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The *En* Mutable System in Maize

III. Transposition Associated with Mutational Events*[1]

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Summary. 1. The mutable allele, $a_1^{m(pa-pu)}$, of the *En* system at the a_1 locus in maize mutates somatically and germinally to pale, colorless, and purple.

2. Colorless and pale germinal deviants arise at a high frequency. The colorless is more frequent than the pale, and each is more frequent than purple. Frequency is correlated with timing of the somatic mutation event — the earlier colorless sectoring is correlated with the higher frequency of colorless deviants.

3. The regulatory element, *En*, has been identified at the a_1 locus. The origin of colorless and pale deviants is accompanied by the transposition of an *En* element away from the a_1 site.

4. The transposing event may lead to implantation of *En* on the same chromosome, on another chromosome, or no implantation occurs. Transposition to a linked site occurs approximately 25% of the time. There is a preference for transposition to sites 6–20 units from a_1.

5. Secondary transpositions of *En* occur, and in one test, approximately 12% of the time, to an independent position. Secondary transpositions take place to new linked sites.

6. Preliminary data indicate that transpositions can occur to both distal and proximal positions on chromosome 3.

7. Since differences exist in the behaviour of elements in transposition, it is likely that the transposition event probably is dependent on the elements of specific mutable systems and differing elements within a system.

8. Theoretical aspects of diverse types of impairment of normal gene function by inserted elements is discussed.

With mutable loci in maize, the mutation event, generally from the recessive (functionless) to the dominant (functioning) condition, frequently is accompanied by the transposition of an element from the affected locus to a new site. This type of mutational change has been documented for the following systems of mutability in maize: P^{vv} (Brink and Nilan, 1952; Brink, 1958; Orton, 1966; Orton and Brink, 1966), *Ac-Ds* (McClintock, 1951, 1956a, 1956b), and *Dt* (Neuffer, 1963). Hypotheses to explain the mechanism of transposition have been presented by Greenblatt and Brink (1962) and Greenblatt (1966, 1968).

The purpose of this report is to describe the transposition of the *En* element which is associated with mutation of $a_1^{m(pa-pu)}$. This allele mutates with a high frequency to several stable forms. Previous references to the activity of the $a_1^{m(pa-pu)}$ allele (Peterson, 1965, 1968a, 1968b), as well as a description of the *En* system at the a_1 locus, have been presented (Peterson, 1961, 1966).

1. Materials, Methods, Explanations[2]

(i) The $a^{m(pa-pu)}$ allele: This allele originated from the standard color (purple or red, depending on modifying factors) allele A_1 that changed to a_1^{m*}, an allele that backmutates at various rates to A_1, purple phenotype (Peterson, 1961). From the a_1^{m*} allele, a dense type, $a_1^{m(dense)*}$, was isolated. Subsequently a large number of derivative types were obtained, including $a_1^{m(nr)*}$, a colorless type *n*ot-*r*esponding to *En**, and in effect therefore a stable allele. From $a_1^{m(dense)}$, another allele was isolated that also mutates to colorless forms but is distinguished by its mutability to various levels of pale coloration in the aleurone. It was identified as $a_1^{m(pa-pu)}$ and is described in section 3. Additional symbols have been described previously (Peterson, 1961, 1966, 1968c).

(ii) Testers: The *En* tester, the *En* line, and the testcross parent ($a_1\ sh_2/a_1\ sh_2$) have been described previously (Peterson, 1966). (*sh* – refers to sh_2 – on chromosome 3; phenotype, shrunken; the subscript is omitted in some tables for clarity; Sh, phenotype, plump.)

(iii) Explanations of terms: (see table 1 for explanation of gene symbols): *Mutability* refers to instability of an allele and, in the present context, usually is manifest as colored spots or "dots" on a colorless background in the aleurone tissue of a kernel.

A *mutable* allele is one that exhibits mutability.

Timing of mutation refers to the time when the event occurred during ontogeny of the tissue; an early mutation yields large sectors, while a later occurring change yields smaller mutant sectors.

States refer to the phenotypic patterns and are dependent on timing and frequency of mutation events (Peterson, 1961, 1966).

Recombination data were obtained from the cross of $\frac{a_1^{m(r)}\ En}{a_1^{dt}} \times \frac{a_1^{dt*}}{a_1^{dt}}$. Mutable and nonmutable kernels are found in the progeny. The mutable kernels have both $a_1^{m(r)}$ and *En*. All other combinations are nonmutable (i. e., colorless). From the ratio of mutable to colorless, the position of *En* can be determined. If the number of mutable kernels is 25% of the total, *En* is assorting inde-

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[2] See table 1 for a further explanation of symbols and terms — identified with an asterisk*.

Table 1. *Designation of symbols and terms*

Allele or Element	
P^{vv}	= A mutable allele at the *P* locus; variegated cob and pericarp
Mp	= In this context, a regulatory element of P^{vv}; in the specificity test (see regulatory elements) triggers the mutability of *Ds* controlled allele and by definition, similar to *Ac*.
En	= Enhancer, a regulatory element necessary for mutability of *En* system alleles: for example, can be located at the a_1 locus or at a position independent of a_1; exists in several states such as $En^{(slow)}$ and $En^{(crown)}$ (Peterson, 1966), with a^{m-1}, acts similarly to *Spm* as bifunctional unit, *Sp* suppresses the action of the a_1^{m-1} allele until *m* causes a mutation event to occur (McClintock, 1956a, 1961)
Dt	= also a regulator element however specific for mutability of the a_1^{dt} allele
Ds	= dissociator — controlling element that acts like *I*, however it is triggered only by *Ac*, (Activator)
a_1^{m-1}	= one of the alleles at the a_1 locus which is colored (non-variegated) in the absence of *Spm* and variegated (colored spots on a colorless background) in the presence of *Spm* (McClintock, 1956a): represents a tester for the presence of *Spm* and *En*
$a_1^{m(r)}$	= a colorless allele that responds to *En*: with *En*, gives purple colored sectors on a colorless background in the aleurone
$a_1^{m(nr)}$	= a colorless allele originating in the *En* system that does *not* respond to *En*
a_1^{dt}	= a controlled colorless allele, and mutability triggered only by *Dt*
a_1^{m}	= in this report, the general designation of all alleles of the *En* system at the a_1 locus
$a_1^{m(dense)}$	= a mutable allele that results in the "dense" phenotype; the original a_1^{m} allele of the *En* system; *En* normally located at the a_1 locus
Controlling element	= elements such as *Ds* and *I* that suppress gene function when in cis position to the locus
regulatory elements	= elements such as *Ac*, *Spm*, and *En* trigger controlling elements such as *Ds* and *I* so that the locus under control becomes functional
controlled allele	= such as $a_1^{m(r)}$ that expresses a mutant phenotype since it is "controlled" by the presence of a controlling element.

