

The fact that several mutants (B and a) appear to have some size may also be due to the heterogeneous backgrounds of the mutants. One cannot discount, however, the possibility that it is actual. If the genesis of a mutant were functional locus + gene controlling element within the locus, then the mutant would appear as a block in studies of this type. Several waxy mutants which are known to have had such an origin have been included in our crosses, and data should soon be available on this point.

Functional complementation if it occurs should be revealed in the endosperm of the seed resulting from the cross between two mutants. Analyses are not complete as yet, but interactions of a magnitude which would suggest that the two mutants crossed are located in different cistrons have not been observed. Still measurable interactions are present. The percentage of amylose in all crosses involving wx^a , for example, is substantially greater (100%) than the percentage of amylose in either parent. More data on amylose percentage in various crosses are being obtained.

The details of the technique used may be pertinent. The tassel samples are collected as mentioned in 70% alcohol. A "curing" period of several weeks is desirable since newly collected microspores do not stain as readily with a standard strength stain as do those which have been collected longer. The standard stain formulation is 25 ml. of water, 250 mg. of potassium iodide, and 45 mg. of iodine. The stain is mixed approximately 20 hours before use and placed on a shaker overnight. One hour before use, 1 drop of "Tween 80" is added and then 0.5 grams of Baker's gelatin. The mixture is heated for 5 minutes on a warm hot plate.

In preparing the slides, 24 anthers are selected--the 3 anthers from the more mature floret of 8 glumes which are just beginning to open. These are placed in the small stainless steel cup of a Virtis Microhomogenizer together with 0.8 ml. of the stain. The mixture is homogenized for 2 minutes after which it is strained through cheesecloth onto the surface of a lantern slide. The microspores are dispersed as evenly as possible and covered with a 50 x 75 mm cover slip. After the mixture has set, the edges of the cover slip are coated with colorless nail polish. Such preparations will keep for several days without desiccation and can be scored at any time in that period.

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2. Gene controlling elements of the a_1^{pm} system.

Notes dealing with a mutable a_1^p allele have appeared in the Newsletter for the past several years. By and large they have been concerned with the analysis of A_1 locus components through the use of patterns of mutation presented by this mutable locus. This letter, on the other hand, will deal with the gene controlling elements involved.

Variegated Phenotypes

Producing basically pale aleurone color, this unstable allele is capable of mutating to a higher level resulting in the production of deep aleurone tissue, or to a lower level giving colorless tissue. These mutations may occur at any time during the development of the tissue, hence, the deep sectors may be large if they occur early, small if they occur late, or they may be non-existent. Similarly the colorless areas may be small, large, or may, in fact, render the majority of the aleurone tissue colorless. Different combinations of direction of mutation and time of mutation result in a number of different variegated patterns.

One of the most common mutable types in this material is called "coarse pale mosaic". In this form colorless mutations occur fairly early giving moderately large colorless interruptions in the pale background. A tendency for few and late deep mutations results in infrequent deep dotting. In a second common mosaic type, aleurone tissue which is mostly colorless results from very early mutations to the null level. Changes to the deep level are more common here, so that the resulting pattern has been called "white plus dots". A third form which resembles stable pale alleles frequently appears. This state, called "apparent pale self" is considered to be a mutable type because its progeny mutate at high rates to other mosaic forms instead of breeding true for the uniformly pigmented condition. In a fourth state, designated "light pale plus dots", rare mutations to the colorless level result in an almost uniform pale background. In addition, a large number of deep dots arise from frequent late changes to the higher level. A number of other mosaic forms arise, but these will not be considered here.

A Closely Linked Controlling Element

Several cases of variegated phenotypic expressions in maize have been attributed to gene controlling elements. Since mosaicism in the present case may occur in the plant tissue and extend into the sporogenous tissue, it can be shown that this mosaicism results from mutation at the A_1 locus. In crosses variegation segregates with the pale allele with which it was introduced, but if this variegation is separated from a_1^P , the frequency of the event is quite low. Since the mosaic effect comes about by mutation at a_1^P , and since the effect has been shown to be linked closely with a_1^P , it seems logical to conclude that this unstable allele, like others described earlier, results from a gene controlling element present at a_1^P and acting upon it.

The effect of this mutable locus on crossing-over in the A- Sh_2 region has been studied (M. N. L., 1956). Stocks carrying the mutability factor show recombination rates which are significantly different from the control rates. Both increases and decreases in the rate of recombination within the A- Sh_2 region were brought about by the influence of the mutability factor. In addition, in stocks carrying the gene controlling element high rates of somatic losses of the linked Sh_2 gene were observed (M. N. L., 1956). That a_1^{pm} can influence the rate of crossing-over in an adjacent

region as well as the somatic loss of a linked gene, two characteristics which have been shown to hold for other gene controlling elements, supports the idea that a typical mutability factor is present at the a^P locus.

Other Controlling Elements

Changes from one mutable form to another occur both at "low" rates typical of mutation and at high rates which could be explained only by segregation of an independent but influential factor. Table 1 illustrates the types of progeny the various mosaic types produce. It is apparent from the first group of four ears that the coarse pale mosaic form often mutates to other mutable types. There is, with a few exceptions, in each of the mutable forms the capability of mutating to the other states. Superimposed upon these mutational events, are changes of a much higher frequency. Ears of this type are illustrated in the second group under each of the kernel classes. Ears which show the 1 to 1 ratio between two mutant forms probably result from the segregation of a controlling element, the presence of which is necessary for the expression of one form, while the other form appears only in its absence. If this is the case, then controlling elements responsible for the following changes in form must exist.

