unequivocally establish the presence of an independent element, a different approach was undertaken. It depended on the idea that if self-coloured mutants are due to a $p^m \rightarrow P^r$ change in germinal cells, at least some of those plants should still carry the controlling element, if such an entity exists. Also, if an independent controlling element regulates the instability at a repressed p^m, then there should be two types of colourless segregants in F_2 progeny obtained from the tester – variegated crosses; one with p^m ($p^m p^m$ and $p^m p$) but without the controlling element, and the other with the controlling element but without the mutable allele (*pp*). By crossing the first category of colourless plants with self-coloured mutants, it should be possible to reconstitute variegated phenotypes (p^m with one or two controlling elements) at least in some of the F_2 progenies. (F_1 itself will be fully coloured since all other phenotypes will be masked by P^r.) To test this hypothesis, self-coloured mutants which arose in homozygous variegated lines were reciprocally mated with F_2 colourless segregants. (Colourless segregants were also selfed to make certain that they breed true). As can be seen from Table 7 several variegated plants did appear in the F_2 from a majority of these crosses whose colourless parents therefore carried the p^m separated from its controlling element, a copy of which was supplied by the self-coloured parent. The presence of an independent controlling element is thus established, which in future will be referred to as *M*.

A limited number of crosses between F_2 colourless segregants was also performed to see whether variegated types can be reconstituted by reuniting p^m and *M*. A small number of F_1 plants did show the variegated phenotype confirming the presence of a controlling element.

Furthermore, all reconstituted plants displayed all three modes of mutability in the same characteristic way as the original parent. Variation in mutability level among the reconstituted variegated plants was no more than that observed in unstable parental lines. Also, on selfing, the reconstituted plants produced a variable number of colourless and uniformly coloured individuals along with the variegated.

3.6 Stability of Colourless Sectors

The prevalence of large colourless areas in several heterozygous plants raised two important questions. Is there any pattern, with reference to morphology of the plant, in the appearance of colourless branches? Such a pattern has been demonstrated but the details are discussed elsewhere (Sastry et al. 1980). The second question concerns the genetical stability of the colourless character itself. To clarify this, 18 different colourless sectors were propagated by cuttings which gave 66 plants. Several hundred flowers from these plants were examined throughout their lives and all of them were colourless. To test the inheritance through the sexual cycle, progeny from the selfed seed of 9 sectors with variegated flowers and of 8 with wholly white flowers were examined. Of these, progeny of two variegated

Table 7. Reconstitution of variegated phenotypes. As explained in the text, 3 self-coloured mutants (isolated from homozygous variegated line) were crossed (a) with 8 F_2 colourless segregants and (b) with *L L H H p p* tester. Where the coloured parent was heterozygous ($P^r p^m$), two types of F_1 were produced but in any case only self-coloured F_1 plants were selfed to generate the following F_2 progeny

Self-coloured parent		Colourless segregant	F_2 Progeny[a]	
Pedigree	Genotype		Colourless	Variegated
829-3	$P^r P^r$	794-14	88	13
829-6	$P^r p^m$	790- 9	12	28
		792- 6	12	12
		793-10	8	0
		793-11	6	0
		794- 6	4	2
		794- 4	24	3
		796- 8	19	4
829-8	$P^r P^r$	796- 8	37	0
829-6	$P^r P^m$	*L L H H p p* tester	62	0

[a] Self-coloureds which were approximately ¾ of the total progeny were not taken into account

sectors produced only colourless plants and progeny of one colourless branch showed a small proportion of variegated plants. The rest of the progeny reflected phenotypes of the sectors from which they were derived. (The exceptional sectors might have been periclinal chimaeras, seed originating from a layer separate from floral epidermis.)

4 Discussion

Results presented in the preceding section established clearly that the unstable phenotype in *I. balsamina* is caused by an interaction of two independent genetic entities: a controlling element *M* which evokes various somatic and germinal changes at the independent, anthocyanin-governing locus when a special allele p^m is present at that site. Although it is not clear what makes p^m respond to the action of *M*, it is a special property which distinguishes the mutable allele from *p* and P^r. Drawing a parallel with the cases investigated in maize (see Fincham and Sastry 1973), it is tempting to postulate that p^m is P^r repressed by a closely linked or an integrated entity which may be either inactivated or removed by the action of *M* during different stages of plant development resulting in a variegated phenotype; such a change in germinal cells results in fully coloured plants.