pendently of $a_1^{m(r)}$. If *En* is very close to $a_1^{m(r)}$, 50% of the progeny will be mutable. Values between 25–50% signify varying degrees of linkage. The recombination frequencies listed in table 7 were obtained in the following manner. The percent of mutable kernels ($a_1^{m(r)}$ + *En*) minus 50% equals 1/2 the recombinants. (For example, if $a_1^{m(r)}$ is linked to *En*, $a_1^{m(r)}$ without *En* represents the separation of $a_1^{m(r)}$ from *En* and, in effect, 1/2 of the recombinant events between a_1 and *En*. The reciprocal event cannot be recognized.) Doubling this value yields the recombinant value [therefore, (50% − % of mutable kernels) × 2 = % recombination]. Alternatively, with a_1^{m-1}, $\left(\frac{a_1^{m-1}\ sh\ En}{a_1^{dt}} \times \frac{a_1^{dt}}{a_1^{dt}}\right)$, only 1/2 of the progeny are considered since the parental strand (a_1^{m-1}**En*, and mutable) can be distinguished from the recombinant (a_1^{m-1}, no *En* and pale colored). The percentage of the pale colored among the sum of mutable plus pale colored kernels is an estimate of the recombination between a_1 and *En*.

In order to test the homogeneity of the individual progenies arising from a series of crosses to determine a_1–*En* linkage the parental and recombinant classes were arranged in a two-way table for a contingency chi-square test.

2. Locus Composition Involving Mutable Loci

In the origin of a mutable locus in maize, a functioning locus, such as A_1 (one of several loci responsible for anthocyanin formation), changes to a_1, a recessive nonfunctioning allele. Back mutations of the recessive a_1 allele to A_1 are expressed in somatic tissue as purple dots on a colorless background. Evidence from the study of several mutable systems indicates that the change from A_1 to a_1, for example, follows the insertion of a controlling regulatory element at the locus or at a site inseparable from the locus (McClintock, 1951, 1953, 1956a; Peterson, 1961, 1963, 1968c). This change to the functionless allele is caused by the blockage of gene activity just as any insertion within a cistron would inhibit gene function (ex., the insertion of material — foreign to the locus — causing the extreme polar mutants at the *gal* locus in *E. coli* — Jordan, Saedler, and Starlinger, 1968; Michaelis, Saedler, Venkov and Starlinger, 1969; Shapiro, 1969). Removal of the inhibiting element restores gene activity (McClintock, 1951; Brink and Nilan, 1952) and a mutable phenotype is produced when it occurs sporadically in somatic tissue.

In addition to such changes that restore gene function, there are derived "controlled"* alleles referred to in this context as $a_1^{m(r)*}$. This type of allele responds to the presence of a second element by mutating to the functioning form of the allele. Such an allele is indicative of a system represented by two types of elements — a controlling element that resides at this locus and blocks the functioning of a "controlled" gene, and a second regulatory element that triggers the mutability of the controlled allele at a relatively high rate (Peterson, 1966). In the absence of the regulatory element, the $a_1^{m(r)}$ allele is stable.

Systems of gene control unique to maize: There are a number of alleles at the a_1 locus that have similar properties; under some conditions, they are colorless and stable but will mutate at varied rates in the presence of a second element. One may consider these "controlled" alleles because some element at the locus responds to a specific second element. This second element (the regulatory element in this context

— *En*), however, is specific and will affect only those alleles at a controlled locus which share a common origin. This is illustrated by the following example. In the origin of an unstable allele, a regulatory element (eg., *En*) is found at the mutating locus (Peterson 1961, 1968c). From this original unstable locus, a colorless, nonvariegated form ($a_1^{m(r)}$) arises which is unstable, only in the presence of a specific regulatory element; namely, the one that was originally inserted at the locus. It appears that there was a partial removal of the regulatory allele from the site of the original instability, and it is the response of the portion of *En* remaining at the controlled locus to *En* that results in a mutable phenotype. However other regulatory elements such as *Ac* or *Dt* have no effect on this allele. The response is specific and identifies the elements as the basis of instability rather than the locus itself. From this, it is hypothesized that the regulatory element alone (in this context, *En*) can be the source of a controlling and regulatory element (Peterson, 1970). Thus, the sequence of events would appear as follows:

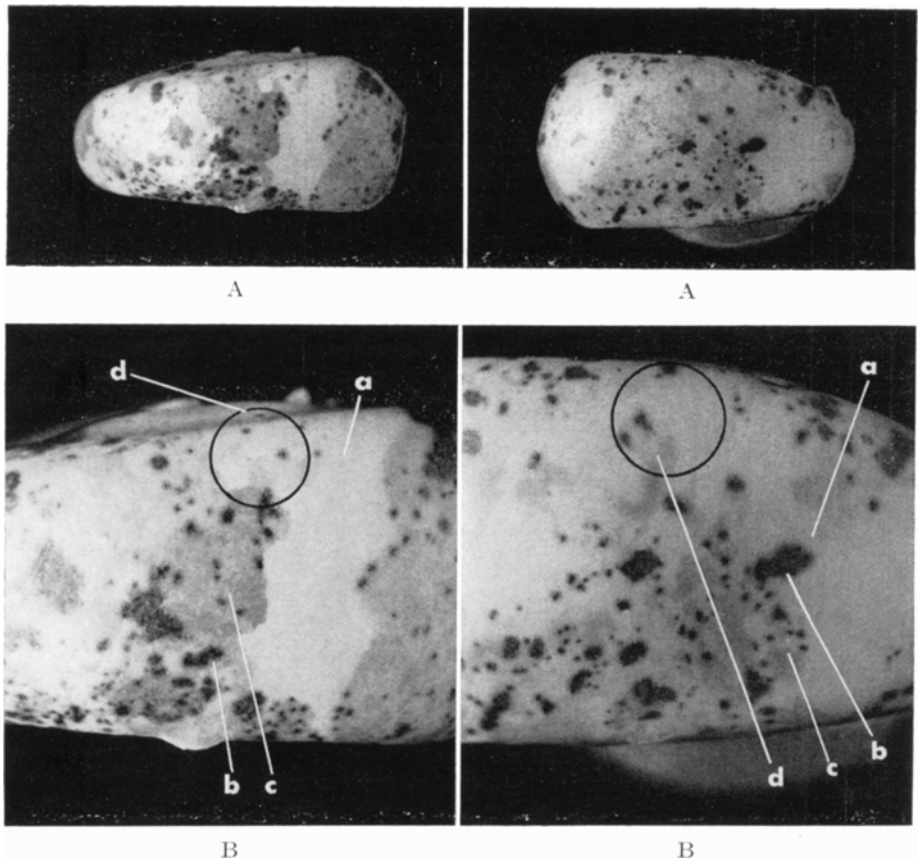

Fig. 1. (A) Two $a_1^{m(pa-pu)}$ kernels. Genotype: $a_1^{m(pa-pu)}/a_1^{m(pa-pu)}/a_1$ — (B) Enlarged sections of the kernels shown in A. (a) colorless; (*b*) purple, (*c*) pale, (*d*) area of a mutation of one $a_1^{m(pa-pu)}$ allele to colorless and other mutating to pale, purple. (Further explanation in text, section 3)

	phenotype
En becomes inserted at the A_1 locus = A_1En	= colored spots on a colorless bkgd.
En transposes away from locus = A_1 + *En*	= colored
or	
En transposes but leaves a residue considered to be *I*	

A_1I = colorless in absence of *En*
A_1I = mutable in presence of *En*

3. Description of the $a_1^{m(pa-pu)}$ Allele

The mutability of the ($a_1^{m(pa-pu)}$ allele is readily observed in the aleurone tissue (Figure 1). Large sectors (representing early mutations) of both the colorless (a) in figure 1 B and pale (c) in figure 1 B occur. The purple spots, (b) in figure 1 B, are smaller and represent later events. The genotype of the kernels, with respect to a_1, is $a_1^{m(pa-pu)}/a_1^{m(pa-pu)}/a_1^{dt}$. The large colorless area is a consequence of a mutation to nonmutating colorless in both $a_1^{m(pa-pu)}$ alleles. In the area outlined in d of figure 1 B, there has been a subsequent change in one of the $a_1^{m(pa-pu)}$ alleles since the purple spots in this area represent mutation to purple. A similar explanation accounts for the purple spots on the pale background (b in figure 1 B). This expression of mutability in somatic tissue (the aleurone) is correlated with the occurrence of stable colorless and pale kernels appearing in the testcross progeny of this allele.

The distribution of phenotypic types found in 18 testcrosses ($a_1^{m(pa-pu)}$ $Sh_2/a_1sh_2 \times a_1sh_2/a_1sh_2$) is given in table 2. The following four classes of progeny appear among the *Sh* kernels: with purple spots, stable colorless, stable pale and, in some of the progenies, purple. The shrunken kernels were not always counted since because of the close linkage of the a_1 and sh_2 genes (0.25 units), the Sh_2 allele marks the kernels receiving the mutable allele. The frequency of the parent allele among the *Sh* progeny varied from 24% to 85%, averaging 55%. The deviant types which included the nonvariegated pale, colorless, and purple

Table 2. *Distribution of progeny types from the cross* $a_1^{m(pa-pw)}Sh/a_1sh$ *or* $a_1^{m-1}sh \times a_1\,sh/a_1sh$

Item	$a_1^{m(pa-pw)}$ parent Pedigree #'s	Genotype	Progeny: Round pale and purple	%	Stable colorless	%	pale	%	purple	%	Total	Shrunken Cl*	dotted	%w/En among sh
1	'3 99B-1	$a_1^{m(pa-pw)}Sh/a_1sh$	84	56.0	31	20.6	35	23.3	0	0	150	—	—	—
2	'3 99B-4	,,	77	55.7	41	29.7	20	14.4	0	0	138	—	—	—
3	'4 67-1	,,	38	24.6	74	48.0	33	21.4	9	5.8	154	—	—	—
4	'4 67-2	,,	30	42.8	23	32.8	16	22.8	1	1.4	70	—	—	—
5	'4 67-4	$a_1^{m(pa-pw)}/a_1^{m-1}sh$	104	55.0	64	33.8	21	11.1	0	0	189	165	20	10.8
6	'4 67-6	,,	59	37.1	56	35.2	38	23.8	6	3.7	159	137	19	12.1
7	'4 67-8	,,	98	49.4	51	25.7	43	21.7	6	3.0	198	no count		
8	'4 68-1	,,	39	48.7	18	22.5	23	28.7	0	0	80	71	13	15.4
9	'4 68-2	,,	73	84.8	4	4.6	9	10.4	0	0	86	74	8	9 5
10	'4 68-4	,,	58	36.2	62	38.7	40	25.0	0	0	160	—	—	—
11	'4 68-5	$a_1^{m(pa-pw)}/a_1sh$	85	50.2	47	27.9	29	17.1	8	4.7	169	—	—	—
12	'4 34-42	,,	64	79.0	11	13.5	6	7.4	0	0	81	—	—	—
13	'4 192-1 × 812	,,	80	66.6	23	19.1	17	14.1	0	0	120	—	—	—
14	'4 192-2 × × 812	,,	55	59.7	19	20.6	18	19.5	0	0	92	—	—	—
15	'4 367-2 × × 812	,,	75	72.8	18	17.4	10	9.7	0	0	103	—	—	—
16	'5 667-1 × × 384	,,	107	56.0	52	27.2	27	14.1	5	2.6	191	—	—	—
17	'5 667-2 × × 385	,,	145	55.5	76	29.1	40	15.3	0	0	261	—	—	—
18	'5 667-4 × × 384	,,	128	64.0	41	20.5	31	15.5	0	0	200	—	—	—