Coarse pale mosaic	----	Apparent pale self
White plus dots	----	Light pale plus dots
Coarse pale mosaic	----	Light pale plus dots
Apparent pale self	----	Light pale plus dots

Ears which illustrate 3 to 1 ratios could result from either the segregation of two independent but similar controlling elements, or from linkage of the controller to a_1^{Pm} . The 1 to 3 ratios can also be explained by linkage, if the controlling element in these cases is located on the homologous chromosome. Under the linkage explanation ratios which vary significantly from 3:1 and 1:3 can be explained by different degrees of linkage.

On the other hand, a linkage hypothesis is not the only possible explanation for the 1:3 ratios seen in Table 1, since they could also arise from the segregation of an element which suppresses the gene controller. In a_1^{Pm} a consistent but low proportion of the apparent pale self types breeds true. More commonly, however, the apparent pale self form gives rise to other mutable forms by mutation and segregation. If the true breeding self types result from loss of the gene controller from the genome, the mutating and segregating apparent pale selfs might arise from the presence of a gene controller suppressor. A unit of this type would allow the a^{pm} locus to be unaffected by the gene controlling element so that the kernel would appear self colored, and yet retain the potentialities to produce other mutable forms. Postulating different numbers of gene controlling elements and gene controller suppressors, one can explain an array of different ratios.

Similar interpretations of shifts between mutable forms could be postulated for the other cases presented in Table 1. Differences in the

coarse pale mosaic - apparent pale self and the white plus dots - light pale plus dot changes could be due merely to different states of the gene controllers, while the rest of the mechanism is essentially the same.

It is apparent that this mutable system is similar but not identical to the $a_1^{m-1}\text{-Spm}$ system investigated by McClintock. A brief comparison of the two systems is presented below.

a_1^{pm}

1. Originated from a^p allele.
2. Self-colored pale kernels arise: a few of these are stable, but most of them subsequently give rise to mutable forms.
3. Segregation of the gene controlling element may result in two variegated classes or in one self-colored and one variegated class. (Pale self-colorless plus dots segregations are rare if they ever occur.)
4. The segregating classes form poor 1:1, 3:1, and 1:3 ratios which may vary from 1:6 to 5:1.
5. Ears sectored for coarse pale mosaic and white plus dot phenotypes are common.
6. Two ears produced on the same plant may differ in the ratios of their segregating classes, due to gain or loss of a gene controlling element.
7. Germinal mutations give rise to uniformly pigmented alleles some of which may be very stable, and others which mutate at rather high rates.

$a_1^{m-1}\text{-Spm}$

1. Originated from A allele.
2. Self-colored kernels arise when Spm is lost from genome and are stable as long as Spm is absent.
3. Segregation of Spm results in production of a self-colored and a variegated kernel class. (Pale self-colorless with dots segregation is common.)
4. The segregating classes form very good 1:1 or 3:1 ratios depending upon whether one or two Spm units is involved.
5. Ear sectors of self colored areas (loss of Spm) on a variegated ear occur.
6. Two ears produced on the same plant may differ in their Spm constitution, hence differ in the ratios of their segregating classes.
7. Germinal mutations occur in the presence of Spm and result in stable alleles.

Table 1. Ear types produced by various mutable states

Cross: $\frac{a^{pm} Sh_2}{a sh_2} \times \frac{a sh_2}{a sh_2}$ (Only Sh_2 kernels counted)

Type of Ear	Frequency	Pale mosaic	Kernel Type					
			Pale self	Wh.+ dots	Lt.pale + dots			
<u>Planted Pale Mosaic</u>								
Most kernels of the parental type	57% of 35 ears classified		252	2	3	0		
			163	0	3	0		
			305	18	2	2		
			388	0	2	23		
Many of the kernels of the parental type	43% of the 35 ears classified	1:1	88	102	0	4		
			111	94	0	17		
			146	71	3	6		
		3:1	77	29	0	0		
			49	125	0	0		
		1:3	66	151	1	8		
<u>Planted Pale Self</u>								
Most kernels of the parental type	53% of the 28 ears classified		0	177	0	0		
			0	122	0	0		
			4	200	0	1		
			1	142	0	14		
Many of the kernels of the parental type	47% of the 28 ears classified	1:1	87	99	0	7		
			95	103	0	4		
			113	57	0	0		
		3:1	109	22	0	1		
			34	122	0	0		
		1:3	33	206	0	1		
<u>Planted White + Dots</u>								
Most kernels of the parental type	12% of the 34 ears classified		5	4	146	8		
			0	14	192	7		
			0	6	188	26		
Many of the kernels of the parental type	62% of the 34 ears classified	1:1	0	5	130	114		
			0	0	129	142		
			1	3	127	44		
		3:1	0	9	137	58		
			0	1	45	183		
		1:3	0	2	44	186		
			0	46	31	142		
			0	56	27	138		
		Few kernels of the parental type	26% of the 34 ears classified		131	3	3	73
					116	10	2	108
	159			20	6	0		
	79			28	1	2		