In the above context two important aspects should be pointed out:

(i) None of the self-coloured mutants isolated from the unstable genotype showed any differences in the intensity of pigmentation. These mutants were indistinguishable from the plants carrying wild type P^r. This is in spite of the fact that sectors of different intensity were observed in almost all variegated flowers (Table 5). When the progeny was obtained from the seed set by the flowers wholly pigmented at intermediate level, they were either normally mutable, colourless or fully coloured with the same intensity as the wild type P^r. This is a rather surprising result since both in maize and in *Antirrhinum* where, in my experience, pale somatic sectors are relatively uncommon, germinal mutations with graded series of pigmentation have been isolated (McClintock 1965; Fincham and Harrison 1967).

(ii) Self coloured mutants (hence P^r) have never reverted to an unstable condition either somatically or germinally. Several thousand flowers from $P^r p^m M$ and $P^r P^r M$ plants were examined for such changes but without any success. Hence the loss of repressor activity at the mutable allele or whatever happens during the p^m to P^r change appears to be rarely, if ever, reversible.

On the basis of the model under consideration the origin of occasional colourless individuals from $p^m p^m$ *M M* and frequent colourless sectors in $p^m p M$ plants can be explained as being due to germinal or somatic loss or inactivation of *M*. However, changes at the p^m itself – loss of sensitivity to the action of the controlling element, for instance – cannot be completely ruled out at present.

Alston and Hagan (1958) showed that the presence of *P* is quite sufficient to produce enough pelargonidin to result in pink flowers and coloured stems. A large

quantity of pelargonidin (hence red flowers) is produced when *H* is also present. *L* converts pelargonidin into malvidin by methylation. In *L h P* plants almost all the pelargonidin is converted into malvidin causing purple flowers. *L H P* plants produce a large quantity of of pelargonidin because of the presence of *H*, but only some of it is methylated by *L*, resulting in a mixture of pelargonidin and malvidin to produce magenta flowers. Preliminary paper-chromatographic analysis and phenotype comparisons suggested that p^m frequently changes to a *P'* allele with a low efficiency. In LHp^m plants, for instance, when p^m changes to such a P', *L* methylates all the pelargonidin to malvidin causing a pale purple sector but when p^m or *P'* change to a fully efficient *P*, a dark magenta sector will be caused. Failure to obtain germinal mutations with an intermediate level of pigmentation might either be related to its labile nature or even more interestingly (if it can be proved conclusively) it might reflect some gene regulatory process in somatic cells. Whatever it is, the mode of instability which is unique to the *Impatiens*- system appears to be an inherent characteristic of p^m and/or *M:* segregation data in reconstitution experiments indicate that no other genes are involved.

The phenomenon of shifting of instability from one frequency to another as described in *Antirrhinum* (Sastry et al. 1980; Jeffries and Sastry 1981) was investigated in the present system using a "late" variant line. In the plants belonging to this line, p^m changes at a late stage in the floral development producing small dots (both pale and dark) on a colourless background (Fig. 3) unlike the large sectors produced by the standard early type. Accurate estimates of mutational events can be obtained in the late line by counting the number of dots. Although the investigation revealed occasional changes in the spot frequency within individual plants, the degree of shifting was in no way near to that found in *Antirrhinum* and certainly the changes were not cyclic.

Fig. 3. Late mutation pattern produced by a variant line derived from the standard type shown in Fig. 1. (Large sector seen in the Fig. is an exception rather than the rule)

Genetic regulation of instability in *I. balsamina* resembles to some extent the type described in *Mirabilis jalapa* (Spitters et al. 1975). It operates in a "two-element" mode originally described in maize (see Fincham and Sastry 1974). During the early stages of the work, I hoped the present system would lend itself to the investigation of transposition of the controlling element since evidence for such a phenomenon is absent from plants other than maize. This hope, however, did not materialise due to the paucity of genetic markers. Several cases of large colourless sectors described in section 3.6 might have involved transposition of the controlling element but under existing conditions it can never be distinguished from somatic change or a loss of the element. However, some $p^m p\, M$ plants such as the one which produced F_2 family 52-404 (Table 6) with a 3 : 1 (variegated : colourless) ratio instead of 9 : 7, do suggest that some kind of transposition does take place in *I. balsamina.*

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