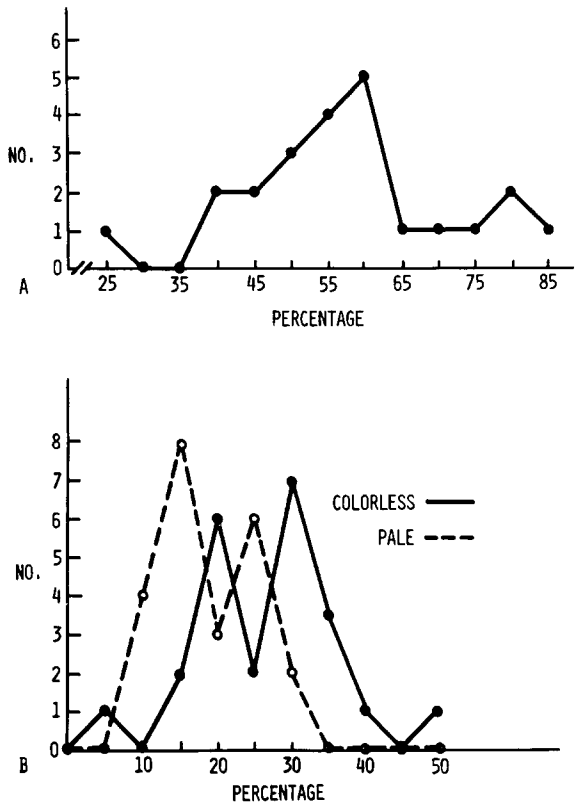

Fig. 2. The distribution of parental mutable frequencies (A) and colorless and pale deviant frequencies (B) among 23 segregating progenies of the testcross of the $a_1^{m(pa-pw)}$ allele ($a_1^{m(pa-pw)}\,sh/a_1\,sh \times a_1sh/a_1sh$)

types, ranged in frequency from 76% to 15% (average 44.7%, table 2, part B). Among all 18 progenies, the colorless were more frequent than the pale types (except for items 8, 9, table 2), and each was more frequent than the purple types. The individual frequencies are in agreement with the timing of the changes in somatic tissue, where, the large size of the colorless nonvariegated sectors indicates that the mutational events occurred early in development while the smaller size of the purple areas suggests later mutations. In all, the frequency of nonparental deviants is indicative of a very high rate of occurrence of changes from $a_1^{m(pa-pw)}$ to the various alleles

Table 2. (*cont.*) *part B. Summary of the frequency of deviants appearing in the testcrosses cited in part A*

deviant	percent: average value	percent: range
colorless	26.1	4.6—48.5
pale	17.4	7.4—28.7
purple	1.1	1.4—5.8
colorless and pale	43.5	
all exceptions	44.7	

*Cl = colored

(approximately 1 of 2 mutable alleles changes to a new form). Figure 2 illustrates the distribution of the various kernel types among 18 progenies.

The excess of colorless deviants over pale varied from 1.5 × to 2× and, in one instance, 3× (item 5, table 2). The frequency of purple was low when compared with the frequency of colorless and pale.

In view of the earliness of the mutation events observed in somatic tissue (aleurones of individual kernels), it is surprising that no ear sectors were observed, though no special effort was made to testcross a large number of ears for this event. The frequency of colorless kernels in one cross (item 3, table 2) was quite excessive, though they did not appear as a sector. The average value for the colorless is 26.1% and, for pale, 17.4% (Table 2,B).

4. *En* content of Deviant Types

(a) Verification of the En content of the $a_1^{m(pa-pu)}$ *stock*

Many of the derivatives were isolated from crosses in which the parent $a_1^{m(pa-pu)}$ mutating allele was heterozygous with $a_1^{m-1}sh_2$ ($a_1^{m(pa-pu)}Sh_2/a_1^{m-1}sh_2$). These plants were testcrossed by a_1sh_2/a_1sh_2. If *En* was present in a heterozygous condition and unlinked to the a_1 locus, half the shrunken kernels in the testcross would be mutable. No deviants were isolated and tested from such a cross since the presence of the independently assorting *En* would complicate the identification of the transposed *En* following the origin of the deviant type. In this manner, any crosses with *En* in the genome, other than at the a_1 locus were discarded. The remaining deviants analyzed were isolated from crosses in which the parent $a_1^{m(pa-pu)}$ allele was heterozygous with a_1sh_2 ($a_1^{m(pa-pu)}Sh_2/a_1sh_2$). Though an independent *En* is not detectable in this heterozygote, each of the $a_1^{m(pa-pu)}$ alleles used had been tested in a cross with a_1^{m-1}, and parents showing an independent *En* were rejected. Examples illustrating the appearance of *En* among usable parental genotypes are shown in items (5, 6, 8, 9 of table 2).

(b) En content of the $a_1^{m(pa-pu)}$ *allele*

The presence of *En* at the $a_1^{m(pa-pu)}$ allele is verified by forming the heterozygote $a_1^{m(pa-pu)}Sh_2/a_1^{m(r)}Sh_2$. The *En* effect on $a_1^{m(r)}$ can be readily distinguished from the mutability pattern of the $a_1^{m(pa-pu)}$ allele (Figure 3) since the mutability pattern of the former lacks the clear areas observed in the $a_1^{m(pa-pu)}/a_1sh_2$ kernels illustrated in Figure 1. In addition, there is a high frequency of purple dots uniformly distributed over the kernel (effect of *En* on $a_1^{m(r)}$) not evident in kernels lacking $a_1^{m(r)}$ (compare with Figure 1). Further, when such a heterozygote is testcrossed by a_1sh_2/a_1sh_2, approximately half the progeny are colorless, indicating that the *En*, along with the $a_1^{m(pa-pu)}$ allele, disjoined from the $a_1^{m(r)}$ allele. This would verify the composition of the $a_1^{m(pa-pu)}$ allele with regard to *En* as A_1En.

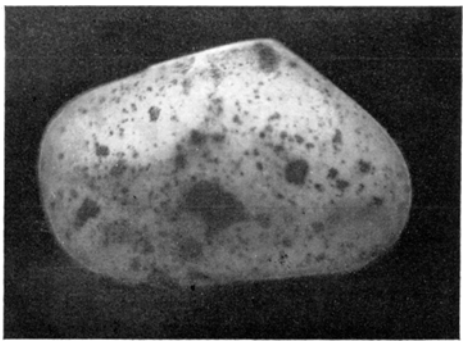

Fig. 3. Aleurone of $a_1^{m(pa-pu)}/a_1^{m(pa-pu)}/a_1^{m(r)}$ from the cross $a_1^{m(pa-pu)}/a_1 \times a_1^{m(r)}/a_1^{m(r)}$

(c) Differentiating the deviant types with respect to En

Since the parent $a_1^{m(pa-pu)}$ allele is associated with *En*, the question was asked as to the disposition of *En* in the changes from $a_1^{m(pa-pu)}$ to nonmutating colorless or pale. This can be tested by crossing the deviant colorless and pale types to one of the two *En* testers, $a_1^{m(r)}$ or a_1^{m-1}. Two phenotypes are observed: (1) kernels with no mutability, indicating the absence of *En*, and (2) kernels with mutability, signifying the presence of *En* (Figure 4).

The presence of *En* in a pale or colorless deviant is an indication that these are $a_1^{m(nr)}$, nonresponsive to *En*, and they are distinguishable from $a_1^{m(r)}$, the responding types. The occurrence of *nr* types has previously been reported for the *En* system (Peterson, 1961, 1968c). The following discussion will be concerned only with tests of the nonmutating pale or colorless types.

(i) The absence of *En*. As indicated, no mutability was observed in some of the crosses of the pale deviants with an *En* tester $\left(\frac{a_1^{m(pale)}}{a_1}\frac{Sh_2}{sh_2} \times \frac{a_1^{m(r)}}{a_1^{m(r)}}\frac{Sh_2}{Sh_2}\right)$. The presence of *En* in the pale parent would have triggered mutability of the $a_1^{m(r)}$ allele. In these crosses, no mutability indicates the absence of *En* in the derivative pale or colorless kernel. The frequency of this (no-*En*) class among the pale and colorless types is listed in column 3 of table 3.

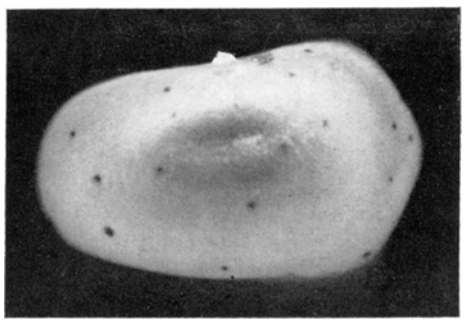

Fig. 4. Kernel type originating from a cross of a stable pale deviant with $a_1^{m(r)}$. Kernel genotype, $a_1^{m(pale-nr)} - En/a_1^{m(pale-nr)} - En/a_1^{m(r)}$. Spot frequency dependent on new *En* as well as state of the $a_1^{m(r)}$ allele

Table 3. *Disposition of the deviant types with respect to En originating from a testcross of the parental* $a_1^{m(pa-pu)}$ *allele* ($a_1^{m(pa-pu)}$ *Sh*/a_1*sh* × a_1*sh*/a_1*sh*)

A	1 deviant	Distribution of *En* 2 Total no.	3 none	4 independent	5 linked	6 % linked
	non-variegated pale	136	51	52	33	24.26
	non-variegated colorless —	147	56	45	46	31.29
	Totals	283	107	97	79	27.9 (ave.)

B			Distribution of *En*							
			non-variegated pales				non-variegated colorless			
$a_1^{m(a-pu)}$ Sh_2/a_1sh_2*		gave rise to	Total no.	none	independ.	linked	Total no.	none	independ.	linked
1	'4 68-4	'5 357	10	2	7	1	18	4	13	1
2	'4 68-5	'5 422-2	7	3	3	1	14	12	1	1
3	'4 68-2	'5 354		none tested			3	9	1	2
4	'4 68-1	'5 432	9	2	7	0	7	2	5	0
5	'6 0858-10	'6 0858-10	11	9	1	1	14	10	2	2
6	'6 0859-4	'6 0859-4	10	6	3	1	11	3	3	5
7	'6 0856-2	'6 0856-2	12	7	4	1	9	5	1	3

* testcrossed by a_1 *sh*/a_1*sh*

Table 4. *Assorted tests selected to illustrate the determination of the location of En among deviant types originating from the cross* $a_1^{m(pa-pu)}Sh/a_1$ *sh or* $a_1^{m-1}sh$ × a_1sh/a_1sh

Item	Genotypic designation of cross: origin of mutant	Round mutable	Round not.-mut.	Shrunken mutable	Shrunken Cl**	Total	percent mut/total	percent mut. sh./sh
	$a_1^{m(nr)}Sh$ *En*/$a_1^{m(r)}Sh$ × a_1sh/a_1sh							
1	'5 221-1	86	80	—	—	166	51.8	—
	$a_1^{m\,pale\,(nr)}Sh$ *En*/a_1^{m-1} *sh* × a_1sh/a_1sh							
2	'5 348-1	—	—	11	155	166	—	6.6
	$a_1^{m\,pale(nr)}$ *Sh En*/a_1sh × $a_1^{m-1}sh/a_1^{m-1}sh$						Recombinants Total	
3	'5 350	92	18	19	67	196	18.8*	—
4	'5 562-1	24	2	4	19	49	12.2*	—
5	'6 0858-4-1	—	—	8	311	319	—	2.5*
6	-2	—	—	11	147	158	—	6.9*
7	-3	—	—	30	137	167	—	17.9*
8	-4	—	—	179	204	383	—	46.9
9	-5	—	—	59	541	600	—	9.8*
	$a_1^{m(rn)}Sh$ *En*/$a_1^{m-1}sh$ × a_1sh/a_1sh							
10	'5 354-1	87	21	26	97	231	20.3*	—
11	-2	48	53	45	49	195	50.2	—
12	-3	63	38	22	72	195	30.7*	—
	$a_1^{m(nr)}Sh$ *En*/a_1sh × $a_1^{m-1}sh/a_1^{m-1}sh$							
13	'5 457-2	101	26	14	93	234	17.0*	—
14	'5 467-1	113	73	62	127	375	36.0*	—
15	-2	112	63	62	119	356	35.0*	—
16	'5 549-1	58	6	4	42	110	9.0*	—
17	'5 551-1	116	24	22	92	254	18.1*	—
18	-2	—	—	52	54	106	—	49.
19	-3	106	18	16	96	236	14.4*	—
20	'5 554-1	67	10	7	69	153	11.*	—
21	-2	99	10	6	101	216	7.4*	—
22	-3	—	—	54	59	113	—	47.7
23	'6 0858-10-1	35	28	22	30	115	43.4	—
24	-2	27	34	20	28	109	47.7	—
25	-4	21	7	6	18	52	25.*	—
26	-5	37	41	35	22	135	53.3	—
27	-6	30	16	6	24	76	28.9*	—

* Recombination values ** Cl = colored

(ii) The presence of *En*. The presence of *En* could be detected readily by the appearance of mutability in crosses of the deviant kernels to $a_1^{m(r)}$ or a_1^{m-1} (Figure 4). Two types of crosses were utilized.

cross 1

$$\frac{a_1^{m(pale)}\ Sh_2\ En}{a_1 \quad sh_2 +} \times \frac{a_1^{m-1}\ Sh_2}{a_1^{m-1}\ Sh_2}$$

or

$$\frac{a_1^{m(nr)}\ Sh_2\ En}{a_1 \quad sh_2 +} \times \frac{a_1^{m-1}\ Sh_2}{a_1^{m-1}\ Sh_2}$$

cross 2

$$\frac{a_1^{m(pale)}\ Sh_2\ En}{a_1 \quad sh_2 +} \times \frac{a_1^{m(r)} Sh_2}{a_1^{m(r)} Sh_2}$$

or

$$\frac{a_1^{m(nr)}\ sh_2\ En}{a_1 \quad sh_2 +} \times \frac{a_1^{m(r)}\ Sh_2}{a_1^{m(r)}\ Sh_2}$$

Because the two crosses are handled differently, each of the crosses will be discussed separately. In the following discussion, remember that sh_2 is closely linked to a_1 and, therefore, that the plump (Sh_2) kernels provide a means of following a_1.

The use of cross 1 leads to an immediate determination of the location of *En*. The presence of an independently assorting *En* (i. e., independent of the a_1 locus) results in mutability in approximately half the shrunken kernels (items 8, 18, 22, 23, 24, 26 of table 4). Values significantly less than 50% mutable shrunken kernels are an indication of linkage of *En* with a_1 (table 4, items with an asterisk).

The use of cross 2 to detect *En* does not lead to an immediate appraisal of *En* location, as in cross 1, if a colorless deviant is being tested, but a linkage value can be obtained if a pale deviant is tested (table 5). If *En* is present in a colorless deviant, half the progeny will be mutable irrespective of location. When mutable kernels ($a_1^{m(nr)}Sh_2/a_1^{m(r)}$, *En*) are testcrossed by a_1sh_2/a_1sh_2. 1/4 of the resulting progeny (only $a_1^{m(r)}$ plus *En*) will be mutable if *En* is independent. Any deviation less than 25% would be an indication of the linkage of a_1 with *En* (for example, from the genotype $\frac{a_1^{m(nr)}\ Sh_2\ En,}{a_1^{m(r)}\ Sh_2 +}$ only $a_1^{m(r)}\ Sh_2\ En$ arising from a crossover will be observed as mutable).

When a pale deviant is being tested, however, the use of cross 2 leads to a more direct means of locating *En*. If *En* is assorting independently, the progeny from such a cross will yield equal numbers of mutable and stable with a pale background and of mutable and stable with a colorless background.

If *En* is linked with the $a_1^{m(pale)}$ deviant, the recombinants are detectable among the progeny (cross 2). A typical cross is illustrated in table 5A. Examples of two isolated deviants (table 5A) show distance values of 10.3 and 15.4 between a_1 and *En*. The 10.3 value of isolate − 1 is confirmed by testcrossing with a_1sh_2/a_1sh_2 (table 5B). In this cross, only one of the recombinant classes can be identified; namely, the mutable with a colorless background. The reciprocal product, the stable pale with no *En*, cannot be distinguished from the stable pale with *En* (pale is an *nr* type). Assuming that the recognizable recombinants (mutable, colorless background) represent half the total recombinant events, then the resulting figure of this event (6.78% − 19/280) is doubled (13.5), and this is in fair agreement with the 10.3 value obtained in the original test.

Identification of these classes of exceptions, which originated as products of a testcross of the $a_1^{m(pa-pu)}$ allele, differentiates the three classes — no *En*, an independent *En*, and a linked *En*. It has previously

Table 5. *A selected cross to illustrate the determination and confirmation of a pale deviant crossed to an En tester*

A phenotype — nonvariegated pale × colorless

genotype — $\frac{a_1^{m(pale)}Sh_2\ En}{a_1 \quad sh_2 +} \times \frac{a_1^{m(r)}Sh_2}{a_1^{m(r)}Sh_2}$

progeny	colorless bkgd.		pale bkgd.		
	stable	variegated	stable	variegated	
	1	2	3	4	%
	P	R	R	P	R/T
-1	139	15	19	156	10.3
-2	119	24	21	128	15.4
genotypes	$\frac{a_1 \quad sh_2}{a_1^{m(r)}\ Sh_2}$	$\frac{a_1sh_2\text{-}En}{a_1^{m(r)}Sh_2}$	$\frac{a_1^{m(pale)}Sh_2}{a_1^{m(r)}Sh_2}$	$\frac{a_1^{m(pale)}En}{a_1^{m(r)}Sh_2}$	

B confirmation of -1

phenotype variegated pale

the cross $\frac{a_1^{m(pale)}Sh_2\ En}{a_1^{m(r)} \quad Sh_2 +} \times \frac{a_1sh_2}{a_1sh_2}$

progeny	124	19	137	6.78 = 1/2 of value
assumed genotypes	$a_1^{m(r)}Sh_2$-no *En*	$a_1^{m(r)}Sh_2$-*En*	$a_1^{m(pale)}Sh_2$-*En*	
			$a_1^{m(pale)}Sh_2$-no *En*	2 × 6.7 = 13.5%

been shown that the mutable parent was used only if it did not contain an independently assorting *En*. Thus, the instances of deviants containing an independent or a linked *En* represent coincident events associated with the origin of the deviant kernels. Further, the wide variety of a_1En linkages arising from a single parental allele provides ancillary support for the hypothesis that each *En* location represents a new insertion associated with the origin of the deviants. If one were to argue that *En* was in such a linked position initially, the derivatives arising from one parent should all show *En* to be positioned similarly. Further, the other two classes of exceptions would not be expected in the frequency in which they occur. The number found with an independent *En* and those without *En* are indicative of the diverse events associated with the origin of these derivatives, incorporation at a linked chromosomal site, at an independent position or nonincorporation.

(d) Analysis of the classes of deviant types

By utilizing the procedures just described to locate *En* among the tested deviant types, it is evident that approximately 27% contain a linked *En* (table 3 A, column 6). The frequency of linked *En* among the colorless, although slightly higher than that observed among the pale class, is not significantly so. Thus, it appears that the incidence of an *En* linked to its former site at the a_1 locus (after emission from that locus) is approximately equal among the colorless and pale kernel types.

The approximate equality of the independent *En* class and the class without *En* (table 3, columns 3 and 4) could be accounted for by segregation of *En* during meiosis. When individual progenies are examined, however, it is evident that they show a *non-uniform* distribution of the types. As an example, the progeny of two sib plants, representing a part of the data contributing to table 3 A, show a disparate distribution (items 1 and 2, table 3 B). Although both show a low incidence of a linked *En*, '4 68—4 has a large number of *En* locations that are independent, but 468—5 is mostly without an *En*. Recall that each of the deviants results from an individual event. Although the remaining progenies in table 3 B are small, it is obvious that the classes are not uniform with respect to the disposition of *En* following a mutation event.

(iii) Secondary transposition of *En*. Emission of *En* from the locus at the time of the origin of deviants is not unexpected. When *En* is tested in linkage tests at the new site, it is necessary to first link it with $a_1^{m(r)}$ or a_1^{m-1}.

The linkage of *En* with a_1^{m-1} (a_1^{m-1} — *En*) reveals the kinds of observable events associated with changes of *En*. The standard phenotype of the a_1^{m-1} allele is pale colored but becomes colorless and shows purple colored spots in the presence of *En*. Thus, kernels containing a_1^{m-1} and *En* show a mutability pattern of purple spots on a colorless background, (a) in figures 5 A and 5 B. Gene functioning of the a_1^{m-1} allele (pale coloration) becomes suppressed (colorless background, figures 5 A and 5 B), and the allele mutates to purple in the presence of *En*. During development of the aleurone, however, pale color reappears in the form of various sized sectors (b in figures 5 A and 5 B). The pale spots express the a_1^{m-1} phenotype and are due to the absence of *En* activity and may occur as relatively late, small pale spots, (b) in figure 5 A. Occasionally, relatively early changes occur (large pale spot — b in figure 5 B). The colorless sectors within the pale area, (c) in figure 5 B, are either the result of a reactivation of *En* (thereby suppressing the a_1^{m-1} gene activity) or the loss of the a_1^{m-1} allele, which permits the expression of the colorless a_1 allele.

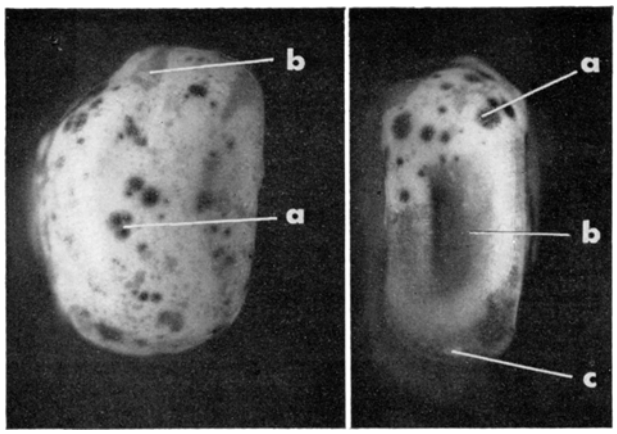

Fig. 5. Kernels of the endosperm genotype a_1^{m-1} *sh En*/a_1^{dt} *sh* + a_1^{dt} *Sh* +. (a) purple spots from the mutability of the a_1^{m-1} allele, (b) pale coloration expressing the a_1^{m-1} phenotype (c) non-colored sectors in the midst of pale coloration (explanation in text, section 4, iii)

5. The Linked *En*

Several questions arise relative to the transposition event associated with the newly inserted *En*. First, is there a preferred site, or does *En* become inserted at a random position in chromosome 3? And with what permanence does *En* reside at this new location? The following results are preliminary.

With regard to the first question, the positions of the linked *En* recovered from isolates obtained in 1964 and those from 1965 that have been verified with further tests are as follows:

linkage distance	0—5	6—10	11—15	16—20	21—25
number	3	13	18	10	7
linkage distance	26—30	31—35	36—40	40—45	
number	5	7	3	1	

Most of the new insertions are distributed between 6 and 20 units from the a_1 locus. Only three are lo-

cated close to the a_1 locus, and there is a relatively even distribution beyond 16 units. Since these are two-point linkages, the direction away from a_1 cannot be verified. It is reasonable to assume, however, that those linkages beyond 20—30 represent proximal positions of *En* with respect to the A_1 locus. It appears likely that there are approximately 20 units to the distal terminus from a_1 on chromosome 3. If one considers that the distal marker, *et*, is 13 units from a_1 and three crossover units proximal to a break at position .95 in the long arm (Rhoades and Dempsey 1953, 1966) the distance from the a_1 point to the .95 position on 3L is 16 units (13 + 3). Although there is no way of determining the amount of exchange in the distal. 05 portion of 3L it appears from preliminary three-point tests that there are from 4 to 10 units in this region. The determination of the exact location of the newly inserted *En* must await the results of 3-point linkage tests, which are currently being analysed. It is probable that the cross-over values above 30 units occur when *En* is at sites proximal to a_1.

On the other hand, the precise location of an *En* close to a_1 is difficult to accurately ascertain since any loss of *En* will be registered as a cross-over, thus lengthening the a_1-*En* distance. Although the exact frequency of *En* losses is not readily determined, phenotypic evidence indicates that loss does occur (Figure 5).

There are two ways to consider the question on the permanency of the *En* position. Change from a linked to an independent position can be detected. For one isolate, '4 36—4, 44 separate tests were made to determine the linked position. Among these, five showed *En* transposition to an independent position. From this small test, it is evident that 1 of 8 linked *En*'s transposes to an independent location. A similar example for a progeny test of a deviant ratio is illustrated in table 6 for '4 36—8.

A second way to record changes in initial insertion is to look for new linkages. In the verification of a linkage, an array of values is obtained. If these are subjected to a contingency X_2 test, the X_2 may or may not be significant. If it is significant, the array is examined for obvious deviant values. If these are removed and the contingency X_2 test is based on the remaining data, the X_2 is not significant. Arrays of 6 sets of data are presented in table 7. Included are the values that were obtained for several isolated *En* positions. The deviant values that resulted in significant X_2 tests are indicated by an asterisk, and these were removed for the final assay. These deviant values are currently being tested in progeny tests for verification of their deviation from the average of the tested isolate.

6. Discussion

In the mutability of the $a_1^{m(pa-pu)}$ allele, the transposition of a locus-situated regulatory element, *En*,

Table 6. *Data illustrating the identification and verification of a change of a linked En to an independent position. Original En isolate-'4 36-8.*

The cross $\frac{a_1^m shEn}{a_1^{dt}\ +} \times \frac{a_1^{dt}}{a_1^{dt}}$	
	% (mutable/total)*
'8 0434-1	47.3
-2	39.9
-3	22.0 — independent
-4	40.0
-5	37.7
-6	45.7
-7	48.7
	mutable kernels from '8 0434-3 were testcrossed
'9 0436-1	26.9 }
-2	27.7 } independent
-3	27.0 } verified
-4	23.4 }
	mutable kernels from '8 0434-5 testcrossed
'9 0437-1	24.9 — independent
-2	42.7
-3	35.1
-4	41.6
-5	45.3
-6	26.2 — independent

*relation to the recombination value is decribed in section 2.

Table 7. *Data illustrating the array of recombination values obtained from the testcross of an En linked to a_1 and tested for uniformity with a contingency chi square test*

isolated strand	pedigree of test	a_1-*En* distance*	isolated strand	pedigree of test	a_1-*En* distance*
'4 36-4	'8 0422	12	'4 36-8	'7 0406	14.1
		16			8.7
		16			8.5
		9.0			16.3
		9.5			ns
		9.6			
		(4.6)**			
		ns			
'4 36-5	'8 0425	14.4	'2 106B	'9 0323	17.2
		20.7			14.0
		10.2			15.1
		(7.1)			
		(4.4)			(32.0)
		(25.7)			ns
		ns			
'4 36-6	'7 0401	16.7	'4 67-3	'8 1456	30.3
		11.8			30.7
		15.5			21.4
		13.5			30.7
		10.4			30.4
		16.6			31.0
		21.2			30.1
		16.2			ns
		12.9			
		ns			

* distance obtained from the number of mutable as described in section 2.

** values in parenthesis are deviant values and are not included in the contingency chi square test for homogeneity.

is coincident with the origin of colorless and pale deviants. It is one of the more unorthodox aspects of mutable genes in maize that an element that has the capacity to be inserted in the linear continuity of the chromosome can be emitted from one site and transposed to a different position. For the a_1^{m-1} allele, the transposed *En* becomes linked to a position on the same chromosome approximately 25% of the time. The remaining transpositions of *En* are to an independent location, or *En* is lost by not becoming implanted. There is direct observational evidence for the loss of *En*. The frequency of actual insertion, therefore, is not in agreement with results obtained with P^{vv}, where obligate implantation of the transposed *Mp* occurs (Greenblatt, 1966, 1968). Diverse destiny of the transposed element also has been found in the *Ac-Ds* system (McClintock 1956a, 1956b).

An additional feature associated with transposition involves site preference. Van Schaik and Brink (1959) reported a preference of the transposed *Mp* for the same chromosome as that emitting *Mp*. Further, preference for a distal position of insertion on the chromosome was predicted, on the hypothecated pattern of chromosome replication (Greenblatt and Brink, 1962) which increased the opportunity for both emission and implantation (Greenblatt 1966, 1968). Although transposed *En* shows a preference for chromosome 3 versus the other nine chromosomes (i. e., 25% transposition to chromosome 3 and the remainder to the other nine chromosomes or nonimplanted), the incidence of transposition on the same chromosome is much less than in the *Mp* cases. That chromosomes can replicate from a distal to a proximal position (Holliday 1965) would permit proximal positions to be sites of implantation, as found for *En*.

Transpositions have been reported for the *Dt* systems (Neuffer, 1963; Doerschug, 1968). In Doerschug's studies, the two transposed *Dt* elements could be differentiated by their predilection for change to *dt*. In both reports (Neuffer 1963; Doerschug, 1968) *Dt*'s occur at new sites while the original site remains seemingly unchanged. Numerous instances of a second and third *En* have been found among the a_1-linked *En* testcross progeny (Peterson, unpublished) again indicating the origin of a second element without an observable change in the first.

The new site for *En* is not necessarily a permanent position. As evident in table 6, there is recurrent transposition when transpositions to an independent position are considered, and perhaps they would be noted even more frequently if all transpositions could be readily registered.

Another feature evident from the study of $a^{m(pa-pu)}$ is the occurrence of qualitatively differentiated alleles appearing at mutable loci. Usually, these differentiated phenotypes express a graded series in a continuous spectrum of mutants (in plants, Demerec, 1935; Rhoades, 1941; McClintock, 1948, 1951; Fincham and Harrison, 1967; and in bacteria, Lederberg, 1952; Sander-Tabaczynska, 1969). Fincham (1967) proposed that the graded phenotypes could be explained by adopting Callan's master-slave model. The applicability of this model to such instances was questioned by Peterson (1969). McClintock (1948) proposed that the phenotypic differences appearing as products of mutation events at the c^{m-2} locus represent the presence of quasi-complementary, tandem units. The effect of the controlling elements on one or the other of these units leads to the differences in phenotypic expression. Alternatively, it can be postulated that these loci may represent aggregates of functionally related genes similar to the *arom* cluster in *Neurospora* (Rines, Case, and Giles, 1969) and that the insertion of the elements at specific sites could lead to the type of phenotypic deviations observed in these studies. It seems, however, that locus ambivalence with respect to expression is accentuated by the presence of controlling elements.

That integration of foreign elements could lead to subsequent malfunctioning of genes has been amply demonstrated in a number of diverse instances (Taylor, 1963; Beckwith and Signer, 1966; Jordan, Saedler, and Starlinger, 1968; Michaelis, Saedler, Venkov and Starlinger, 1969; Shapiro, 1969). Although these instances of insertion lead to gene malfunction, they, unlike the controlling elements in maize, are relatively stable.

The high frequency of individual kernel mutants and the lack of obvious ear sectoring for the $a^{m(pa-pu)}$ allele suggest restricted timing of the transposition event (just before, during or just after meiosis) to yield mutant kernels. Further, the divergence of results with respect to the ultimate destiny of the transposing *En* may be due to a temporal cellular or chromosomal situation that influences implantation on the same chromosome versus another chromosome or none at all.

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